Chapter I

INTRODUCTION

The plant African marigold, botanically known as *Tagetes erecta* L. native to Mexico and other warmer parts of America and neutralized elsewhere in the tropics and subtropics including India and Bangladesh and locally known as Genda Phool belongs to the family Asteraceae syn. Compositae (Batish *et al.*, 2007). It is a small shrub and bears yellowish orange flowers in abundance during the flowering season which lasts for more than 6-8 month (Vasudevan *et al.*, 1997). Marigold flower production is mainly centred in countries like Mexico, Ecuador, Peru, China and India. According to National Horticulture Database 2014-15, Madhya Pradesh is the leading state in the loose flower production of marigold having 89,000 tonnes production. It is very popular as a garden plant and yields a strongly aromatic essential oil (*Tagetes* oil), which is mainly used for the compounding of high-grade perfumes (Khulbe, 2015).

Marigold is grown as an ornamental crop for its flower, which is sold in the market as loose flowers in bulk, or for making garlands. It has some distinctive characteristics, which make them a very valuable commodity with many industrial uses and applications. Their aromatic value enables them to be used as flavorings in both the food and beverage industries. These oils are also widely used in both the cosmetic and pharmaceutical industries (Wonwood, 1990).The oil carves from marigold can cure skin damages, inflammations, diaper irritation and several skin problems and is considered to be the best baby oil and for skin nourishments (Janakiram and Rao, 1996; Raghava, 1998).

Medicinal plants having high therapeutic value are becoming popular in the area of medicine for its less expense, less side effects etc. compared to modern allopathic drugs (Pavithra *et al*., 2012). The beneficial effect of herbal medicine typically results from the combination of secondary metabolites such as glycosides, alkaloids, flavonoids, tannins, gums etc produced in herb (Ahito, 2015). About 70% of Indian population (approximately 1.1 billion) are depending on non-allopathic system of medicine (Thomas *et al.,* 2006). India harboured around 8% of the medicinal plants diversity which is around 0.126% million species of the world (Patel *et al.,* 2001).

Essential oils are natural products that plants produce for their own needs other than nutrition i.e. protection or attraction and are complex mixtures of organic compounds that give characteristic odour and flavour to the plants (Baser, 2010).

Tagetes oil has a wild, sweet, fruity almost citrus-like smell and is yellow to reddish-amber in colour. The essential oils extracted from leaves, stem, flowers of marigold etc. comprise limonene, ocimene, valeric acid and tagetone which is reported to be ant-haemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, diaphoretic and emmenagogue and is valuable in aromatherapy for its powerful skin healing properties (Kirtikar *et al.,* 1975; Shiva *et al*., 2002). Additionally, the decoctions of the leaves of *Tagetes erecta* and *Tagetes patula* have been traditionally used as ant-malarial and as febrifuge (Rasoanaivo *et al*., 1992).

The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The traditional technologies pertaining to essential oil processing are of great significance and are still being used in many parts of the globe. Conventional solvent extraction method has been implemented for fragile or delicate flower materials, which are not tolerant to the heat of steam distillation. Different solvents

including acetone, hexane, petroleum ether, methanol or ethanol can be used for extraction (Areias *et al.,* 2000; Kosar *et al.,* 2005; Pizzale *et al.,* 2002). Hydro-distillation has become the standard method of essential oil extraction from plant material such as wood or flower, which is often used to isolate nonwater-soluble natural products with high boiling point. The process involves the complete immersion of plant materials in water, followed by boiling (Okoh *et al.,* 2010).

Chemical investigations of essential oils in the nineteenth century revealed that many of the compounds responsible for the pleasant odours contained exactly ten carbon atoms. These ten carbon compounds came to be known as terpenes, if they were hydrocarbons and terpenoids if they contained oxygen and were alcohols, ketones, or aldehydes. Eventually, it was found that there are also minor and less volatile plant constituents with fifteen, twenty, thirty, and forty carbon atoms. Because compounds of ten carbons were originally called terpenes, they came to be called terpenoids if they contained oxygen and were alcohols, ketones, and aldehydes (Pavia *et al*, 2005).

The estimation of chemical components of oil is possible by various methods of which Fourier Transform Infrared (FT-IR) spectroscopy is a high-resolution analytical technique to identify the chemical constituents and elucidate the structural compounds (Hussain *et al.,* 2007).

Direct Analysis in Real Time (DART) is an atmospheric pressure that instantaneously ionizes gases, liquids and solids in open air under ambient conditions. Direct Analysis in Real Time (DART) has been coupled to the AccuTOF atmospheric pressure ionization mass spectrometer to permit high resolution, exact mass measurements of gases, liquids and solids (Cody *et al.,* 2005).

There is a huge demand for essential oils worldwide and they have been traded internationally for several centuries. There is hence, a need to improve the quality and quantity of the essential oils produced as they have a very competitive and profitable market worldwide. The present study was planned with the hypothesis that generally *Tagetes minuta* (wild marigold) is used for extraction of oil and to study their phytochemicals but nowaday's African marigold (*Tagetes erecta* L.) is a very important flower commonly grown as loose flower for use for religious purposes and for decoration in marriages. The flowers are offered to the deities in temples and are thus available in huge quantities as temple waste. Most of these flowers are either dumped by the side of river Ganga or allowed to naturally decay and used as compost. Post harvest processing of marigold for its oil, extracted from its flower as well as its plant parts may enhance value of the crop multifold since marigold is reported as a rich source of bio-colour, pigments and bioactive molecules which may be exploited in the food and pharmaceutical industry. This technology may also be used for waste management of flowers which are generally discarded after their use in temples; marriage decoration etc.

To best of our knowledge, limited work has been reported regarding oil extraction from African marigold plant parts and their phytochemicals screening. Therefore, this study was planned with the following objectives:

1. To standardize the method for extraction of essential oil from marigold plant parts.
2. Qualitative estimation of essential oil from marigold plant parts.
3. Quantitative estimation of essential oil from marigold plant parts.
4. Comparative study of the yield of the essential oil from different flowering seasons.

Chapter II

REVIEW OF LITERATURE

Essential oils are frequently referred to as the “life force” of plants. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total. That's why tons of plant material is required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavour and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics.

This chapter covers up-to-date literatures on essential oils including, extraction methods, bioactivities, and their applications.

The present study on the topic “A Study on the Extraction of Essential Oil from Marigold (*Tagetes erecta* L.) and its Characterization**”** has been designed to investigate the various aspect required for commercialization of this crop for oil extraction. The study is categorized according to the designated objectives on which the literature was reviewed and are quoted below accordingly.

2.1. African marigold

The plant African marigold also a medical plant (Pérez *et al.,* 2006)is classified as: Kingdom: Plantae; Phylum: Angiosperms; Class: Eudicots; Order: Asterales; Family: Asteraceae; Subfamily: Asteroideae; Tribe: Tageteae.**(**Joy,2001).It contains 56 species of herbaceous plant in sunflower family. *Tagetes* genus is

originated in North and South America. Now-a-days it is also cultivated in Asian countries like India, Bhutan, Nepal, and China (Cruz-Ramírez Luís Alfredo, 2006).

2.2. Plant essential oils

Plant essential oils (EOs) also known as volatile oils or ethereal oils are volatile, natural aromatic complexes, formed by certain plants as secondary metabolites (Prabuseenivasan *et al*., 2006). They are usually the complex mixture of natural compounds, both polar and nonpolar compounds (Masango, 2005).

Essential oils are isolated from various parts of the plant, such as leaves (basil, patchouli and cedar), fruits (citrus), bark (cinnamon), root (ginger), grass (citronella), gum (myrrh and balsam oils), berries (pimenta), fruits (bergamot, orange, lemon, juniper), seed (caraway), flowers (rose and jasmine), twigs (clove stem), wood (amyris), heartwood (cedar), rhizomes (ginger, calamus and curcuma) and saw dust (cedar oil) (Burt, 2004; Hussain *et al*., 2008 and Chi, 2013). They are not produced by all plants but rather, their occurrence is restricted to well over 2000 plant varieties from about 60 different families. However, only about 100 varieties are the basis for the economically important production of essential oils in the world (Chi, 2013).

Essential oils have been studied extensively for their antimicrobial properties among other biological activities. Essential oils containing mixtures of volatile substances, such as monoterpenes, sesquiterpenes and/or phenylpropanoids, esters, alcohols, and terpenoids(Bakkali *et al.*, 2008 and Mohamed *et al.*, 2010) among other constituents have been reported to have antibacterial, antifungal, antiviral, nematicidal and insecticidal properties (Centeno *et al*., 2010; Silva *et al.*, 2012 and Pooja *et al.*, 2013). Since essential oils could contain up to 100 different phytochemicals, they have multiple modes of action against bacteria (Lambert *et al*., 2001). Their antibacterial modes of action includes; interference and destabilization of the

phospholipids bi-layer of the cell membrane, enzyme systems, and genetic material (Kim *et al*., 1995). Fungal growth inhibition by essential oils just like in the case of bacteria involves multiple modes of action such as prevention/reduction of hyphal growth and sporulation, alteration of cell wall composition, induction of lysis and cytoplasmic evacuation (Kishore *et al.*, 2007).

2.2.1. Essential oil from leaves of marigold

The leaf oil of *Tagetes erecta* was later reported to contain a total of forty four constituents with major constituents being limonene, terpinolene, (Z)-myroxide, piperitone and piperitenone (Krishna *et al*., 2004). The essential oil of the leaves of *Tagetes erecta* showed the presence of d-limonene, α-pinene,(Baslas *et al*., 1981)β-pinene, dipentene, ocimene, β-phellandrene, linalool, gereniol,(Baslas and Singh, 1980) menthol, tagetone, nonanal and linalyl acetate. It also contain camphene, sabinene, myrecene, (z)-β-ocimene,(Gupta and Bhandari,1975)(E)-β-ocimene, ΄Υ-terpinene, terpinolene, p-mentha1,3,8-triene,terpinen-4-ol, p-cymen-9-ol, piperitone, thymol, indole, carvacrol, piperitenone, geranyl acetate, belemene, cyperene, β-caryophyllene , (E)-β-farnesene, ΄Υ-muurolene, ΄Υ-elemene, and nerolidol(Machado *et al*., 1994).

About 19 phytochemicals were identified from methanol extract sample of leaves of *Tagetes erecta*. The major bioactive compound present are Tetra decanoic acid, 2,6,10- Trimethyl 14 – ethylene – 14 – Pemtadecme, N – Hexadecanmic acid, 15-Hydroxy penta decanoic acid and Stigmasterol. About 31 phytochemicals were identified from methanol extract sample of flowers, the major are Hexadecanoic acid, 7-Tetra decenal (z), Vitamin E and Norolean – 12 – Ene (Devika and Justin, 2014). The major biocomponent of flowers of *Tagetes erecta* is carotenoid; includes all trans

and cis isomers of zeaxanthines (5%), all trans and cis isomers of lutein, and lutein esters (88%) (Leigh *et al.,* 1999).

Dhingra and Dhingra, (1956) reported that the essential oil of the leaves, flowers and stems of *Tagetes patula* was reported to contain ocimene, limonene, linalool, linalyl acetate and tagetone. The steam distillation of fresh leaves offer 0.3% of essential oil with a strong, sweet lasting odor and contains d-limonene, ocimene, l-linalyl acetate, l-linalool tagetone, n-nonyl aldehyde, lutein (Ghosh,.2004).

Six compounds were identified from the stem and leaves of *Tagetes erecta* plant as 4'-methoxy-eupatolitin-3-O-glucoside, kaempferitrin, rutin, beta- sitosterol, daucosterol and gallic acid (Zang and Zhang, 2010). Tygadlo *et al.,* (1994) investigated that both the flowers and leaves of *Tagetes* contain terpenoids, the component and composition of the extracts varying with the species. The characteristic components of essential oil of *T. erecta* were piperitone, limonene, dehydrotegetone, (Z)-tagetone, terpinolene, E-β-ocimene, linalool, methyl eugenol.

2.2.2. Essential oil from flowers of marigold

The oil from the flowering shoots of *Tagetes erecta* contained piperitone and caryophyllene.(Hethelyi *et al*., 1987), d-limonene, ocimene, 1-linalyl acetate, 1linalool, tagetone and n-nonyl aldehydes(Sharma *et al*., 1961),aromadendrene, phenylethyl alcohol, salicylaldehyde, phenylacetaldehyde, 2-hexen-1-al, eudesmol, tagetone, ocimene, linalyl acetate(Handa *et al*., 1963),. 3,7-dimethyloct-1-en-6-one, 3,7-dimethyl-5-hydroxyoct-1-en-6-one and 3,7-dimethyloct-1,7-dien-6-one(Garg *et al*., 1998),myrecene, caryophyllene, p-cymene, d-carvone and eugenol (Baslas *et al*., 1981). *Tagetes lucida* was reported to contain linalool, estragol and methyl eugenol, *Tagetes minuta* contain β-ocimene, dihydrotagetone, tagetone and ocimenones.

geraniol (Razdan, 1984),(Z)-β-ocimene(Chalchat *et al*., 1995),trans-ocimenone, *cis*-ocimene, dihydrotagetone, *cis*-tagetone, *cis*-ocimene, *cis*-ocimenone, *trans*-ocimenone, limonene, allocimene and *cis*-tagetone (Hadjiakhoondi *et al*., 2008).*Tagetes patula* contain limonene, α-terpinolene, tagetone, ocimenone, piperitone, piperitenone and caryophyllene (Hethelyi *et al*., 1988).

Twenty two naturally occurring phytoconstituents were isolated from the various fractions of ethanolic extract of flower. They were β – sitosterol(Kojima *et al.*, 1990), β- daucosterol (Zhou *et al.*, 2007), 7-hydroxysitosterol (Greca *et al.*, 1990), lupeol (Liu and Kong, 2005; Xue *et al.*, 2008), erythrodiol (Antonio *et al.*, 1981), erythrodiol-3-palmitate (Shaheen and Aneela, 2004),1-[5-(1-propyn-1-yl)-[2,2-bithiophen]-5-yl]-ethanone (Tsumotu *et al.*, 1986; Wei *et al.*, 1997; Hai and Yue, 2008), α- terthienyl (Coogan and Horn, 1965), quercetagetin (Huang, 2006), quercetagetin- 7-methyl ether (Vilegas *et al.*, 1999), quercetagetin-7-O-glucoside (Nair *et al.*, 1995), kaempferol (Xio *et al.*, 2006), syringic acid (Yang *et al.*, 2003), gallic acid (Huang, 2007), 3-β-galalctosyl disyringic acid (Huang, 2007), 3 α galalctosyl disyringic acid (Huang, 2007), 6-ethoxy-2,4-dimethylquinoline (Gallagher and Stahr, 1980), oplodiol (Werner and Kinzo, 1983; Takahashi and Takani, 2000), (3S,6R,7E)-hydroxy-4,7-megastigmadien-9-one (Brigida *et al.*, 2004), palmitin (Wu *et al.*, 2005), ethylene glycol linoleate (Wang, 2007), and n-hexadecane (Huang, 2007).

Marotti *et al*., (2004) stated thirty five compounds present in the essential oil of the leaves and flowers of six *Tagetes* species *viz. Tagetes erecta, Tagetes filifolia, Tagetes lucida, Tagetes minuta, Tagetes patula* and *Tagetes tenuifolia*. Among the six species analyzed, the essential oil of the four species including *Tagetes erecta, Tagetes minuta, Tagetes patula* and *Tagetes tenuifolia* were reported to possess the

same pool of components *viz.* dihydrotagetone, tagetones, ocimenones and piperitone, while remaining *Tagetes* species: *T. filifolia* and *Tagetes lucida* had methyl chavicol as the main constituent.

Eighteen components were reported from the essential oil of the aerial parts of *Tagetes maxima* and *Tagetes multiflora* whereby (Z)-tagetone, dihydrotagetone and (E)-ocimenone were identified as major components in the oil of *Tagetes maxima* while the oil of *Tagetes multiflora* contained (Z)-tagetone,(E)-ocimenone and (Z)-β – ocimene as major components (Pichette *et al*., 2005).

De Feo *et al*., (2005) stated sixty five compounds were identified in the essential oil of the aerial parts of *Tagetes terniflora* with the main components being tagetones, ocimenes, ocimenones, (E)-β-ocimene, *trans*tagetone, limonene, isomenthone, spathulenol, *cis*anethole and *trans*anethole. Maurer and Hauser (1984) reported that a monoterpenoid, 5-isobutyl-3-methyl-2-furancarbaldehyde was present in the essential oil of the flowers of *Tagetes glandulifera.*

2.3. Bioactive Phytochemical Constituents

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man and animals. Although nutrients elicit pharmacological or toxicological effects when ingested at high dosages (e.g. vitamins and minerals), nutrients in plants are generally not included in the bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. Thus, a definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals (Bernhoft,2010).

The phytochemical studies of the plants in *Tagetes* L. could be traced back to 1920s. Till now, about 126 secondary metabolites with various carbon skeletons,

phenolic derivatives, phenylpropanoids, thiophene derivatives, benzofunan derivatives, triterpenoids, steroids, alkaloids, flavonoids, carotenoids, and others have been obtained from the species of *Tagetes* L. Some of them showed potent activities as leading compounds of the new drugs.A variety of chemical constituents have been isolated from *Tagetes* species and their structures elucidated. They belong to the classes as essential oils, thiophenes, flavonoids, carotenoids and phenolic compounds. A preliminary phytochemical screening of the crude successive extracts of the roots of *Tagetes erecta* revealed the presence of sterols, glycosides, gums and mucilages (Gupta *et al*., 2009).

Compounds and aroma of essential oils can be divided into 2 major groups: terpene hydrocarbons and oxygenated compounds.

2.3.1. Terpene hydrocarbons

The hydrocarbons are the molecule, constituting of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5 carbon base units, C_5), which are the combinations of 2 isoprene units, called a "terpene unit." Essential oils consist of mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}), which are hydrocarbons with the general formula $(C_5H_8)_n$. The diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) exist in essential oils at low concentration (Mohamed *et al.*, 2010). Terpenoids (a terpene containing oxygen) is also found in essential oils (Burt 2004). Essential oils mostly contain monoterpenes and sesquiterpenes, which are $C_{10}H_{16}$ (M_W136 amu) and $C_{15}H_{24}$ (M_W 204 amu), respectively. Although sesquiterpenes are larger in molecules, structure and functional properties of

sesquiterpenes are similar to the monoterpenes (Ruberto and Baratta, 2000). For diterpenes, triterpenes, and tetraterpenes, they have the larger molecule than monoterpenes and sesquiterpenes, but they are present at very low concentration in essential oils (Bakkali *et al*., 2008).

2.3.2. Oxygenated compounds

These compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils. Oxygenated compounds can be derived from the terpenes, in which they are termed "terpenoids." Some oxygenated compound prevalent in plant essential oils are shown as follows:

2.3.2.1. Phenols

Hydrocarbons in which one or more hydrogen atoms are replaced by hydroxy group (-OH) are named as alcohols and phenols like compounds thymol, eugenol, carvacrol, chavicol and thymol, In other words, the hydroxy derivatives of hydrocarbons are named as alcohols and phenols. Out of alcohols and phenols, alcohols are hydroxy derivatives of aliphatic hydrocarbons, whereas phenols are hydroxy derivatives of aromatic hydrocarbons. Phenols have wide applications in different fields like medicines. Like exachlorophene which is a phenolic compound is antiseptic in nature and acts as a main constituent of mouthwashes, deodorant and medicinal skin cleansers.

Four phenolic derivatives viz, syringic acid (Tripathy *et al.,* 1991), 3,4-dihydroxybenzonicacid, gallic acid and 3,4-dihydroxy-5-methoxy-benzoicacid(Huang *et al.,*2007) have been isolated from the species of *Tagetes* L. Among them, the positions C-1 and C-4 are substituted by –COOH and -OH, respectively, while no substitution occurs at the position C-2 or C-6. The positions C-3 and C-5 are often substituted by -OMe or -OH. Some other phenolic compounds like ethyl gallate and

methyl-3,5-dihydroxy-4-methoxy benzoate are also found in the Tagetes (Tripathi *et al*., 1992).

2.3.2.2. Alcohols

An alcohol is any organic compound in which the hydroxyl functional group is bound to a saturated carbon atom.

Monoterpene alcohol: borneol, isopulegol, lavanduol, *α*-terpineol, and so on.

Sesquiterpenes alcohol: elemol, nerolidol, santalol, *α*-santalol and so on.

2.3.2.3. Aldehydes

An aldehyde or alkanal is an organic compound containing a functional group with the structure −CHO, consisting of a carbonyl center (a carbon double-bonded to oxygen) with the carbon atom also bonded to hydrogen and to an R group, which is any generic alkyl or side chain. The group—without R—is the aldehyde group, also known as the formyl group. Aldehydes are common in organic chemistry. Many fragrances are aldehydes e.g. citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde etc.

2.3.2.4. Ketones

A ketone (alkanone) is an organic compound with the structure RC (=O) R', where R and R' can be a variety of carbon-containing substituents. Ketones and aldehydes are simple compounds that contain a carbonyl group (a carbon-oxygen double bond). They are considered "simple" because they do not have reactive groups like −OH or −Cl attached directly to the carbon atom in the carbonyl group, as in carboxylic acidscontaining −COOH e.g. carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone etc.

2.3.2.5. Esters

Ester is a chemical compound derived from an acid (organic or inorganic) in which at least one –OH (hydroxyl) group is replaced by an –O–alkyl (alkoxy) group. e.g. bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, etc.

2.3.2.6. Oxides

An oxide is a chemical compound that contains at least one oxygen atom and one other element in its chemical formula. "Oxide" itself is the dianion of oxygen, an O2– atom.e..g. 1,8-cineole, bisabolone oxide, linalool oxide, sclareoloxide etc.

2.3.5.7. Ethers

Ethers are a class of organic compounds that contain an ether group - an oxygen atom connected to two alkyl or aryl groups. They have the general formula R–O–R′, where R and R′ represent the alkyl or aryl groups. e.g.1,8-cineole, anethole, elemicin, myristicin etc.

Different constituents in essential oils exhibit varying smell or flavour (Burt 2004). Also, the perception of individual volatile compounds depends on their threshold.

2.3.3. Other bioactive phytochemicals

2.3.3.1. Flavonoids

Flavonoids are the main components within the genus *Tagetes* L., and may have the meaning of chemosystematic interpretations up to some extent. Several flavonoids have been identified from the genus *Tagetes* L., which within this genus in the free or glycoside form.

Steinmetz, *et al*., (1991) stated that among the plant secondary metabolites flavonoids constitute the major component, and they are found in all parts while several other flavonoids were identified from the aqueous methanolic extract of the defatted flower heads and leaves of *Tagetes erecta* including quercetagetin and 6-hydroxykaempferol-7-O-glucoside. Additionally, the extract of the leaves particularly yielded kaempferol, kaempferol-7-O-rhamnoside and kaempferitrin. Quercetin was also found in the dried flowers of *Tagetes erecta* (Tripathy *et al.,* 1991).

2.3.3.2. Alkaloid

Alkaloids are any of a class of nitrogenous organic compounds of plant origin which have pronounced physiological actions on humans. They include many drugs (morphine, quinine) and poisons (atropine, strychnine).

Alkaloids are a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties.

Faizi and Naz, (2002) reported two alkaloids (6-ethoxy-2, 4-dimethylquinoline and luteolin) are found from *Tagetes* genus. Jafrine (Faizi and Naz, 2002) is an inherently unstable and structurally novel tetra hydro-β-carboline alkaloid.

2.3.3.3. Carotenoids

Carotenoids are the important components from the petals extracts in the species of *Tagetes* L. Carotenoids within this genus mainly composed of all-*trans*-lutein, β-carotene, zeaxanthin and lutein esters. Lutein, bearing one hydroxyl group at each ionone ring, could be esterifies with the saturated fatty acids, resulting in mono- and diacylated derivatives, such as lutein dipalmitate diesters, lutein myristate palmitate diesters and lutein violaxanthin monoesters.

2.3.3.4. Quinones

The *quinones* represent a class of organic compounds that are formally "derived from aromatic compounds [such as benzene or naphthalene] by conversion of an even number of –CH= groups into –C (=O) – groups with any necessary rearrangement of double bonds", resulting in "a fully conjugated cyclic dione structure".

Janiszowska *et al*., (1976) stated that quinones were isolated from different parts of *C. officinalis.* They included plastoquinone, phylloquinone, α-tocopherol and ubiquinone.

2.3.3.5. Tannin

A yellowish or brownish bitter-tasting organic substance present in some galls, barks, and other plant tissues, consisting of derivatives of gallic acid.

2.4. Pharmacological Actions

Genus *Tagetes* has been investigated by many workers for its various pharmacological effects *viz.* antiplasmodial, antioxidant, antidepressant and antimicrobial etc. which exhibit a broad range of biological effects, some of which are very interesting for possible future development.

2.4.1. Antimicrobial Activity

The essential oil of the leaves of *Tagetes erecta* exhibited moderate antimicrobial activity against *Bacillus subtilis* and *Bacillus anthraces* while slight activity was observed against *Escherichia coli, Klebsiella pneumoniae, Salmonella pullorum, Salmonella richmond, Salmonella newport, Salmonella stanley, Salmonella typhimolium, Staphylococcus aureus, Proteus vulgaris, Pseudomonas agalactiae, Apergillus niger, Aspergillus fumigatus, Aspergillus flavus, Rhizophus stolonifer, Fusarium* sp., *Penicillium digitatum* and *Candida albicans* (Grover and Rao, 1978).

Garg and Dengre, (1986)investigated that the essential oil of the leaves and stems of *Tagetes erecta* showed noticeable antibacterial activity against four gram positive and fifteen gram negative pathogenic bacteria *viz. Staphylococcus aureus, Bacillusmycoides, Bacillus pumilus, Bacillus subtilis, Salmonella paratyphi A, Salmonella paratyphi B, Salmonellaparatyphi C, Salmonella typhi H, Salmonella enteritides, Salmonella flexneri, Salmonella typhimurium, Shigellasonnei, Shigella schimizii, Shigella shiga, Vibrio cholerae Inawa, Vibrio cholerae Ogawa, Vibrio cholerae Eltor* and *Xanthomonas campestris.*

The alcoholic extract of *Tagetes erecta* revealed slight antifungal activity against *Penicillium chrysogenum, Penicillium notatum, Aspergillus niger* ISO-I, *Aspergillusniger* ISO-II and *Mucor* species (Nanir and Kadu, 1987).

Romagnoli*et al*., (1994)found essential oil of the flowers heads of *Tagetes minuta* revealed noticeable antimicrobial activity against *Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Aspergillus niger* and *Trichodermaviride* but was found ineffective against *Microsporum gypseum.*

Gupta and Vasudeva, (2010) conducted studies on five successive extracts *viz.* petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of the roots of *Tagetes erecta* and extracted a new bithienyl compound: 2-hydroxymethyl-non-3-ynoic acid 2-[2,2']-bithiophenyl-5-ethyl ester from the roots of the plant exhibited significant antimicrobial activity against three Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*), two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial and two fungal (*Candida albicans* and *Aspergillus niger*) strains with minimum inhibitory concentrations (MIC) for the extracts ranging between12.5-100 µg/mL

Rhama and Madhavan (2011) reported the anti-bacterial activity of different solvents of *Tagetes erecta* flowers against *Alcaligens faecalis, Bacillus cereus, Campylobacter coli, Escherchia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus mutans* and *Streptococcus pyogenes.* The flavonoid possesses antibacterial activity against all the tested strains and shows maximum zone of inhibition for *Klebsiella pneumonia* (29.50 mm). The flavonoid-Patulitrin is one of the potential elements for its anti-bacterial activity.

Gupta *et al*., (2012) studied on the antibacterial effect of Mexican marigold (*Tagetes erecta*) leaf extract at room temperature against 10 gram positive multidrug resistant bacterial isolates including *Enterococcus faecalis, Enterococcus faecium. Staphylococcus epidermidis, Staphylococcus aureus, Bacillus cereus, Erysipelothrixrhusiopathiae, Propionibacterium acne, Streptococcus pneumoniae, Streptococcus agalactine, Staphylococcus saprophyticus* and 6 gram negative multidrug resistant bacterial isolates including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas auregenosa, Salmonella enteriditis, Acinetobacter baumannii, Alcaligen faecalis* were studied by well diffusion method. The maximum antibacterial effect of Mexican Marigold leaf extract among those micro-organism was obtained for *Acinetobacter baumannii* (Acitvity Index = 0.913333333) and *Propionibacterium acne* (Acitvity Index = 0.906666667) and minimum was for *Streptococcus pneumonia* (Activity Index = 0.026666667). The results suggest that species of Mexican marigold i.e. *Tagetes erecta* has antibacterial effect against airborne disease causing gram positive and gram negative bacteria and mainly against skin infection causing bacteria, and hence can be useful in developing drugs for diseases like dermatitis, acne, skin races and also can be developed as antiseptic.

Kiranmai and Mohammed (2012) investigated the antibacterial effect of different extracts of leaves and flowers of *Tagetes erecta* Linn. After performing preliminary phytochemical screening and thin layer chromatography, antibacterial study was conducted according to the agar diffusion method by using gram positive *B. cereus, S. aureus* and gram negative *E. coli, P. aeruginosa*. This study was shown that petroleum ether extract of leaves and ethyl acetate extract of flower of *Tagetes erecta* significantly inhibit the growth of bacteria dose independently.

Iauk*et al.,* (2003) found that the methanol extract and 10 % decoction of the plant's flowers assessed for their activity against anaerobic and facultative aerobic periodontal bacteria, namely, *Porphyromonos gingivalis, Prevotella spp., Furobacterium nucleatum, Caphocytophaga gingivalis, Veilonella parvula, Eikenella corrodens, Peptostreptococcus micros* and *Actinomyces odontolyticus* showed marked inhibition against all tested microorganisms with MIC ≥2048 mg/L.

2.4.2. Anti-oxidant activity

Basavaraj *et al.*,(2011**)** stated that the antioxidant studies on the ethanolic extract of *Tagetes erecta* flowers by three different assays like DPPH, reducing power and super oxide radical scavenging activity at different concentrations were used. In all the three assay, *Tagetes erecta* showed better reducing power than the standard (i.e. ascorbic acid), and super oxide anion scavenging activity and DPPH antioxidant activity showed less than standard. However, ethanolic extract of *Tagetes* erecta demonstrated antioxidant property in all the in Vitro models.

Tripathi *et al.,* (2012)conducted a trial to study the essential oil extracted by hydro distillation of the aerial parts (leaves, flowers and shoots) of *Tagetes erecta* (Var. Pusa Narangi Genda) of Asteraceae family, was analyzed by gas chromatography–mass spectrometry and evaluated for antimicrobial and antioxidant

properties. In the aerial part extract forty three constituents were identified, representing more than 83% of the total detected. The major components were identified as *cis*-ocimene (18.46%), (E)- oscimene (8.65%), l-limonene (11.16%), (E)-tagetone (10.56%), b-caryophyllene (6.9%) and dl-limonene (4.16%). The essential oil was evaluated *in vitro* for antioxidant activity using specific assays. A significant antioxidant and radical scavenging activity was observed. The oil also exerted significant antifungal activity against the phyto pathogenic fungi, *Rhizoctoniasolani*, *Sclerotium rolfsii* and *Macrophomina phaseolina* and antibacterial activity against *Xanthomonas oryzae*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

A 70 % methanol extract of the plant was successively extracted with ether, chloroform, ethyl acetate and n-butanol leaving a residual aqueous extract which was assayed for antioxidant activity by liposomal lipid peroxidation-induced Fe2+ and ascorbic acid. The ether, butanol and water extracts, containing flavonoids, showed antioxidant activity (Popovic *et al.,* 1999).

Carotenoids such as lutein extracted from marigold (*Tagetes erecta*) revealed significant antioxidant activity whereby they were reported to scavenge superoxide radicals generated by photoreduction of riboflavin, hydroxyl radicals generated reaction and inhibited *in vitro* lipid peroxidation (Sindhu and Kuttan, 2007).

2.4.3. Insecticidal Activity

Saxena *et al*., (1992**)** reported that the acetone extract of the whole plant was reported for the growth inhibitory and juvenile hormone mimicking activity against the larvae of *Culex quinquefasciatus.*

The hexane, benzene, ethyl acetate and methanolic extracts, myristic and dodecanoic acids isolated from the hexane extract and the essential oil of *Tagetes*

erecta flowers revealed significant antianemic activity against the root knot nematode *Meloidogyne incognita* juveniles (Ray *et al*., 2000).

The essential oils of *Tagetes pusilla* seeds and *Tagetes minuta* aerial parts exhibited significant insecticidal activity against *Aedes aegypti* larvae (Chantraine *et al*., 1998).

Siddiqui and Alam, (1988)investigated thatthe aqueous extracts of flowers, seeds, roots and leaves of *Tagetes lucida* revealed significant nematicidal effect against *Meloidogyne incognita, Rotylenchulusreniformis, Tylenchorhynchus brassicae, Hoplolaimus indicus, Helicotylenchus indicus and Tylenchus filiformis*. Among all the fractions, the flower extract caused greatest nematode mortality followed by seed, leaf and root extracts.

2.4.4. Antiplasmodial Activity

Five successive extracts *viz.* petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of the roots of *Tagetes erecta* and a new bithienyl compound: 2-hydroxymethyl-non-3-ynoic acid 2-[2,2']-bithiophenyl-5-ethyl ester from the roots of the plant exhibited significant schizonticidal activity against chloroquine sensitive and resistant strains of *Plasmodium falciparum* (Gupta and Vasudeva, 2010).

2.4.5. Antidepressant Activity

The essential oil of the aerial parts of *Tagetes minuta* was reported to exert antidepressant activity via negative modulation on GABAergic function (Martijena *et al*., 1998).

2.5. Methods of Essential Oil Extraction from *Tagetes erecta* L.

Essential oils are extracted from oil sacs in flowers, leaves, stems, roots, seeds, wood, bark and other plant organs. There are numerous methods available for the extraction of EOs such as steam distillation, hydro distillation, maceration, solvent extraction and cold pressing also known as expression among other methods (Schmidt, 2010). The choice of a particular method will depend on a number of factors such the plant material as well as the desired end-product (Kabura, 2009). Furthermore, the isolation of an essential oil will be facilitated by its various properties as vapour pressure, solubility and polarity among other chemical and physical properties (Okoh, 2010). Steam distillation is undoubtedly one of the most popular methods of essential oils extraction (Schmidt, 2010). Steam distillation as a technique involves distillation of two immiscible liquids, for which steam provides one of the immiscible phases. The two substances i.e. water and essential oils mix in the gaseous phase and co-distill, but when cooled the two components separates since they are immiscible (Pavia *et al*., 2005). In this method of essential oil extraction, the plant material is packaged into a still where pressurized steam from boiling water passes through. The heated steam causes globules of oils in the plant to burst and the oils to evaporate. The essential oils and steam vapours passes through the top of the still into a condenser where they are condensed back to liquids with the oils separating from the water and floating on the top (Raaman, 2006). The fundamental nature of this technique is that it enables a compound or mixture of compounds to be distilled at temperature considerably below that of the boiling point(s) of individual constituent(s) (Kumar, 2010). Essential oils typically consist of individual components with boiling points of 200°C and above. However, in the presence of pressurized steam, these constituents are volatilized at temperature close to 100°C, at atmospheric pressure (Rao and Pandey, 2007).

2.5.1. Hydro distillation

Hydro distillation has become the standard method of essential oil extraction from plant material such as wood or flower, which is often used to isolate nonwater-soluble natural products with high boiling point. The process involves the complete immersion of plant materials in water, followed by boiling. This method protects the oils extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. The steam and essential oil vapour are condensed to an aqueous fraction. The advantage of this technique is that the required material can be distilled at a temperature below 100 °C.

Baser, (2010) reported hydro-distillation, a method of essential oil extraction which allows for the separation of slightly volatile, water-immiscible substances by means of low temperature distillation, being of particular use when the components boil at high temperature (higher than 100ºC) and are susceptible to decomposition below this temperature.

Sefidkon *et al.,* (2004) observed that hydro-distilled volatile oils from the leaves and stems and the flowers of *Tagetes erecta* L. were analyzed by a combination of GC and GC/MS. Thirty-three components in leaf and stem oil and 34 components in flower oil were identified. The main characterized constituents were β -caryophyllene (8.5 and 35.2%), terpinolene (18.4 and 6.3%), (E)-ocimenone (12.6 and 9.8%), (Z)-β -ocimene (10.4 and 13.7%), piperitenone (10.4 and 2.6%), (Z)-ocimenone (5.5 and 7.7%) and limonene (6.2 and 2.5%) in leaf and stem and flower oils respectively.

Wong *et al.,* (2014) elucidated that *Cinnamomum zeylanicum* is one of the herbs and spices plants that come from cinnamon family which contains high quality of essential oil. In this study, the essential oil from plant *Cinnamomum zeylanicum*

was extracted using two methods which were steam distillation and soxhlet extraction. Steam distillation produced high quality essential oil extraction using separatory funnel. Soxhlet extraction produced essential oil in crude from using rotary evaporator to purify the extracted product. Cinnamon essential oil contains high cinnamaldehyde content which is the main component in cinnamon. The percentage of cinnamaldehyde in essential oil from steam distillation was about 90% and 62-73% from Soxhlet extraction. Cinnamon essential oil has high antimicrobial properties which formed clear zone when tested with gram positive bacteria *Bacillus subtilis spp.* and a gram negative bacterium *Escherichia coli.* It also showed antimicrobial properties with two unknown bacteria with unknown characteristics. Cinnamaldehyde contains high antibiotic quality since it is the main compound in cinnamon.

Hydrodistilled volatile seed oil composition of commonly growing ornamental *Tagetes patula* L. was analyzed for its constituents by GC/MS. Forty constituents were identified, comprising 94% of the total oil. Sesquiterpene hydrocarbons (52.7%) and oxygenated sesquiterpenes (15.8%) were the main subclasses of volatile oil components followed by monoterpene hydrocarbons (12.6%). The principle constituents of the volatile oil were (E)-caryophyllene (44.6%) caryophyllene oxide (14.8%), germacrene D (3.8%), (Z)-β-ocimene (3.8%) and limonene (3.7%). From chemical point of view, oxides (15.7%) were the predominant group of components with caryophyllene oxide as their main representative. α-terthienyl (3.8%) comprised partially large amount in the volatile oil content despite of its polar and less-volatile nature. Taking into account the volatile oil profile, the chemical composition of the volatile seed oil of commonly growing ornamental *T. patula* L. was characterized as sesquiterpene and α-terthienyl rich one probably with appreciable biocidal (Insecticidal and nematicidal) and pharmacological potential.

2.5.2. Solvent extraction

Conventional solvent extraction has been implemented for fragile or delicate flower materials, which are not tolerant to the heat of steam distillation. Different solvents including acetone, hexane, petroleum ether, methanol, or ethanol can be used for extraction (Areias *et al*., 2000; Pizzale *et al*., 2002; Kosar *et al*., 2005 and Li *et al*., 2009).

Agarwal *et al.,* (2012) mentioned that Soxhlet extraction is one of the traditional methods used for the isolation of metabolites from plant material. Analytes with medium to low volatility which may play a role for the aroma and quality of oil extracted from the plant material are extracted with this technique. The correct choice of solvent is important in order to obtain a good yield from the extraction as well as to prevent the loss of volatiles. The solvent used in this method is indicative of the polarity of the compounds extracted. The extraction is usually carried out for a long period. The disadvantage of this technique is that, due to the long heating period, the analytes are exposed to high temperatures, which may lead to thermal degradation of some compounds. The recovered sample is diluted and has to be concentrated further, by evaporation. It is during this step that loss of volatiles can take place (Govender, 2010).

2.6. Qualitative and Quantitative estimation of essential oil

2.6.1. Qualitative estimation by FT-IR Spectroscopy

In many ways, mid-infrared spectroscopy would appear to be the ideal technology for on-line chemicals analysis. After all, IR spectroscopy is the only analytical method which provides both ambient temperature operation and the ability to directly monitor the vibrations of the functional groups which characterize molecular structure and govern the course of chemical reactions. In principle, IR also

offers the advantages of continuous (near real-time) operation and low maintenance compared to gas chromatography and low cost and structural specificity compared to mass spectroscopy.

The term “infrared” generally refers to any electro-magnetic radiation falling in the region from 0.7 mm to 1000 mm. However, the region between 2.5 mm and 25 mm (4000 to 400 cm^{-1}) is the most attractive for chemical analysis. This “mid-IR” region includes the frequencies corresponding to the fundamental vibrations of virtually all of the functional groups of organic molecules. These spectral lines are typically narrow and distinct, making it possible to identify and monitor a band corresponding to the specific structural feature that is to be modified by a reaction. As a result, quantitative calibrations performed in the mid-IR are usually straightforward and robust, being largely immune to the effects of spurious artifacts (Baughman *et al*., 1989; Kelly *et al*., 1989; Miller *et al*., 1990; Lodder *et al*., 1988).

In principle, the intensity of a given spectral point could be determined by simply passing the electrical signal obtained from the IR detector through a narrow band electronic filter and the complete spectrum could be measured by varying the filter frequency. A much more rapid approach is to use a digital computer to perform a Fourier transformation of the inter ferogram, thereby directly yielding the composite spectrum of the source, the instrument, and any sample interposed in the optical path. This is the basis of all modern rapid-scan FT-IR spectrometers (Hussain *et al*., 2007).

2.6.2. Quantitative estimation by Direct Analysis in Real Time (DART) Mass Spectrometry

Direct Analysis in Real Time (DART) is an atmospheric pressure that instantaneously ionizes gases, liquids and solids in open air under ambient conditions. Direct Analysis in Real Time (DART) has been coupled to the AccuTOF atmospheric

pressure ionization mass spectrometer to permit high resolution, exact mass measurements of gases, liquids and solids (Cody *et al.,* 2005; Kim *et al.,* 2011).

Okoh *et al*., (2008) stated that *Calendula officinalis* is a medicinal plant whose essential oils are used for various purposes. The oils were extracted by hydro-distillation from fresh leaves, dry leaves and fresh flowers of the herb yielding 0.06, 0.03 and 0.09%, respectively. The analysis of the oils by GC-MS revealed a total of 30, 21 and 24 compounds from the fresh leaves, dry leaves and the flowers in the same order. Sesquiterpenoids dominated the fresh leaves (59.5%) and flowers (26%), while the monoterpenes dominated the oil in the dry leaves (70.3%). T-muurolol (40.9%) predominated in the fresh leaf oil; α-thujene (19.2%) and dcadinene (11.8%) were also present in high quantities. Whereas, 1,8-cineole (29.4%), g-terpenene (11.6%), d-cadinene (9.0%), β-pinene (6.9%) and α -thujene (6.3%) were the major components in the dry leaf oil. In the fresh flower oil, α -thujene (15.9%), d-cadinene (13.1%) and d-cadinene (10.9%) were the major components. The significance of the effect of drying on essential oil composition of this plant is discussed.

Singh *et al*., (2012) investigate to characterize diversity in terms of metabolite profiles of *Cinnamomum tamala* Nees and Eberm genotypes; a newly emerging mass spectral ionization technique direct analysis in real time (DART) is very helpful. The DART ion source has been used to analyze an extremely wide range of phytochemicals present in leaves of *Cinnamomum tamala*. Ten genotypes were assessed for the presence of different phytochemicals. Phytochemical analysis showed the presence of mainly terpenes and phenols. These constituents vary in the different genotypes of *Cinnamomum tamala*. Principal component analysis has also been employed to analyze the DART data of these *Cinnamomum* genotypes. The result shows that the genotype of *Cinnamomum tamala* could be differentiated using DART

MS data. The active components present in *Cinnamomum tamala* may be contribute significantly to high amount of antioxidant property of leaves and, in turn, conditional effect for diabetic patients.

Twenty-one *P. betle* landraces were analyzed using a Direct Analysis in Real Time (DART) mass spectral technique and evaluated on the basis of molecules detected in the leaves. Clustering of landraces based on three well known biologically active phenols (*m/z* 151,165,193) showed two broad groups with high and low phenol contents suggesting differences in their therapeutic potential. Findings of this study could be useful in rapid screening of the landraces for determining their medicinal potential and optimum utilization of the bio resource (Bajpai *et al*., 2013).

Casta *et al*., (2016) found that the application of direct analysis in real time combined with mass spectrometry (DART-MS) for the qualitative analysis of lubricant and oil additives, and the quantitative analysis of a lubricant antioxidant additive. Analysed alone and in the presence of a base oil, from filter paper, glass and steel surfaces, showed the potential of the DART-MS technique for the direct, rapid analysis of lubricant oil additives. The quantitative capabilities of the technique were evaluated for the antioxidant in an oil matrix at concentrations in the range 0.1–8 mg/mL in oil (1–80 μg antioxidant on spot), using a structural analogue of the antioxidant as an internal standard. The linearity (R2 = 0.997), precision (% RSD = 2.6%) and LOD (0.04 mg/mL in oil) of the method demonstrates that DARTMS is capable of the rapid determination of additives in oil without pre-extraction.

Gazim*et al*., (2008) studied on terpenes and aroma volatiles from flowers of *Calendula officinalis* cultivated in southeastern Brazil were obtained by steam distillation (SD), headspace-cold finger (HS-CF) extraction and headspace solid-phase micro extraction (HS-SPME) coupled with gas chromatography and mass

spectrometric analysis. The dried flowers contained 0.1% oil. Kovats indices and mass spectra were used to identify 27 individual components in the various volatile fractions. The main components present in the volatile fractions of the *C. officinalis* flowers, obtained by SD, HS-SPME, and HSCF, were δ-cadinene (22.5, 22.1, and 18.4 %) and γ-cadinene (8.9, 25.4, and 24.9 %) while 20.4 % of α-cadinol was seen only after SD extraction.

Mehran *et al*., (2013) elucidated that *Tagetes* is a genus of 56 species of annual and perennial mostly herbaceous plants in the sunflower family (Asteraceae or Compositae). In this research our goal was recognizing the main components of the plant to extract useful ones. Essential oils of the leaves, seeds and flowers of T*agetes minuta* L., growing wild in Iran, were extracted by hydro distillation and analyzed by GC and GC-MS. Identification of the constituents of the oils was done by comparison of their mass spectra and retention indices with those given in the literature and the authentic samples. Twenty-six components were identified in the essential oils of the investigated organs. The main components extracted from the leaf oils were dihydro tagetone (45.9%), cis-β-ocimene (11.9%) and borneol (11.1%), and those of the seed oils included dihydrotagetone (21.0%) and benzoic acid-4-hydroxy-methyl ester (33.5%) also trans-ocimenone (27.0%), cis-β- ocimene (26.0%) and cis-ocimenone (17.6%) were the major constituents in the flower oils.

Chapter III

MATERIALS AND METHODS

The present investigation entitled "A Study on the Extraction of Essential Oil from Marigold (*Tagetes erecta* L.) and its Characterization" was carried out at the Horticulture Research Farm and Laboratory of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya-Vihar, Rae Bareli Road, Lucknow, Uttar Pradesh during the year 2013 to 2016.The preliminary qualitative screening of phytochemicals in the essential oil of different samples of various parts of marigold was done at the Laboratory of Department of Applied Plant Science (Horticulture).The FT-IR Spectroscopy was performed at University Scientific Instrumentation Centre (USIC), Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rae Bareli Road Lucknow (U.P.) India and the Direct Analysis in Real Time (DART) Mass Spectrometry was done at Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute (CSIR-CDRI), Lucknow, (U.P.) India. The details of materials used and methodologies adopted in the course of investigation have been presented hereunder.

3.1. Experimental Material

Flower and leaf samples of African marigold (*Tagetes erecta* L.) variety Pusa Narangi Genda were taken for the study of phytometabolites in the essential oil of marigold. Seeds of this variety were procured from Indian Agricultural Research Institute, Pusa, New Delhi.

3.2. Preparation of nursery beds and sowing of the seeds

Beds of one meter width and 1.5m length were prepared in the nursery block of the Horticulture Research Farm, Department of Applied Plant Science by digging the bed sand incorporating well rotten FYM. For winter crop, seeds were sown in nursery in the last week of September and for summer crop seeds were sown in the first week of February. Seeds were sown at a row to row distance of 6-8 cm and depth of 2.0cm so that the population of the seedlings would not be thick. The seeds were covered with sieved leaf mold and germinated 4-5 days after sowing. The nursery beds were kept moist during entire period. Standard intercultural operations were done at regular intervals for better development of plant.

3.3. Transplanting

The seedlings became ready for transplanting when they attained3-4 leaf stage, after 25 days of sowing in both the season Seedlings which were healthy and free from insect-pest and diseases were uprooted with utmost care without damaging the roots of plant and then transplanted carefully in the experimental field. A light irrigation was given after transplanting.

The entire study was subsequently divided into the four experiments listed below:

Plate 1: Nursery raising of African marigold (*Tagetes erecta* L.) at Horticulture Research Form

3.4. Experiment Number I

To standardize the method for extraction of essential oil from marigold plant parts:

Fresh and dried, flower and leaf samples of marigold *Tagetes erecta* L were collected for standardizing the method for extraction of essential oil from marigold. Oil extraction from marigold plant parts was done using two methods viz. Hydro-distillation and Solvent extraction.

3.4.1. Collection of sample

For the present experiment, the leaf and flower part of African marigold plant were collected at full blooming stage in the early morning.

Plate 2: Collection of fresh flowers of marigold for oil extraction.

Plate 3: Processing of fresh flowers of marigold for making sample dry for oil extraction.

Plate 4: Collection and preparation of fresh and dry leaf sample of marigold (*Tagetes erecta* L.)

3.4.1.1. Fresh flower and leaf sample

The fresh flowers and leaves of African marigold (*Tagetes erecta* L.) cultivated in Horticulture Research Farm of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Lucknow, U.P. were collected at full flowering stage in early morning in both the seasons i.e. winter and summer respectively.

3.4.1.2. Dry flower and leaf sample

Fresh flowers and leaves collected from the experimental field were processed in the Laboratory of the Department of Applied Plant Science (Horticulture) and subjected to air drying for 24 hours in shade. The dried plant material was subsequently, powdered using mixer and grinder. The samples were protected from direct sunlight and subsequently stored in air tight containers for further study.

3.4.2. Extraction of essential oil

Two methods of essential oil extraction were used from the fresh and dry sample of flowers and leaves of marigold.

a. Hydro-distillation using Clevenger's method
b. Solvent extraction using Soxhlet apparatus

3.4.2.1. Hydro-distillation

The plant parts of African marigold i.e. flower (fresh and dry) and leaf (fresh and dry) were subjected to hydro-distillation using a Clevenger's apparatus. The collection of oil started after a heating time of about 40 minutes and was continued till no more essential oil was obtained after 5-6 hours. After the distillation process was complete, the volatile essential oils were removed from the top of the hydrosol and dried over anhydrous sodium sulphate (Na_2SO_4) (Golfakhrabadi *et al*., 2015) and stored in small sealed tubes at low temperature for preliminary screening of phytometabolites, qualitative screening by FT-IR and quantitative DART-MS analysis.

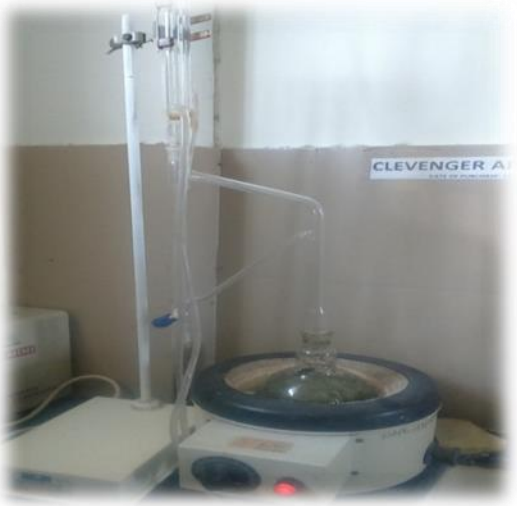


Plate 5: Hydro-distillation using Clevenger's apparatus

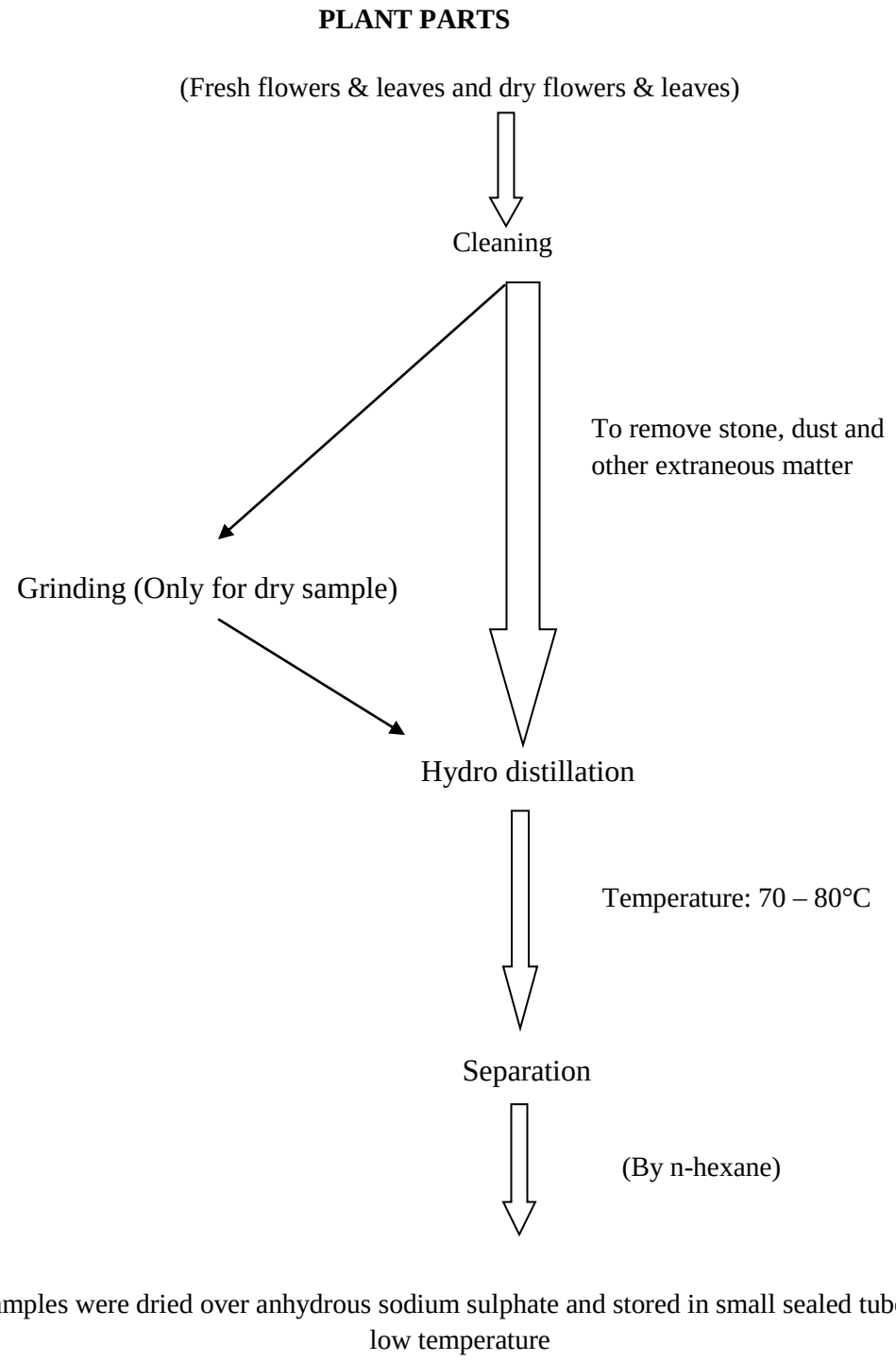


Fig 3.1: Process for extraction of essential oil by hydro-distillation modified from Gavahian *et al.*, 2012.

3.4.2.2. Solvent extraction

Solvent extraction is one of the traditional methods used for the isolation of metabolites from plant material. Analytes with medium to low volatility which may play a role for the aroma and quality of oil extracted from the plant material are extracted with this technique. The correct choice of solvent is important in order to obtain a good yield from the extraction as well as to prevent the loss of volatiles. The solvent used in this method is indicative of the polarity of the compounds extracted (Dutta *et al.,* 2014).The extraction is usually carried out for a long period. About a significant amount of fresh and dry flowers & fresh and dry leaves of African marigold (*Tagetes erecta* L.) were subjected to solvent extraction for 3-4 hours at temperature 60^0Cwith the help of non- polar solvent n-hexane using a soxhlet apparatus. The oil extracted was stored in small sealed tubes at low temperature for preliminary screening of phytochemical, qualitative screening by FT-IR and quantitative screening of oil by DART-MS analysis**.**

Plate: 6 Solvent extractions using soxhlet apparatus.

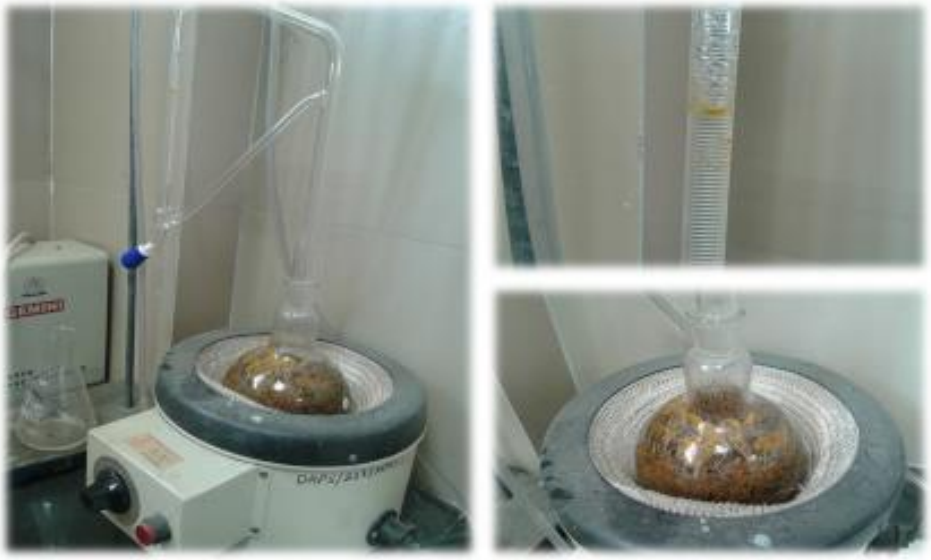


Plate 7: Oil extraction from dry flowers of marigold through hydro-distillation using clevenger's apparatus.

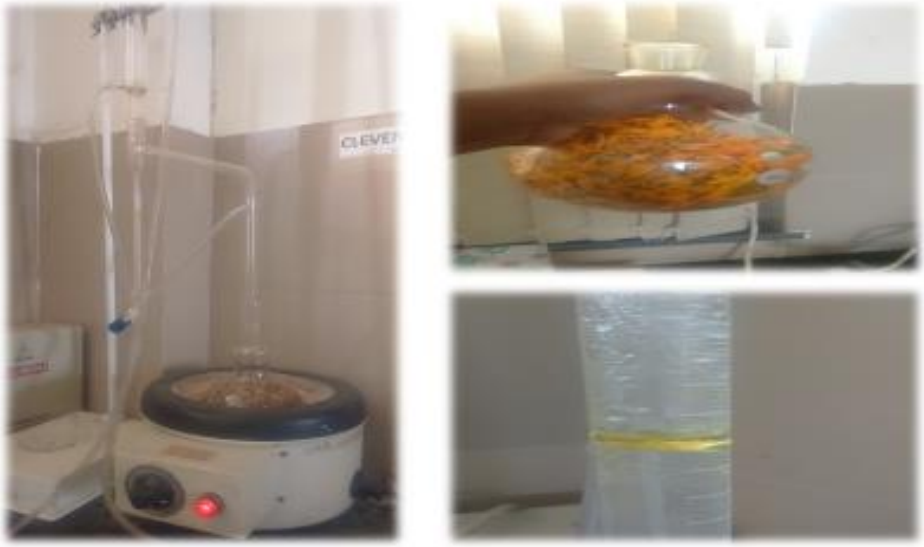


Plate 8: Oil extraction from fresh flowers of marigold through hydro-distillation using clevenger's apparatus.

Plate 9: Oil extraction from dry leaves of marigold through hydro-distillation using clevenger's apparatus.

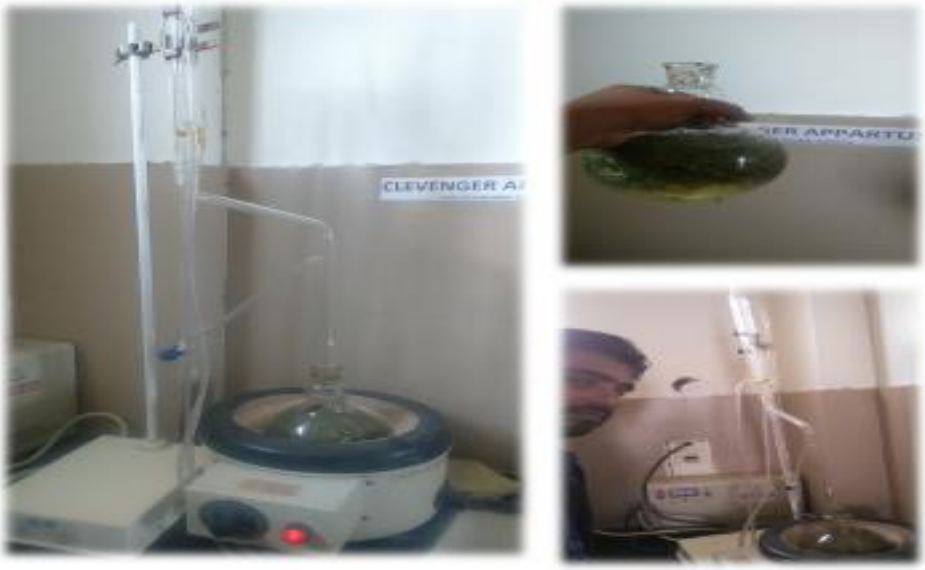


Plate 10: Oil extraction from fresh leaves of marigold through hydro-distillation using clevenger's apparatus.

Plate 11: Solvent extraction of essential oil from fresh flowers of marigold using soxhlet apparatus

30 g fresh and dry sample of flowers& leaves of African marigold (Durling *et al.*, 2007)

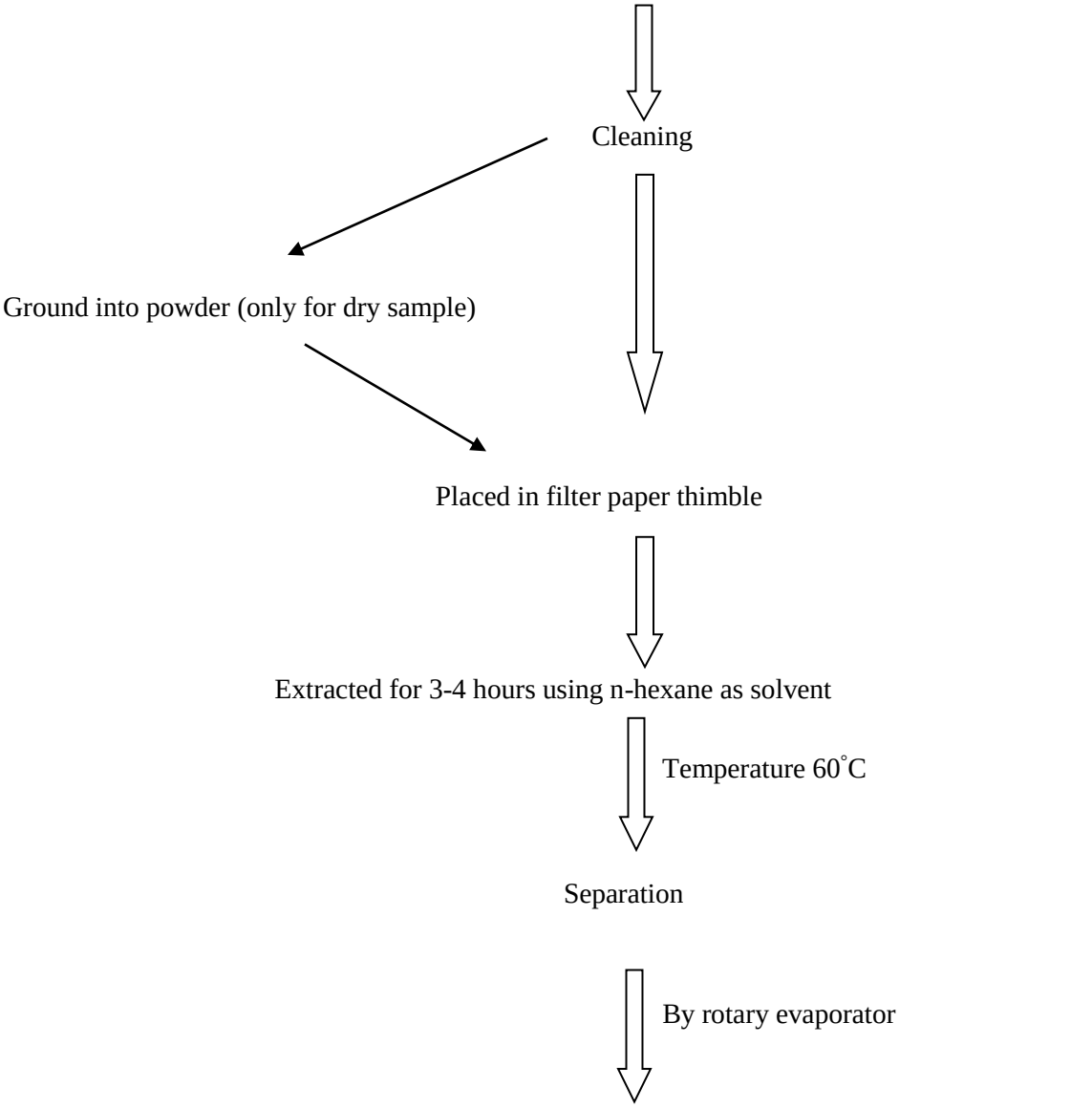


Fig3.2: Process for extraction of essential oil by solvent extraction

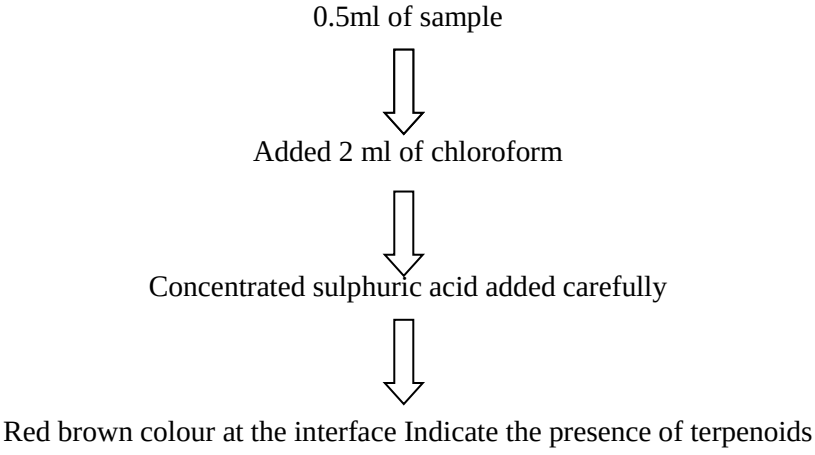
3.5. Experiment Number II

Qualitative estimation of essential oil from marigold plant parts:

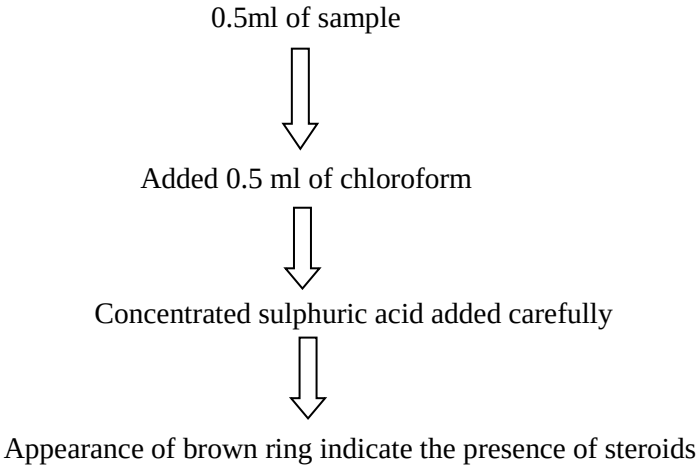
3.5.1 (A). Preliminary screening of phytochemicals

The oil samples extracted from fresh and dry, flowers and leaves of African marigold by using the Hydro-distillation and Soxhlet methods were tested for presence of bioactive phytochemicals using standard procedures as quoted by Mostafavi and Pezhhanfar (2015) to identify the secondary phytometabolites in the oil sample.

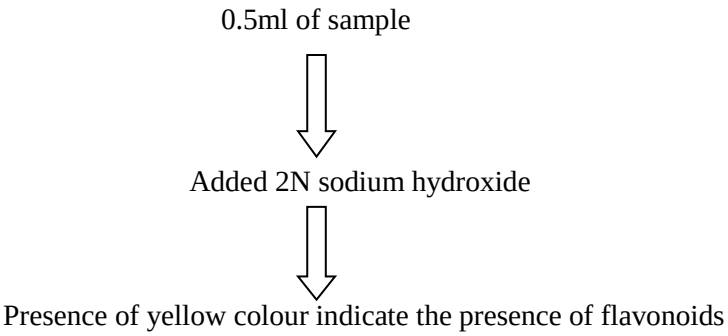
3.5.1.1. Detection of Terpenoids (Chloroform test)



3.5.1.2. Detection of Steroids

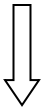


3.5.1.3. Detection of Flavonoids (Alkaline reagent test)

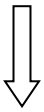


3.5.1.4. Detection of Alkaloids

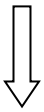
0.5ml of sample



Added 0.5 ml of concentrated hydrochloric acid



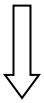
Added few drops of Mayers's reagent



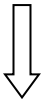
Presence of green colour or white precipitate indicate the presence of alkaloids

3.5.1.4. Detection of Quinones

0.5ml of sample



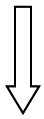
Added 0.5 ml of concentrated sulphuric acid



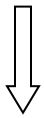
Formation of red colour indicate the presence of Quinones

3.5.1.5. Detection of Glycosides

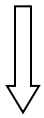
0.5ml of sample



Added 2ml chloroform



10% ammonium solution added



Formation of pink colour indicate the presence of glycosides

3.5.1.6. Detection of Phenols

1 ml of sample

Added 2ml distilled water

Followed by few drops of 10% ferric chloride

Formation of blue or green colour indicate the presence of phenols

3.5.1.7. Detection of Triterpenoids

0.5 ml of sample

Added 0.5 ml Libemann – Buchard Reagent
(aectic anhydride+ concentrated sulphuric acid)

Formation of blue colour indicate presence of triterpenoids

3.5.1.8. Detection of Coumarins

0.5ml of sample

Added 0.5 ml of 10% NaOH

Formation of yellow colour indicate presence of Coumarins

3.5.1.9. Detection of Tannin

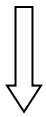
0.5ml of sample

Added 0.5 ml of ferric chloride

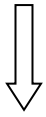
Formation of dark blue or greenish black colour indicate the presence of tannin

3.5.1.10. Detection of Saponins (Froth test)

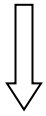
0.5ml of sample



Added 1 ml of distilled water



Shaken in a graduated cylinder for 15minutes lengthwise



Formation of 1cm layer of foam indicate the presence of saponins

3.5.1. (B). Qualitative screening by FT – IR Analysis

The qualitative analysis was done by using FT-IR analysis. Sample were placed into Fourier Transform Infrared (FT-IR) spectrometer(Nicolet TM 6700, Thermo scientific: USA) having a scan range from 400 to 4000 cm^{-1}, at the University Scientific Instrumentation Centre, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rae Bareli Road, Lucknow (U.P.) India. FT-IR Spectrometer was used to identify various types of chemical bonds in a molecule by producing an infrared absorption spectrum. The IR spectra were reported as % transmittance. The functional groups were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph from library of the system and previous literature related to FTIR studies.

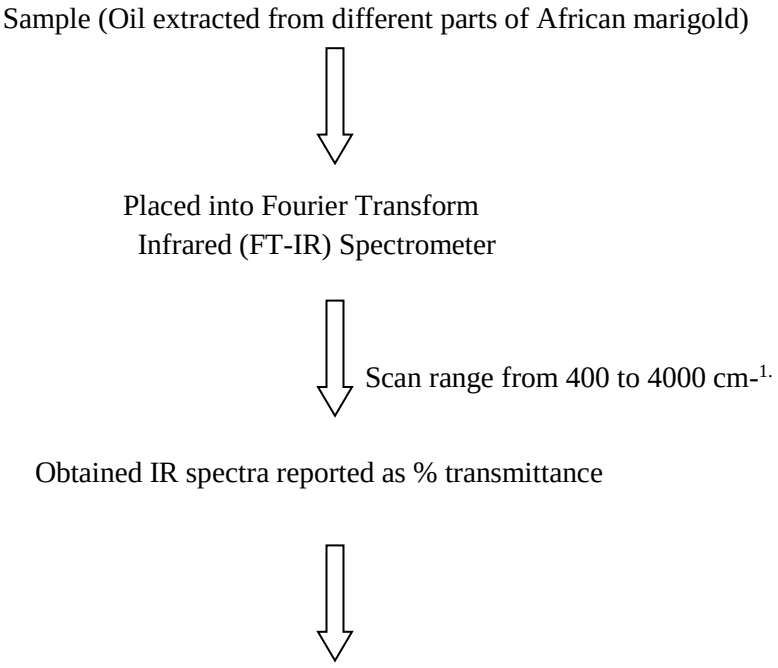


Fig 3.3: Process for Fourier Transform Infrared (FT-IR) Spectrometer Analysis

3.6. Experiment Number III

Quantitative estimation of essential oil from marigold plant parts

The qualitative analysis of fresh and dry, flowers and leaves essential oil extracted by both the hydro-distillation and solvent extraction method was done by using Direct Analysis in Real Time (DART) Mass Spectrometry.The DART-MS was done at Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute (CSIR-CDRI), Lucknow,(U.P.) India. The DART-MS was recorded on a JEOL-AccuTOF LMS-T100LC Mass spectrometer having a DART (Direct Analysis in Real Time) source. The oil samples extracted from flowers and leaves of African marigold by using the Hydro-distillation and Soxhlet methodswere

subjected as such in front of DART source. Dry helium gas was used with 4 LPM flow rate for ionization at 350^0C. Data acquisition was from *m/z* 50.0 to 1000.0. The orifice was set at 28 V and spectra were collected. The components in the oil were identified by comparing their *m/z* values with those of a computer library and with data already published in literature.

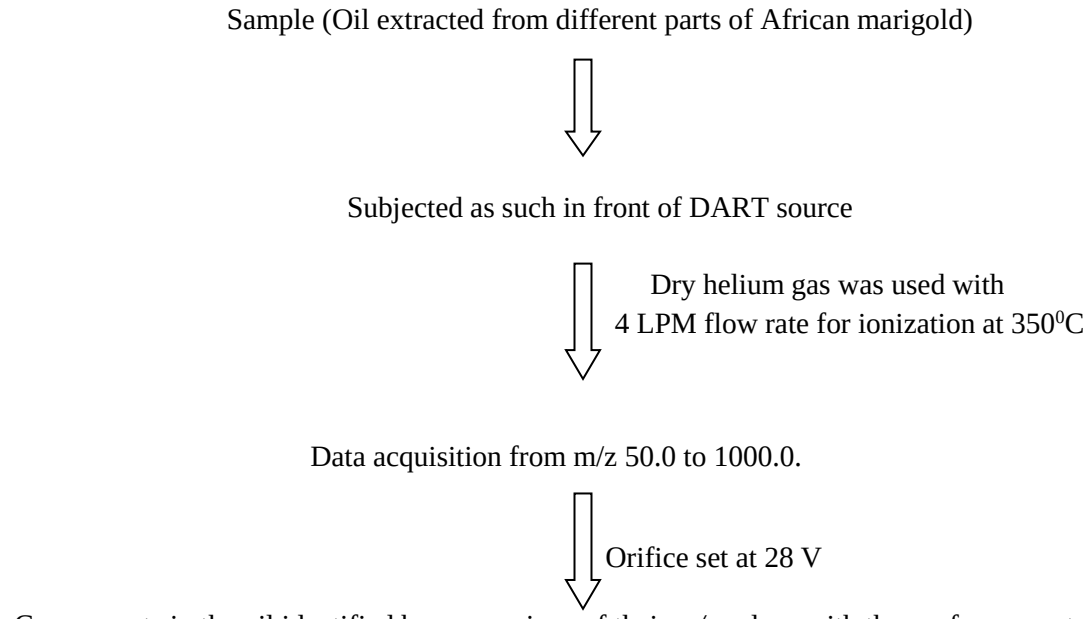


Fig 3.4: Process for Direct Analysis in Real Time (DART) Mass Spectrometry

3.7. Experiment Number IV

Comparative study of the yield of the essential oil from different flowering seasons

Oil was extracted from flower and leaves in different growing seasons by hydro-distillation and solvent extraction fromfresh and dry, flowers and leaves of marigold (*Tagetes erecta* L.) for 4 hours using a clevenger's apparatus and soxhlet apparatus. Polar solvent n-hexane was used for solvent extraction. Solvent was removed from the samples by using rotary evaporator. All extractions were performed in triplicate, and the mean values were reported. The

yield of oil was expressed as a percentage of the weight of oil obtained from extraction relative to the weight of sample used for extraction.

$$\text{Yield of oil} = \frac{\text{Weight of oil extracted}}{\text{Weight of sample used}} \text{ X } 100\ \%$$

3.8. Antioxidant activity (DPPH radical scavenging activity)

DPPH (2, 2 diphenyl1picryl hydrazyl) free radical scavenging assay was measured using the method of Molyneux *et al*., 2004. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (1-16 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm in spectrophotometer. A blank was prepared without adding extract. Ascorbic acid (1%) at various concentrations (1 to16 µg/ml) was used as standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\text{DPPH Scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \text{ x } 100$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. Results are expressed as IC_{50}, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%.

Chapter IV

EXPERIMENTAL FINDINGS

The experimental results obtained from the investigation entitled "A Study on the Extraction of Essential Oil from Marigold (*Tagetes erecta* L.) and its Characterization" performed at the Horticulture Research Farm and Laboratory in the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya-Vihar, Rae Bareli Road, Lucknow, Uttar Pradesh during the year 2013 to 2016 respectively, are presented here under.

4.1: Experiment Number I

To standardize the method for extraction of essential oil from marigold plant parts

The standardization of methods for extraction of essential oil from African marigold (*Tagetes erecta* L.) plant parts having hydro distillation and solvent extraction using n-hexane was done as per the methods described in the previous chapter.

To explore the most suitable method for extraction of the essential oil from African marigold (*Tagetes erecta* L.) plant parts, a comparative study was designed with both hydro-distillation and solvent extraction methods. The Table 4.1 and Fig. 4.1 elucidated the variation in the yield of oil from flowers and leaves extracted by both hydro-distillation and solvent extraction methods from various parts of African marigold plant. The highest yield of essential oil was obtained by solvent extraction method by using n-hexane as a solvent, was recorded (8.33 %) in the Dry Flowers Soxhlet Extract

(DFSE) followed by the Dry Leaves Soxhlet Extracts (DLSE) which showed (6.33 %) oil yield.

The highest yield of essential oil from Hydro-distillation method from various samples of African marigold plant parts was recorded in the Fresh Flowers Clevenger Extract (FFCE) sample (0.45 %) followed by the Dry Flowers Clevenger Extracts (DFCE) which showed (0.42%) oil yield.

Thus, on the basis of the above observations, it can be concluded that solvent extraction exhibited higher extraction efficiency than the hydro distillation method.

Table 4.1: Yield of essential oil extracted from different parts of African marigold (*Tagetes erecta* L.).

S.NO.	SAMPLE	QUANTITY OF SAMPLE (g)	QUANTITY OF OIL (g)	OIL YIELD %
1	Fresh flowers clevenger extract (FFCE)	100	0.45	0.45%
2	Dry flowers clevenger extract (DFCE)	100	0.42	0.42%
3	Fresh leave clevenger extract (FLCE)	100	0.39	0.39%
4	Dry leave clevenger extract (DLCE)	100	0.41	0.41%
5	Fresh flowers soxhlet extract (FFSE)	30	0.14	0.46%
6	Dry flowers soxhlet extract (DFSE)	30	2.5	8.33%
7	Fresh leave soxhlet extract (FLSE)	30	0.12	0.40%
8	Dry leave soxhlet extract (DLSE)	30	1.9	6.33%

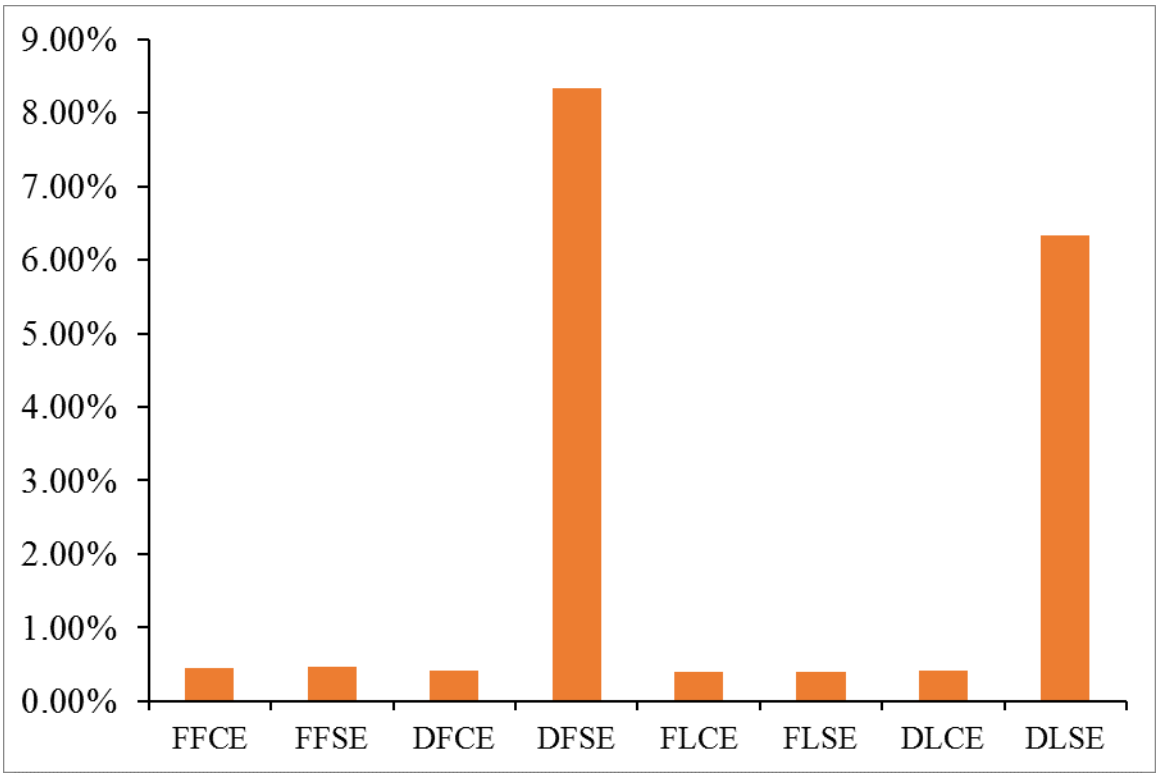


Fig. 4.1: Yield of essential oil extracted from different parts of African marigold (*Tagetes erecta* L.).

4.2. Experiment Number II

Qualitative estimation of essential oil from marigold plant

4.2.1. Results of preliminary screening of phytochemicals

The preliminary phytochemical analysis of various samples of marigold was done by using the methods described in the previous chapter. The results concerning the qualitative screening of the samples are presented in Table 4.2 and results of observation of phytochemicals analysis are presented in Table 4.3.

Terpenoids, flavonoids, alkaloids and coumarins were identified in all the samples of flowers and leaves of *Tagetes* erecta L. essential oil extracted by both Hydro-

distillation and Solvent extraction method. Quinones were present in all samples except in fresh leaves samples, extracted by both methods. Test for phenols were showed positive for all flowers samples extracted by both methods. Triterpenoids were only indentified in the dry flowers. Test for tannins were found positive in fresh and dry flowers oil extracted by hydro-distillation method and dry flowers oil extracted by solvent extraction method. Negative results were obtained for phytochemicals such as steroids, glycosides and saponins for all samples extracted by both methods. Thus, on the basis of the above observations, it was concluded that the identification of the above compounds supports the use of these oils in traditional medicine as these compounds have valuable antifungal, antibacterial and anti-inflammatory properties.

Table 4.2: Qualitative phytochemical screening of various oil samples of African marigold (*Tagetes erecta* L.).

PHYTOCHEMICAL TESTS	HYDRO-DISTILLED OIL				SOXHLET EXTRACTED OIL			
	Fresh flowers	Dry flowers	Fresh leaves	Dry leaves	Fresh flowers	Dry flowers	Fresh leaves	Dry leaves
TERPINOIDS	+	+	+	+	+	+	+	+
STEROIDS	-	-	-	-	-	-	-	-
FLAVONOID	+	+	+	+	+	+	+	+
ALKALOID	+	+	+	+	+	+	+	+
QUINONES	+	+	-	+	+	+	-	+
GLYCOSIDES	-	-	-	-	-	-	-	-
PHENOLS	+	+	-	-	+	+	-	-
TRITERPINOIDS	-	-	-	-	-	+	-	-
COUMANINS	+	+	+	+	+	+	+	+
TANNIN	+	+	-	-	-	+	-	-
SAPONIN	-	-	-	-	-	-	-	-

Table 4.3: Phytochemical analysis of various samples of African marigold (*Tagetes erecta* L.) showing presence of bioactive molecules.

PHYTOCHEMICAL TESTS	HYDRO-DISTILLED OIL				SOXHLET EXTRACTED OIL			
	Fresh flowers	**Dry flowers**	**Fresh leaves**	**Dry leaves**	**Fresh lowers**	**Dry flowers**	**Fresh leaves**	**Dry leaves**
Terpinoids	Red brown colour	Red brown colour	Red brown colour	Red brown colour	Red brown colour	Red brown colour	Red brown colour	Red brown colour
Steroids	No change	No change	No change	No change	No change	No change	No change	No change
Flavonoid	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow	Yellow colour	Yellow colour
Alkaloid	Green colour	Green colour	Green colour	Green colour	Green colour	Green colour	Green colour	Green colour
Quinones	Red colour	Red colour	No change	Red colour	Red colour	Red colour	No change	Red colour
Glycosides	No change	No change	No change	No change	No change	No change	No change	No change
Phenols	Green colour	Green colour	No change	No change	Green colour	Green colour	No change	No change
Triterpinoids	No change	No change	No change	No change	No change	Blue	No change	No change
Coumanins	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow	Yellow colour	Yellow colour
Tannin	Blue colour	Blue colour	No change	No change	No change	Blue colour	No change	No change
Saponin	No change	No change	No change	No change	No change	No change	No change	No change

4.2.2. Determination of the functional groups present using FT-IR Spectroscopy

FT-IR spectroscopic analysis of essential oil from various samples of African marigold extracted by both hydro-distillation and solvent extraction methods was done by using the methods described in the previous chapter. The FT-IR spectroscopic analysis was made based on percentage of transmittance and wave numbers.

4.2.2.1. Qualitative screening of Fresh Flowers Clevenger Extract (FFCE) using FT-IR Spectroscopy

The bonds and the wave numbers (cm^{-1}) of prominent peaks of the major constituents obtained from spectra are described in Table 4.4 and elucidated in Fig 4.2. The analysis of essential oil of Fresh Flowers Clevenger Extract (FFCE) from African marigold has showed the existence of various secondary phytometabolites. The essential oil extracted by hydro-distillation method from fresh flowers showed major peaks primarily at 3361.7 cm^{-1}, 2958.1 cm^{-1}, 2924.0 cm^{-1}, 2863.2 cm^{-1}, 2734.0 cm^{-1}, 2674.6 cm^{-1}, 1629.6 cm^{-1}, 1461.7 cm^{-1}, 1379.3 cm^{-1}, 1342.9 cm^{-1}, 1296.4 cm^{-1}, 1247.0 cm^{-1}, 1135.6 cm^{-1}, 1060.9 cm^{-1}, 885.2 cm^{-1} and 724.2cm^{-1}. The peak at 3361.7 cm^{-1} was assigned to the N-H stretching vibration while that in the range of 2990-2650 cm^{-1} is mainly attributed to the stretching vibration of C-H. In addition, the peak at 1629.6 cm^{-1} is assigned to the N-H bend vibration which indicates that some amide compounds existed in the hydro-distilled essential oil of fresh flowers from African marigold. The alkane peaks at 1461.7 and 1379.3 cm^{-1} and the peak situated at 1342.9 cm^{-1} assigned to N-O symmetric stretching. The peak at 1296.4 and 1247.0 cm^{-1} are due to C-O stretching vibrations. The peak at 1135.6 and 1060.9 cm^{-1} are due to C-N stretching vibrations. The aromatics were present at the range of 890-750 cm^{-1}**.**

Table 4.4: Wave numbers and related functional groups in essential oils extracted by hydro-distillation from various samples of African marigold (*Tagetes erecta* L.) analyzed in FTIR spectrometer.

S.NO.	FREQUENCY (CM-1)	FRESH FLOWERS	DRY FLOWERS	FRESH LEAVES	DRY LEAVES	BOND	FUNCTIONAL GROUP
1	3450-3400	-	3402.8	-	-	O-H stretch	alcohols, phenols
2	3390-3300	3361.7	-	3375.6	3383.4	N-H Stretch	1*, 2* amines, amides
3	2990-2950	2958.1	2957.7	2958.3	2958.2	C-H Stretch	alkanes
4	2950-2900	2924	2924.1	2924.6	2926	C-H Stretch	alkanes
5	2880-2850	2863.2	2863.9	2865.1	2867.7	C-H Stretch	alkanes
6	2750-2700	2734	2733.7	-	-	H-C=O: C-H Stretch	aldehydes
7	2690-2650	2674.6	-	-	-	H-C=O: C-H Stretch	aldehydes
8	1750-1700	-	1708.4	-	-	C=O Stretch	α, β- unsaturated aldehydes, ketones
9	1690-1650	-	-	1670.5	1684.6	C=O Stretch	Carbonyls (general)
10	1650-1600	1629.6	1618.4	1625	1617	N-H Bend	1* amines
11	1550-1500	-	-	-	1514.2	N-O asymmetric stretch	Nitro compounds
12	1490-1450	1461.7	1460.3	1459.9	1454.2	C-H bend	alkanes
13	1390-1350	1379.3	1378.3	1377.8	1375.9	C-H rock	alkanes
14	1350-1300	1342.9	-	-	-	N-O Symmetric stretch	Nitro compounds
15	1300-1250	1296.4	-	1295.4	1268.6	C-O Stretch	carboxylic acid
16	1250-1200	1247	1219.4	1219.8	1219.3	C-O stretch	carboxylic acid
17	1150-1100	1135.6	1135.3	1140	1149.1	C-N Stretch	Aliphatic amines
18	1090-1050	1060.9	1060.8	1062.1	1065.1	C-N Stretch	Aliphatic amines
19	1050-1020	-	-	-	1033.2	C-N Stretch	Aliphatic amines
20	890-850	885.2	890.8	864.3	875.1	C-H “oop”	aromatic
21	850-800	-	802.9	-	812.3	C-H Bend (para)	aromatic
22	730-700	724.2	724.7	723.1	723.4	C-H rock	alkanes

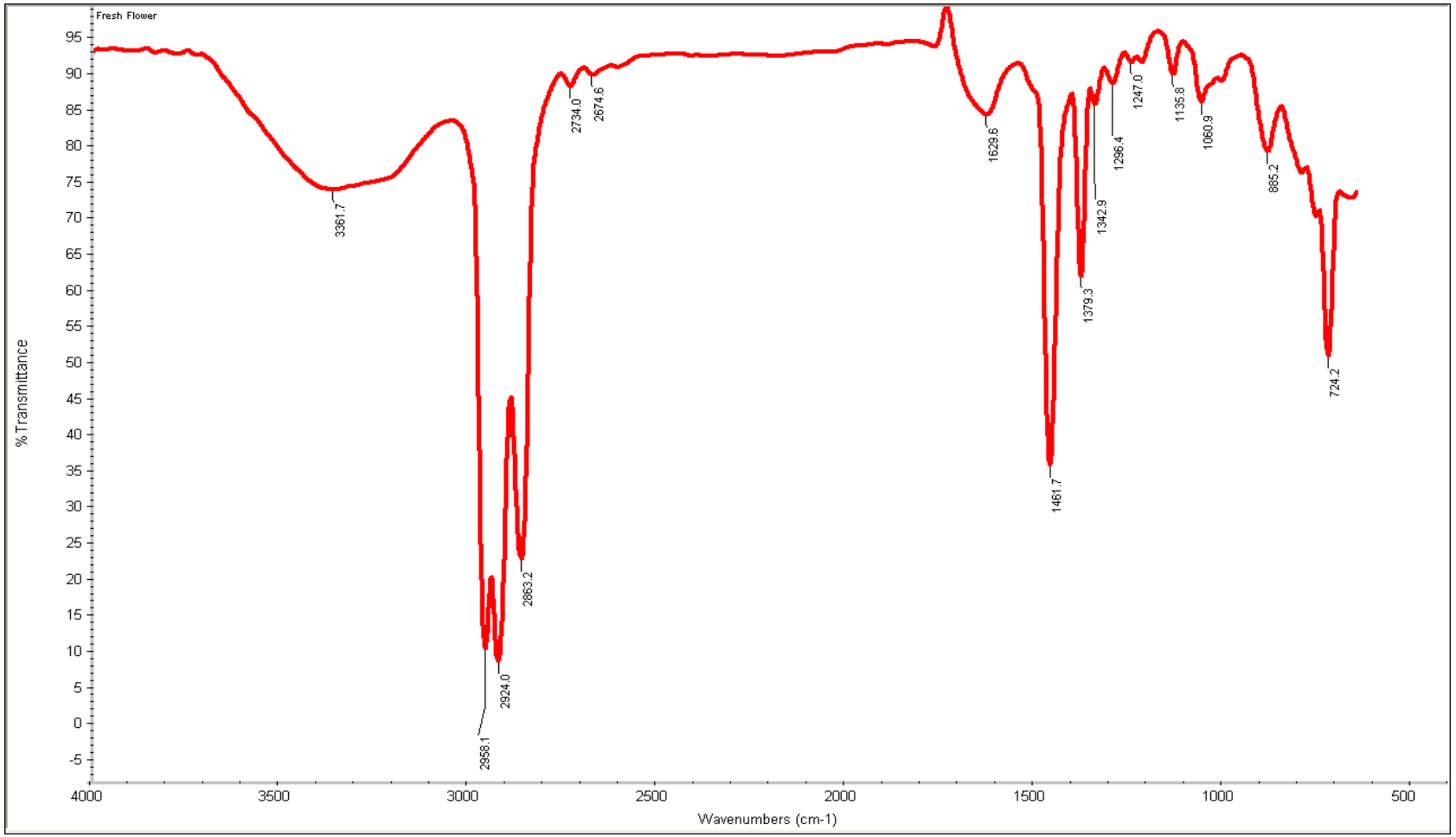


Fig. 4.2: Transmittance spectrum of hydro-distilled essential oil from fresh flowers of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

4.2.2.2. Qualitative screening of Dry Flowers Clevenger Extract (DFCE) using FT-IR Spectroscopy

The bonds and the wave numbers (cm^{-1}) of prominent peaks of the major constituents obtained from spectra are described in Table 4.4 and Fig 4.3. The analysis of essential oil of Dry Flowers Clevenger Extract (DFCE) from African marigold has showed the existence of various secondary phytometabolites. The essential oil extracted by hydro-distillation method from dry flowers showed major peaks primarily at 3402.8 cm^{-1}, 2957.7 cm^{-1}, 2924.1 cm^{-1}, 2863.9 cm^{-1}, 2733.7 cm^{-1}, 1708.4 cm^{-1}, 1618.4 cm^{-1}, 1460.3 cm^{-1}, 1378.3 cm^{-1}, 1219.4 cm^{-1}, 1219.4 cm^{-1}, 1135.3 cm^{-1}, 1060.8 cm^{-1}, 890.8 cm^{-1}, 802.9 cm^{-1} and 724.7cm^{-1}. The peak at 3402.8 cm^{-1} is assigned to the O-H stretching vibration while that in the range of 2990-2700 cm^{-1} is mainly attributed to the stretching vibration of C-H. In addition, the peak at 1708.4 cm^{-1} is assigned to the C=O stretching vibration which indicates that some α, β- unsaturated aldehydes and/or ketones compounds existed in the hydro-distilled essential oil of dry flowers from African marigold.. The alkane peaks at 1460.3 and 1378.3 cm^{-1} and the peak situated at 1219.4 cm^{-1} assigned to C –O stretching. The peak at 1135.3 and 1060.8 cm^{-1} are due to C-N stretching vibrations. The aromatics are present at the range of 800-890 cm^{-1}.

4.2.2.3. Qualitative screening of Fresh Leaves Clevenger Extract (FLCE) using FT-IR Spectroscopy

The bonds and the wave numbers (cm^{-1}) of prominent peaks of the major constituents obtained from spectra are described in Table 4.4 and Fig 4.4. The essential oil extracted by hydro-distillation method from fresh Leaves shows major peaks primarily at 3375.6cm^{-1} 2958.3 cm^{-1}, 2924.6 cm^{-1}, 2865.1 cm^{-1}, 1670.5 cm^{-1}, 1625.0 cm^{-1}, 1459.9cm^{-1}, 1377.8cm^{-1}, 1295.4cm^{-1}, 1219.8cm^{-1}, 1140.0cm^{-1}, 1062.1cm^{-1} ,864.3cm^{-1} and 723.1cm^{-1}. The peak at 3375.6cm^{-1} in fresh

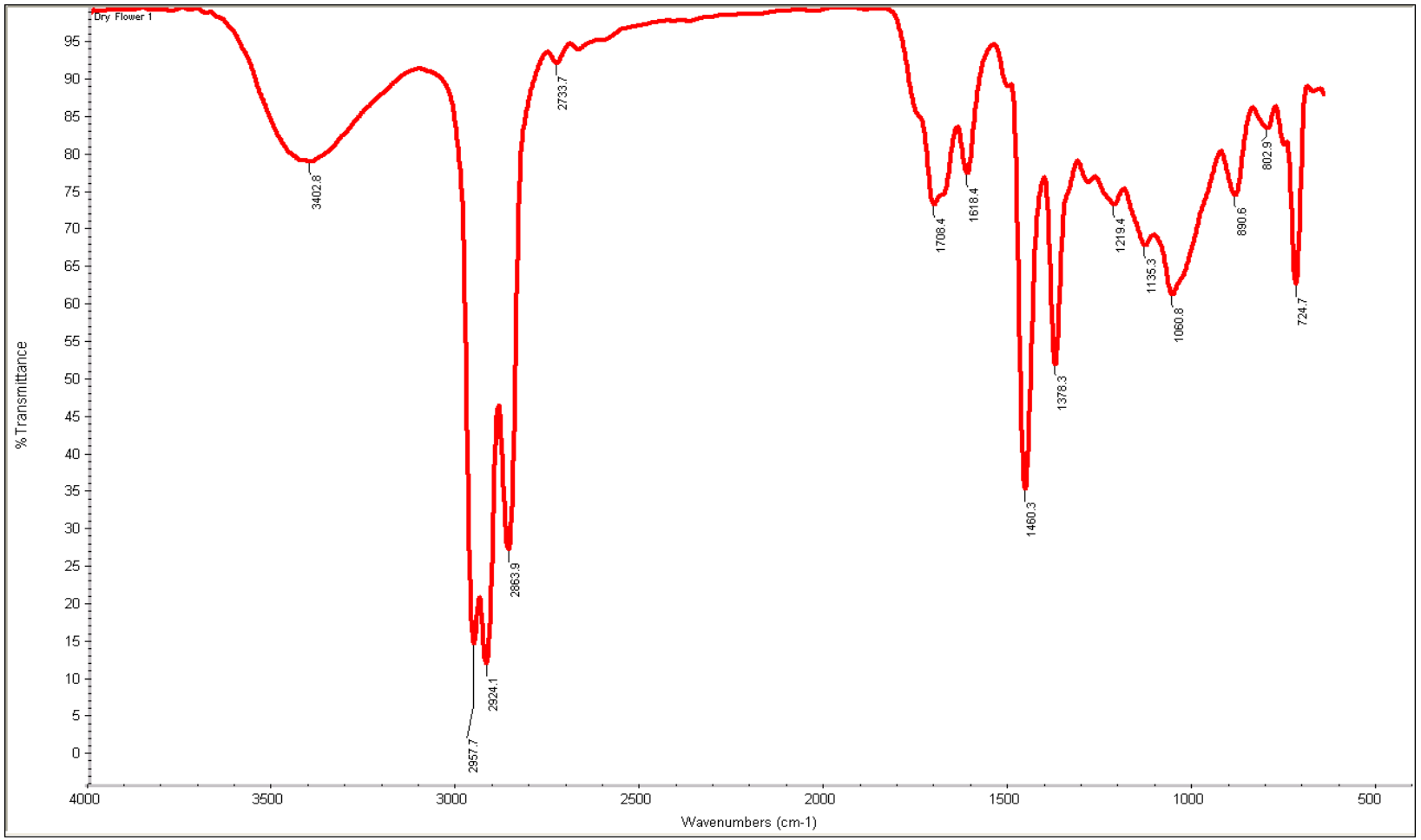


Fig. 4.3: Transmittance spectrum of hydro-distilled essential oil from dry flowers of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

Leaves oil was assigned to the N-H Stretching indicates the presence of amide functional group. The peak at 2958.3 cm^{-1}, 2924.6 cm^{-1} and 2865.1 cm^{-1} was due to C-H Stretching which clearly indicates the presence of alkane in the leaves oil. The peak at 1140.0cm^{-1} and 1062.1 cm^{-1} was indicates the existence of aliphatic amines. In the samples studied, the aromatics were present at the 864.3 cm^{-1} and 723.1 cm^{-1}.

4.2.2.4. Qualitative screening of Dry Leaves Clevenger Extract (DLCE) using FT-IR Spectroscopy

The FTIR spectroscopic analysis was made based on percentage of transmittance and wave numbers. The bonds and the wave numbers (cm^{-1}) of prominent peaks of the major constituents obtained from spectra are described in Table 4.4 and elucidated in Fig 4.5. The essential oil extracted by hydro-distillation method from dry leaves showed major peaks primarily at 3383.4 cm^{-1} 2958.2 cm^{-1}, 2926.0 cm^{-1}, 2867.7 cm^{-1}, 1684.6 cm^{-1}, 1617.0 cm^{-1}, 1514.2 cm^{-1}, 1454.2 cm^{-1}, 1375.9 cm^{-1}, 1268.6 cm^{-1}, 1219.3 cm^{-1}, 1149.1 cm^{-1}, 1065.1 cm^{-1}, 1033.2 cm^{-1}, 875.1 cm^{-1}, 812.3 cm^{-1} and 723.4 cm^{-1}. The peak at 3383.4 cm^{-1} in dry leaves oil was assigned to the N-H Stretching indicates the presence of amide functional group. The peak at 2958.2 cm^{-1}, 2926.0 cm^{-1} and 2867.7 cm^{-1} was due to C-H Stretching which clearly indicates the presence of alkane in the dry leaves oil. The peak at 1514.2 cm^{-1} which was only found in dry leaves oil was assigned to the N-O asymmetric stretch, indicates the presence of nitro compound in the sample. The peak at 1268.6 cm^{-1} and 1219.3 cm^{-1} indicates the presence of carboxylic acid functional group. The C-N Stretching was due to the aliphatic amines at the peak at 1149.1 cm^{-1}, 1065.1 cm^{-1} and 1033.2 cm^{-1}. The aromatics were present at the peak 875.1 cm^{-1} and 812.3 cm^{-1}.

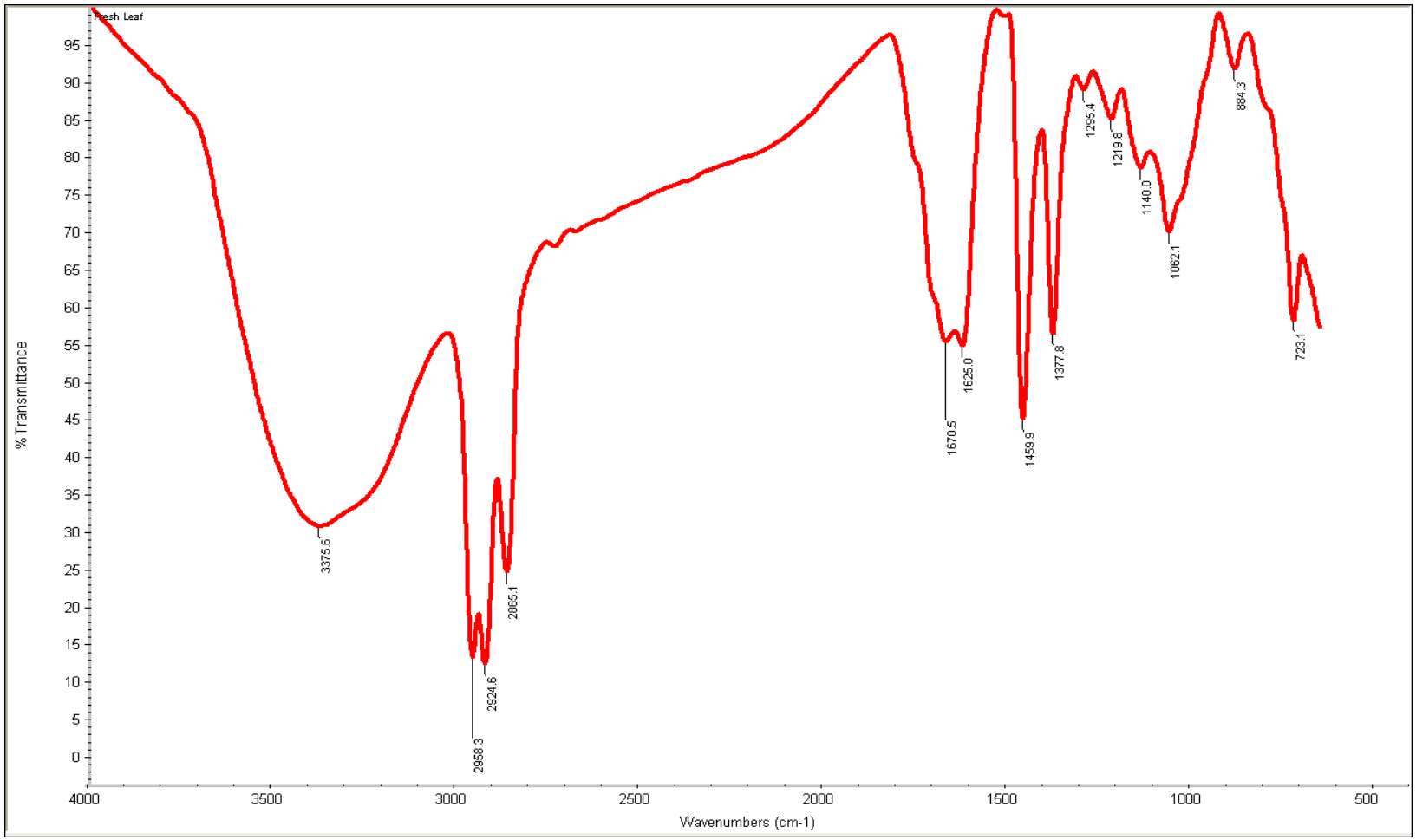


Fig. 4.4: Transmittance spectrum of hydro-distilled essential oil from fresh leaves of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

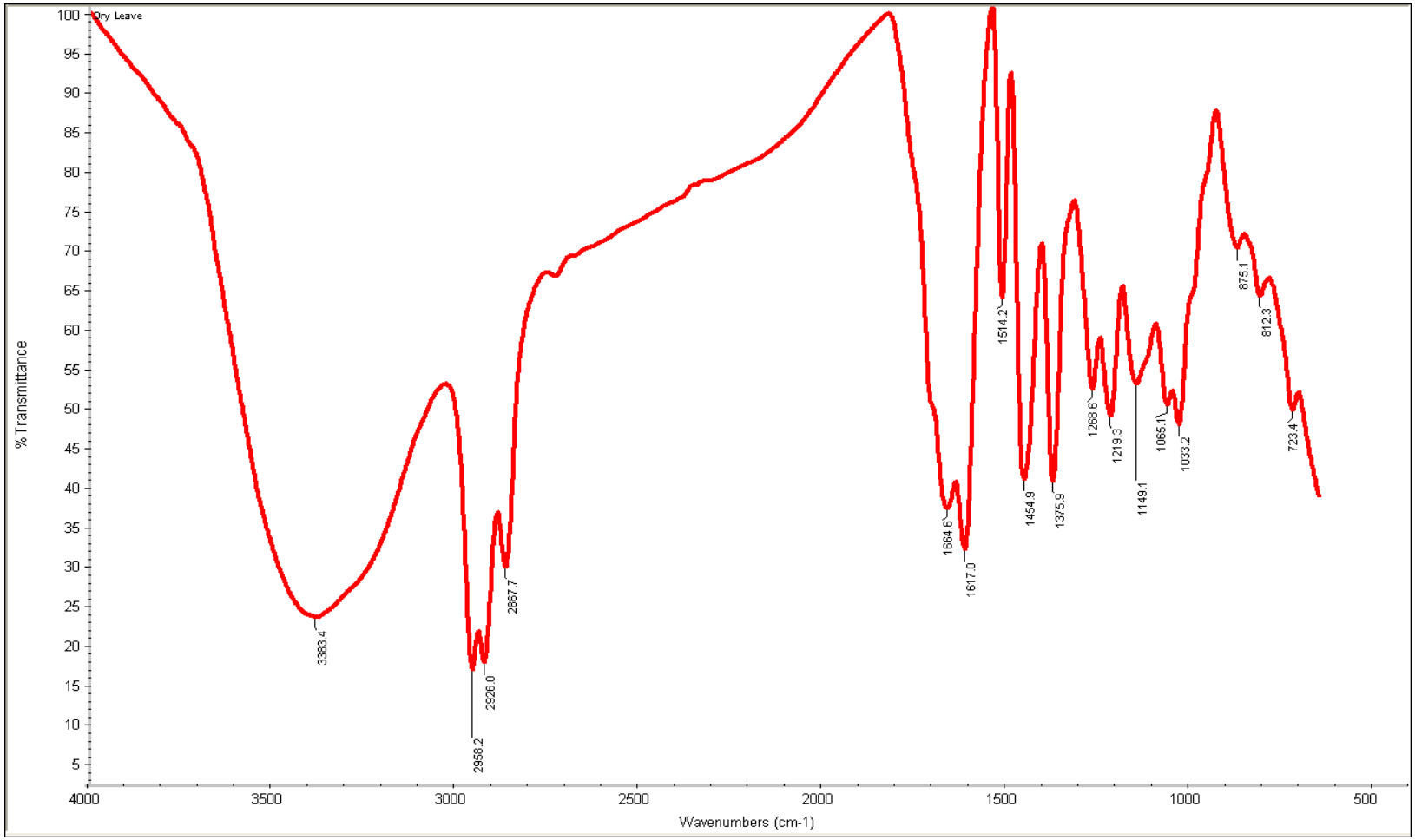


Fig. 4.5: Transmittance spectrum of hydro-distilled essential oil from dry leaves of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

4.2.2.5. Qualitative screening of Fresh Flowers Soxhlet Extract (FFSE) using FT-IR Spectroscopy

FTIR spectroscopic analysis of the Fresh Flowers Soxhlet Extract (FFSE) was revealed the existence of various chemical constituents. The bonds and the wave number (cm^{-1}) of prominent peaks obtained from spectra are elucidating in Table 4.5 and Fig 4.6. The essential oil extracted by Solvent extraction method from fresh flowers of marigold showed major peaks primarily at 3458.2 cm^{-1}, 2923.6 cm^{-1} ,2853.8 cm^{-1}, 1734.4 cm^{-1}, 1461.9 cm^{-1}, 1374.3 cm^{-1}, 1245.6 cm^{-1}, 1174.7 cm^{-1}, 1112.5 cm^{-1}, 980.1 cm^{-1} and 724.1.cm^{-1}. The transmittance frequency at 3458.2 cm^{-1} revealed the presence of O-H stretch for phenol and alcohol. The frequency band at 2923.6 cm^{-1} and 2853.8 cm^{-1} was due to C-H stretch which showed the existence of alkanes in the sample. The peak at 1734.4 cm^{-1} was assigned to α, β- unsaturated aldehydes, ketones due to C=O stretch. The frequency at 1461.9 cm^{-1} and 1374.3 cm^{-1} was for alkanes due to C-H bend and C-H rock respectively. The peak at 1245.6 cm^{-1} indicates the presence of carboxylic acid due to C-O stretch. The peak at 1174.7 cm^{-1}, 1112.5 cm^{-1} and 980.1 cm^{-1} was assigned to aliphatic amines due to C-N stretch. The peak at 724.1.cm^{-1} was due to C-H rock which indicates the presence of alkanes.

4.2.2.6. Qualitative screening of Dry Flowers Soxhlet Extract (DFSE) using FT-IR Spectroscopy

FTIR spectroscopic analysis of the Fresh Flowers Soxhlet Extract (FFSE) was revealed the existence of various chemical constituents. The bonds and the wave number (cm-1) of prominent peaks obtained from spectra are elucidating in Table 4.5 and Fig 4.7. The essential oil extracted by Solvent extraction method from dry flowers of marigold

showed major peaks primarily at 2957.9 cm^{-1}, 2923.8 cm^{-1}, 2862.7 cm^{-1}, 2733.4 cm^{-1}, 2674.1 cm^{-1}, 1461.7 cm^{-1}, 1379.2 cm^{-1}, 1342.6 cm^{-1}, 1296.8 cm^{-1}, 1247.5 cm^{-1}, 1135.8.cm^{-1}, 1060.9 cm^{-1}, 887.3 cm^{-1}, 797.8 cm^{-1} 758.2 cm^{-1} and 724.5 cm^{-1}. The peak at 2957.9 cm^{-1}, 2923.8 cm^{-1}, 2862.7 cm^{-1}, 1461.7 cm^{-1}, 1379.2 cm^{-1} and 724.5 cm^{-1}, assigned to the C-H stretching vibrations which indicates the presence of alkanes in the essential oil of dry flowers of marigold, extracted by solvent extraction method. The absorption band at 2733.4 cm^{-1} and 2674.1 cm^{-1} showed the existence of aldehydes in the essential oil of dry flowers of marigold extracted by solvent extraction method due to H-C=O: C-H Stretching. The nitro compound peat was observed at 1342.6 cm^{-1} and carboxylic acid peat was at 1296.8 cm^{-1} and 1247.5 cm^{-1} was due to N-O Symmetric stretch and C-O stretch, respectively. The peak at 1135.8.cm^{-1} and 1060.9 cm^{-1} was due to C-N stretching vibrations which mean the functional group aliphatic amines were present in the dry flowers of marigold extracted by solvent extraction method. The aromatic compounds were present at the peak 887.3 cm^{-1}, 797.8 cm^{-1} and 758.2 cm^{-1}.

Table 4.5: Wave numbers and related functional groups in essential oils extracted by solvent extraction from various samples of African marigold (*Tagetes erecta* L.) analyzed in FTIR spectrometer.

S.NO.	FREQUENCY (cm^{-1})	FRESH FLOWERS	DRY FLOWERS	FRESH LEAVES	DRY LEAVES	BOND	FUNCTIONAL GROUP
1.	4000-3900	-	-	3925.4	-	-	-
2.	3900-3850	-	-	3869.3	-	-	-
3.	3850- 3800	-	--	3824.1	-	-	-
4.	3800-3700	-	-	3752.3	-	-	-
5.	3480-3400	3458.2	-	3430.8	3471.1	O-H stretch	alcohols, phenols
6.	3390-3300	-	-	-	-	N-H Stretch	1*, 2* amines, amides
7.	2990-2950	-	2957.9	-	-	C-H Stretch	Alkanes
8.	3050-3000	-	-	3009.5	3009.8	=C-H stretch	Aromatic
9.	2950-2900	2923.6	2923.8	2920.4	2921.0	C-H Stretch	Alkanes
10.	2880-2850	2853.8	2862.7	2852.1	2852.7	C-H Stretch	Alkanes
11.	2750-2700	-	2733.4	-	-	H-C=O: C-H Stretch	aldehydes
12.	2690-2650	-	2674.1	-	-	H-C=O: C-H Stretch	aldehydes
13.	2400-2350	-	-	2363.2	2396.4	-	-
14.	2050-2000		-	2037.2		-	-
15.	1750-1700	1734.4	-	1739.0	1735.2	C=O Stretch	α, β- unsaturated aldehydes, ketones

				1715.2			
16.	1690-1650	-	-	1652.0	-	C=O Stretch	Carbonyls (general)
17.	1650-1600	-	-	-	1635.0	N-H Bend	1* amines
18.	1550-1500	-	-	1545.7	-	N-O asymmetric stretch	Nitro compounds
19.	1490-1450	1461.9	1461.7	1461.3	1458.5	C-H bend	alkanes
20.	1390-1350	1374.3	1379.2	1377.3	1366.8	C-H rock	alkanes
21.	1350-1300	-	1342.6		-	N-O Symmetric stretch	Nitro compounds
22.	1300-1250	-	1296.8		-	C-O Stretch	carboxylic acid
23.	1250-1200	1245.6	1247.5	1243.1	1231.5	C-O stretch	carboxylic acid
24.	1200-1150	1174.7	1135.8	1167.1	-	C-N Stretch	Aliphatic amines
25	1150-1050	1112.5	1060.9	1095.4	1093.7	C-N Stretch	Aliphatic amines
26	1050-900	980.1	-	-	-	C-N Stretch	Aliphatic amines
27	890-850	-	887.3	840.3	-	C-H “oop”	aromatic
28	850-800	-	-	-	835.4	C-H Bend (para)	aromatic
29	800-760	-	797.8	-		C-H “oop”	aromatic
30	760-720	-	758.2	722.9	728.7	C-H Bend (ortho)	aromatics
31	720-700	724.1	724.5	-	-	C-H rock	alkanes
32	600-550	-	-	582.4	592.9	C-Br stretch	Alkyl halides

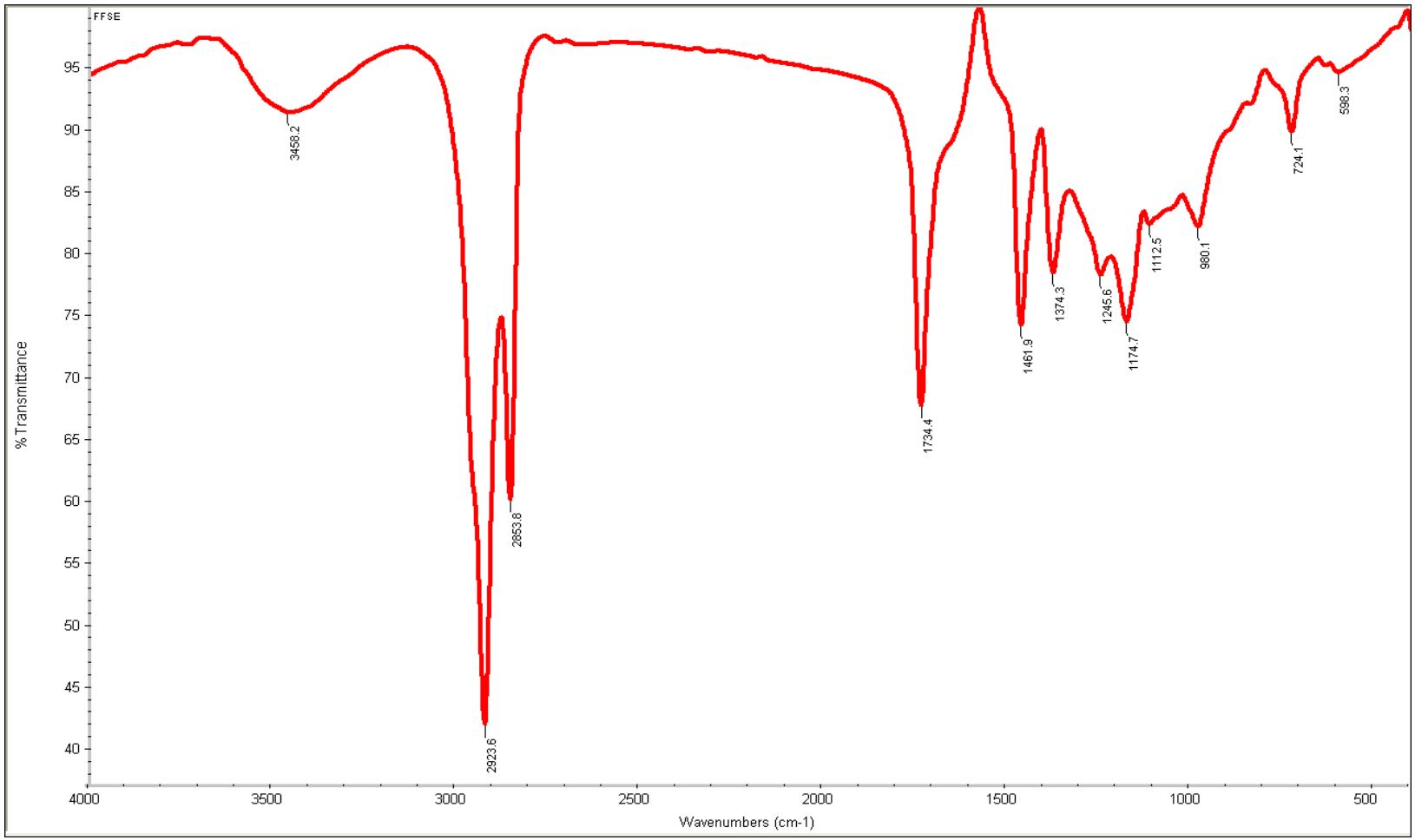


Fig. 4.6: Transmittance spectrum of essential oil extracted by solvent extraction from fresh flowers of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

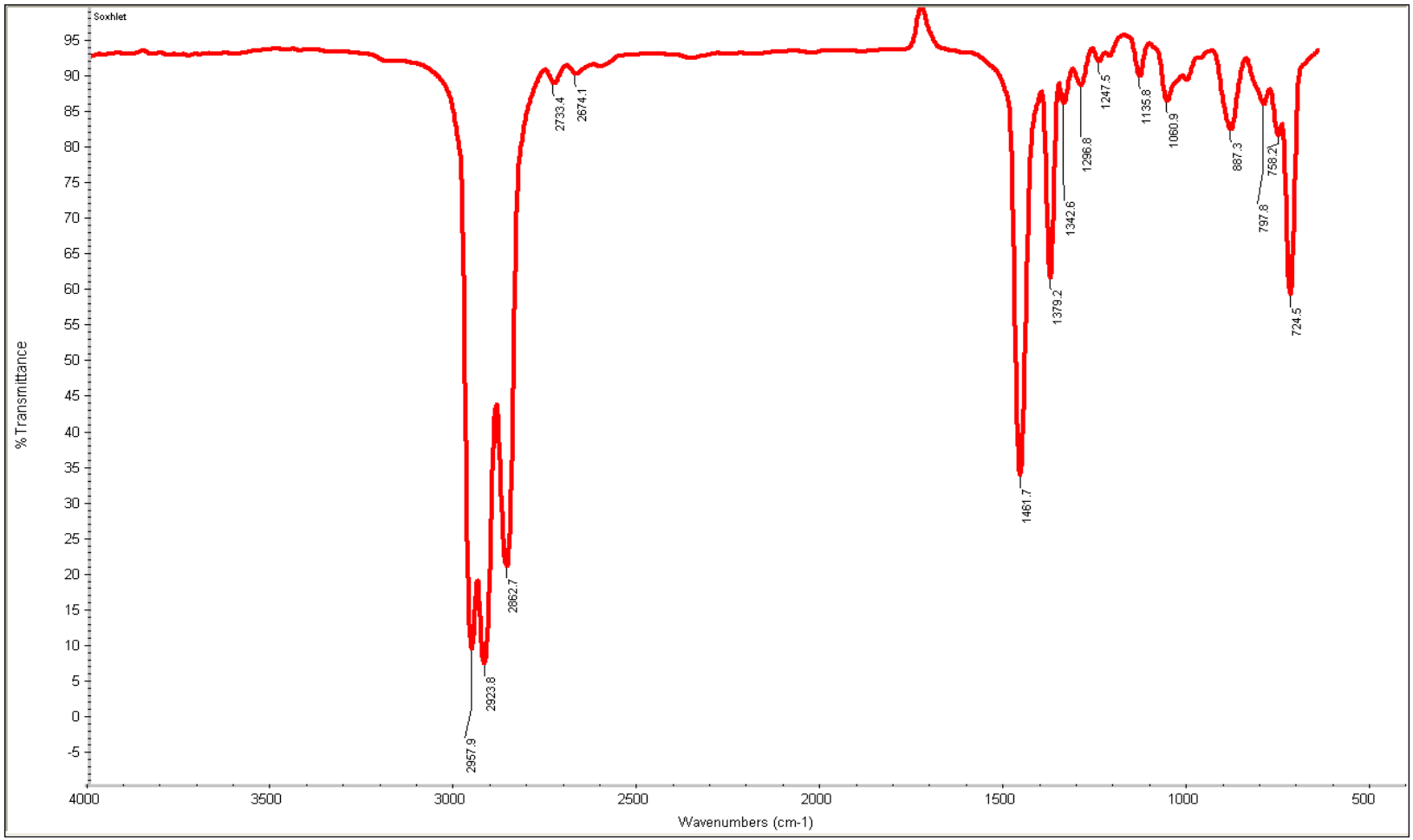


Fig. 4.7: Transmittance spectrum of essential oil extracted by solvent extraction from dry flowers of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

4.2.2.7. Qualitative screening of Fresh Leaves Soxhlet Extract (DFSE) using FT-IR Spectroscopy

FTIR spectroscopic analysis of the Fresh Leaves Soxhlet Extract (FFSE) was revealed the existence of various chemical constituents. The bonds and the wave number (cm^{-1}) of prominent peaks obtained from spectra are elucidated in Table 4.5 and Fig 4.8. The essential oil extracted by Solvent extraction method from dry leaves of marigold showed major peaks primarily at 3925.4cm^{-1}, 3869.3cm^{-1}, 3824.1cm^{-1}, 3752.3cm^{-1}, 3430.8cm^{-1}, 3009.5cm^{-1}, 2920.4cm^{-1}, 2852.1cm^{-1}, 2363.2cm^{-1}, 2037.2 cm^{-1}, 1739.0 cm^{-1}, 1715.2 cm^{-1}, 1652.0 cm^{-1}, 1545.7 cm^{-1}, 1461.3 cm^{-1}, 1377.3 cm^{-1}, 1243.1 cm^{-1}, 1167.1 cm^{-1}. 1095.4 cm-1, 840.3 cm^{-1}, 722.9 cm^{-1}.and 582.4 cm^{-1}. The peaks at 3925.4cm^{-1}, 3869.3cm^{-1}, 3824.1 cm^{-1}, 3752.3cm^{-1} 2363.2 cm^{-1} and 2037.2 cm^{-1} could not be identified. The peak at 3430.8cm^{-1} was due to the stretching vibration of O-H which showed the presence of alcohol and phenolic compounds in the fresh leaves oil of marigold (*Tagetes erecta* L.) extracted by solvent extraction method. The peak at 2920.4 cm^{-1}, 2852.1 cm^{-1}, 1461.3 cm^{-1}, 1377.3 cm^{-1} confirms the existence of alkanes. The aromatics were present at peak 3009.5 cm^{-1}, 840.3 cm^{-1} and 722.9 cm^{-1} which was possible due to C-H ortho and para stretching vibration. The transmittance frequency at 1739.0 cm^{-1} and 1715.2 cm^{-1} was due to stretching vibration of C=O which clearly showed the presence of α, β- unsaturated aldehydes, ketones. The peak at 1652.0 cm^{-1} was assigned due to C=O Stretch which showed the presence of carbonyls (general) functional group in the sample. The N-O asymmetric stretching vibration showed the existence of nitro compound. The peak at 1167.1 cm^{-1} and 1095.4 cm^{-1} confirms the presence functional group aliphatic amine in the fresh flowers oil of marigold. The carboxylic acid group was present at the peak 1243.1 cm^{-1} which was due to the vibration of C-O stretching. The lowest peak 582.4 cm^{-1} of the dry leaves oil of marigold

transmittance spectrum speaks about the existence of Alkyl halides which was due to the vibration of C-Br stretching

4.2.2.8. Qualitative screening of Dry Leaves Soxhlet Extract (DFSE) using FT-IR Spectroscopy

FT-IR spectroscopic analysis of the fresh leaves soxhlet extract (FFSE) has revealed the existence of various chemical constituents. The bonds and the wave number (cm^{-1}) of prominent peaks obtained from spectra are elucidated in Table 4.5 and Fig 4.9. The essential oil extracted by Solvent extraction method from dry leaves of marigold showed major peaks primarily at 3471.1cm^{-1}, 3009.8 cm^{-1}, 2921.0 cm^{-1}, 2852.7 cm^{-1}, 2396.4 cm^{-1}, 1735.2 cm^{-1}, 1635.0 cm^{-1}, 1458.5 cm^{-1}, 1366.8 cm^{-1}, 1231.5 cm^{-1}, 1093.7 cm^{-1}, 835.4 cm^{-1}, 728.7 cm^{-1} and 592.9 cm^{-1}. The peak at 3471.1cm^{-1} was due to the O-H stretching vibration which revealed the presence of alcohol and phenolic compounds in the dry leaves oil of marigold (*Tagetes erecta* L.) extracted by solvent extraction method. The aromatics were present at peak 3009.8 cm^{-1}, 835.4 cm^{-1} and 728.7 cm^{-1} which was possible due to C-H ortho and para stretching vibration. The peak at 2921.0 cm^{-1}, 2852.7 cm^{-1}, 1458.5 cm^{-1} and 1366.8 cm^{-1} confirms the existence of alkanes in the dry leaves oil of marigold (*Tagetes erecta* L.) extracted by solvent extraction method. The transmittance frequency at 1735.2 cm^{-1} was due to stretching vibration of C=O which clearly showed the presence of α, β- unsaturated aldehydes, and ketones. The N-H bend at the peak 1635.0 cm^{-1} confirms the availability of 1* amines in the sample. The carboxylic acid and Aliphatic amines functional group was present in the dry leaves oil at the peak 1231.5 cm^{-1} and 1093.7 cm^{-1} due to C-O Stretch and C-N Stretch respectively. The lowest peak 592.9 cm^{-1} of the dry leaves oil of marigold transmittance spectrum speaks about the existence of Alkyl halides which was due to the vibration of C-Br stretching.

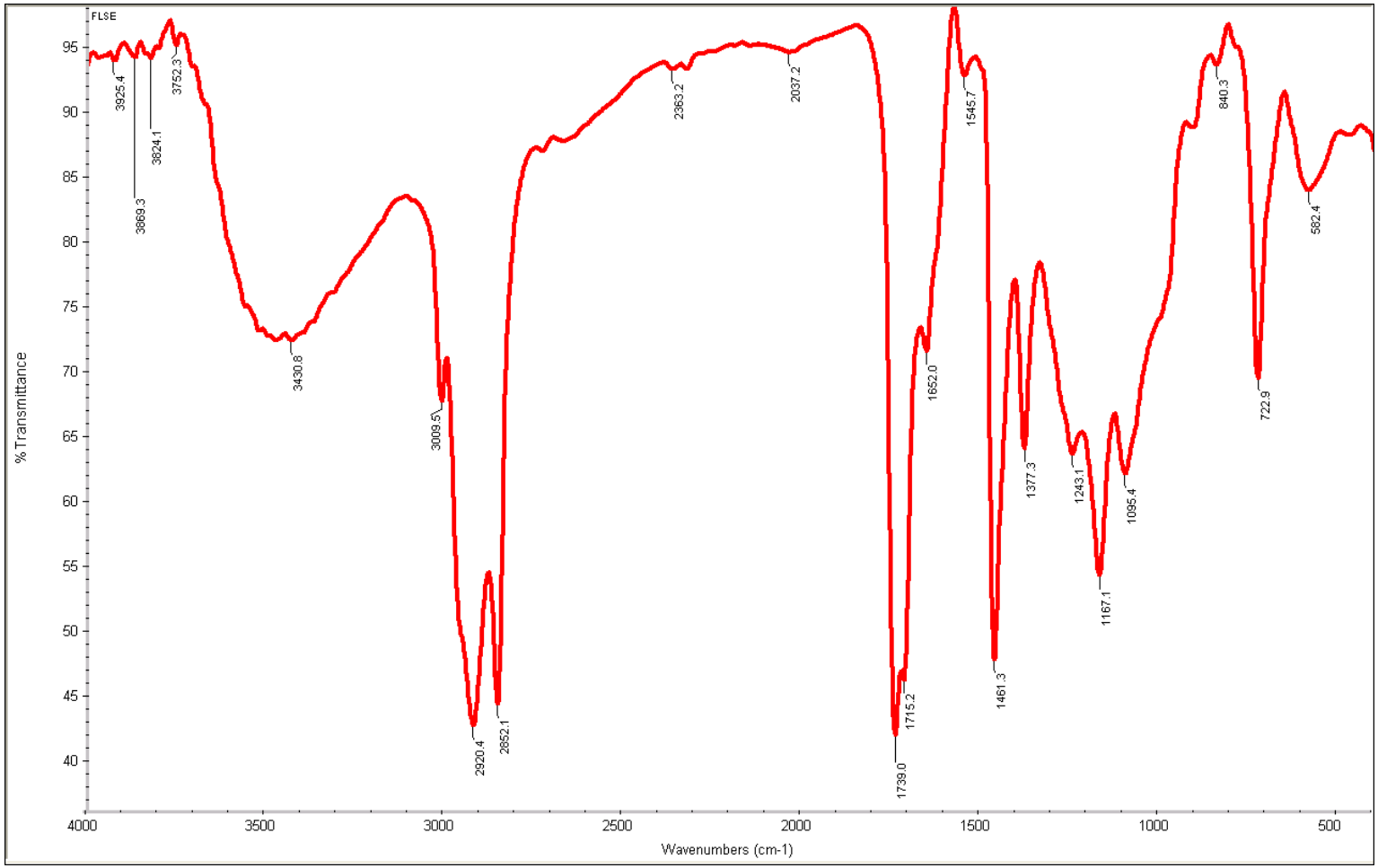


Fig. 4.8: Transmittance spectrum of essential oil extracted by solvent extraction from fresh leaves of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

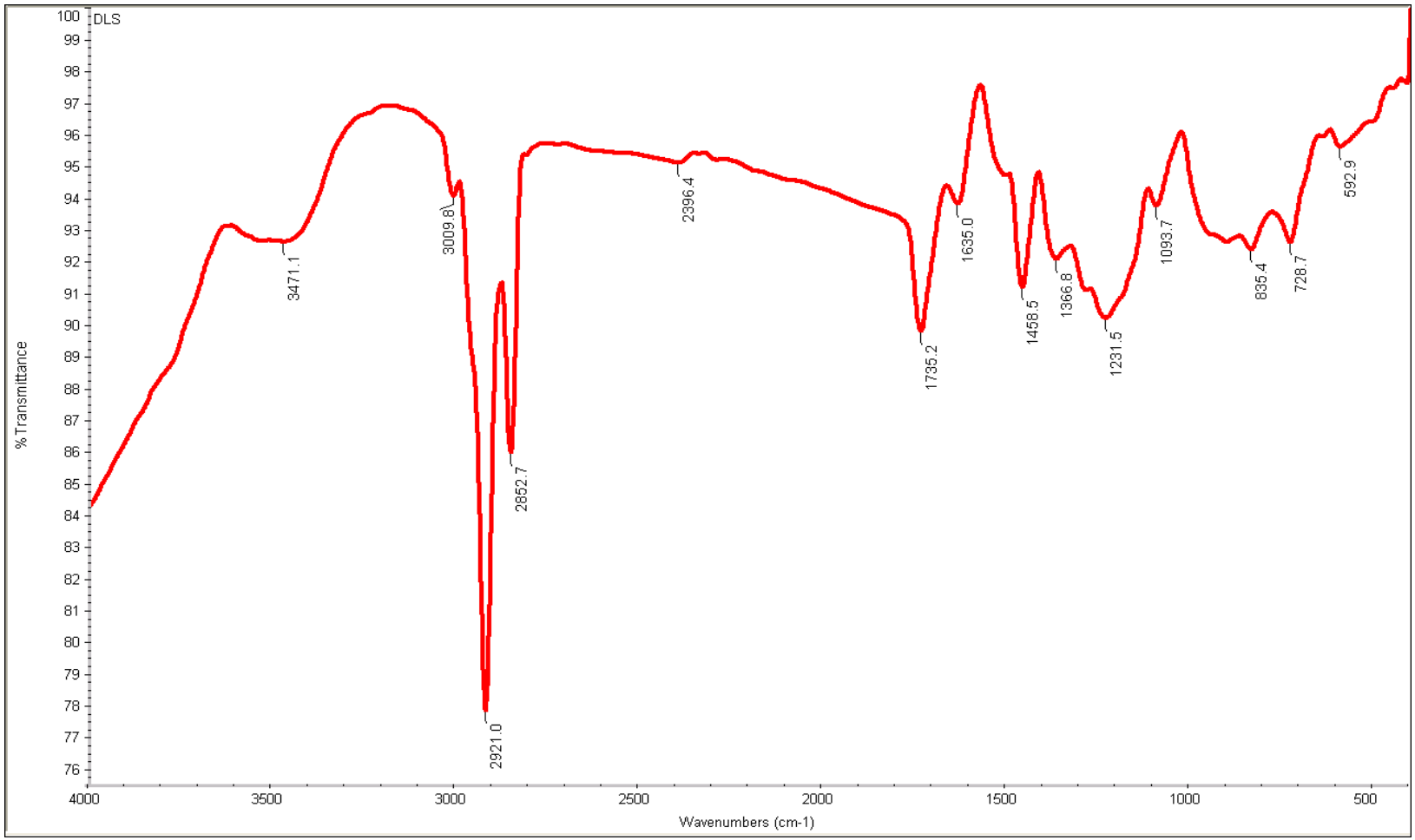


Fig. 4.9: Transmittance spectrum of essential oil extracted by solvent extraction from dry leaves of African marigold (*Tagetes erecta* L.) through FT-IR spectroscopy.

4.3. Experiment Number III

Quantitative estimation of essential oil from marigold plant parts

The quantitative estimation of essential oil from various samples of African marigold extracted by both hydro-distillation and solvent extraction methods was done by using Direct Analysis in Real Time Mass Spectrometry. The DART-MS was done at Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute (CSIR-CDRI), Lucknow; (U.P.) India. The DART-MS was recorded on a JEOL-AccuTOF LMS-T100LC Mass spectrometer having a DART (Direct Analysis in Real Time) source.

4.3.1. Quantitative estimation of hydro-distilled essential oil from fresh flowers of marigold

The phytometabolite components present in hydro-distilled essential oil from fresh flowers of African marigold was subjected to DART-MS and correlates the results obtained by FTIR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of hydro-distilled essential oil from fresh flowers of African marigold is given in Table 4.6.and Fig 4.10. The constituents were identified by matching their mass spectra with those recorded in literature. In fresh flowers essential oil, extracted by hydro-distillation method from African marigold, the peak at *m/z* 114.11 could be due to n-Heptanal or 2Heptanone having relative intensity (R.I % 4), the peak at *m/z* 135.14 best match with the compound terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene, limonene (R.I % 4).The peak at *m/z* 139.18 could be due to Tran-pinane or Cis pinane (R.I % 0.5). However, they have the same molecular formula; a distinction could not be easily made.). Besides this, the peak of other terpenes was

observed at *m/z*, 149.12, 151.13, and 153.15 corresponding to piperitenone, Ocimenone, umbellulone, verbenone (R.I % 10), tagetone (R.I % 90) and Linalool, fenchol, Terpinen-4-ol (R.I % 13) respectively. The peak at *m/z*, 167.12 could be due to Trans Dihydrocarvone epoxide or a-campholenic acid having relative intensity (R.I % 10). The phenolic compound galic acid was present at the peak had *m/z*, 169.14 (R.I % 5). The peak at *m/z* 170.14 and 180.13 best match with the compound 8 Hydroxy linalool, cis or Trans linalool oxide (R.I % 1) and Trans pinocarvyl formate (R.I % 1).The peak at *m/z* 183.12, 185.15 and 196.20 could be due to Mannitol (R.I % 7), 3,4-Dihydroxy-5-methoxybenzoic acid (R.I % 0.5) and Linalyl acetate or Geranyl acetate (R.I % 1), respectively.

Table 4.6: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in hydro-distilled essential oil from fresh flower of African marigold (*Tagetes erecta* L.) subjected to DART –MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	97	97.08	-	1.5%	-
2.	109	109.08	-	12%	-
3.	114	114.11	$C_7H_{14}O$	4%	n-Heptanal, 2Heptanone
4.	123	123.14	$C_8H_{10}O$	4%	p-Methylanisol, 4-Ethylphenol
5.	135	135.14	$C_{10}H_{16}$	4%	terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene
6.	139	139.18	$C_{10}H_{18}$	0.5%	Tran-pinane, Cis pinane
7.	149	149.12	$C_{10}H_{14}O$	10%	piperitenone, ocimenone, umbellulone, verbenone
8.	151	151.13	$C_{10}H_{18}0$	90%	tagetone
9.	153	153.15	$C_{10}H_{18}O$	13%	linalool, fenchol, Terpinen-4-ol
10.	165	165.12	$C_{10}H_{14}O_2$	0.5%	-
11.	167	167.12	$C_{10}H_{16}O_2$	10%	Trans Dihydrocarvone epoxide, a-campholenic acid
12.	169	169.14	$C_7H_6O_5$	5%	galic acid
13.	170	170.14	$C_{10}H_{18}O_2$	1%	8 hydroxylinalool, cis or Trans linalool oxide
14.	180	180.13	$C_{11}H_{16}O_2$	1%	Trans pinocarvyl formate
15.	183	183.12	$C_6H_{14}O_6$	7%	mannitol
16.	185	185.15	$C_8H_8O_5$	0.5%	3,4-Dihydroxy-5-methoxybenzoic acid
17.	196	196.20	$C_{12}H_{20}O_2$	1%	linalyl acetate, geranyl acetate

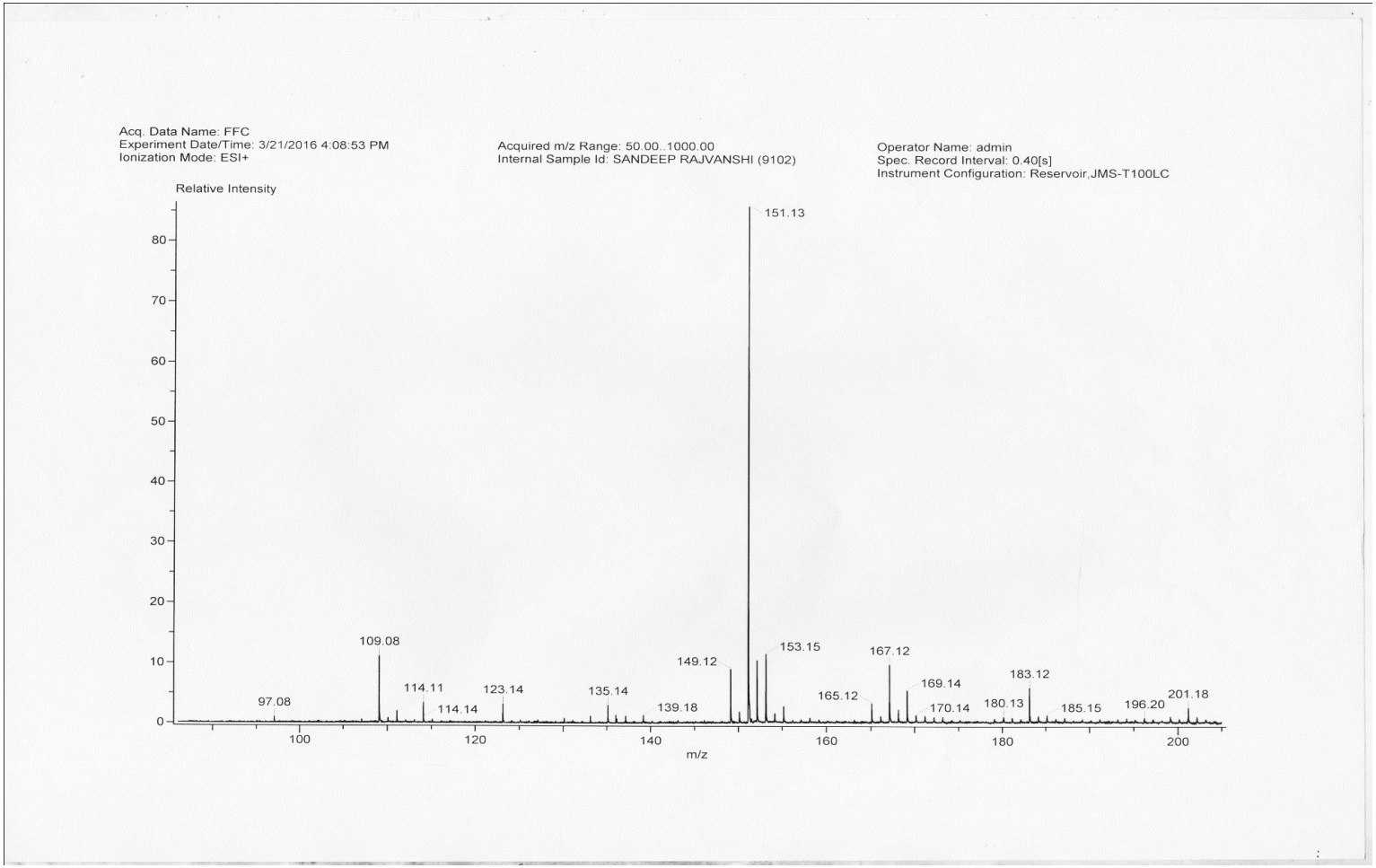


Fig. 4.10: DART-MS spectrum of hydro-distilled essential oil from fresh flowers of African marigold (*Tagetes erecta* L.).

Table 4.7: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in hydro-distilled essential oil from dry flower of African marigold (*Tagetes erecta* L.) subjected to DART – MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	97	97.07	-	1%	-
2.	114	114.11	$C_7H_{14}O$	5%	n-heptanal, 2heptanone
3.	123	123.14	$C_8H_{10}O$	4%	p-methylanisol, 4-ethylphenol
4.	135	135.14	$C_{10}H_{16}$	4%	terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene
6.	139	139.17	$C_{10}H_{18}$	0.5%	tran-pinane, cis pinane
7.	149	149.11	$C_{10}H_{14}O$	11%	piperitenone, ocimenone, umbellulone, verbenone
8.	151	151.13	$C_{10}H_{18}0$	91%	tagetone
9.	152	152.13	$C_{10}H_{16}O$	12%	piperitone, camphor
10.	165	165.11	$C_{10}H_{14}O_2$	4%	furomyrcenol
11.	167	167.13	$C_{10}H_{16}O_2$	16%	trans dihydro carvone epoxide, a-campholenic acid
12.	169	169.15	$C_7H_6O_5$	7%	galic acid
13.	172	172.20	$C_{10}H_{20}O_2$	1%	trans-linalool oxide
14.	181	181.12	$C_{11}H_{18}O_2$	0.5%	geranyl formate
15.	183	183.12	$C_6H_{14}O_6$	10%	mannitol
16.	185	185.16	$C_8H_8O_5$	2%	3,4-dihydroxy-5-methoxybenzoic acid

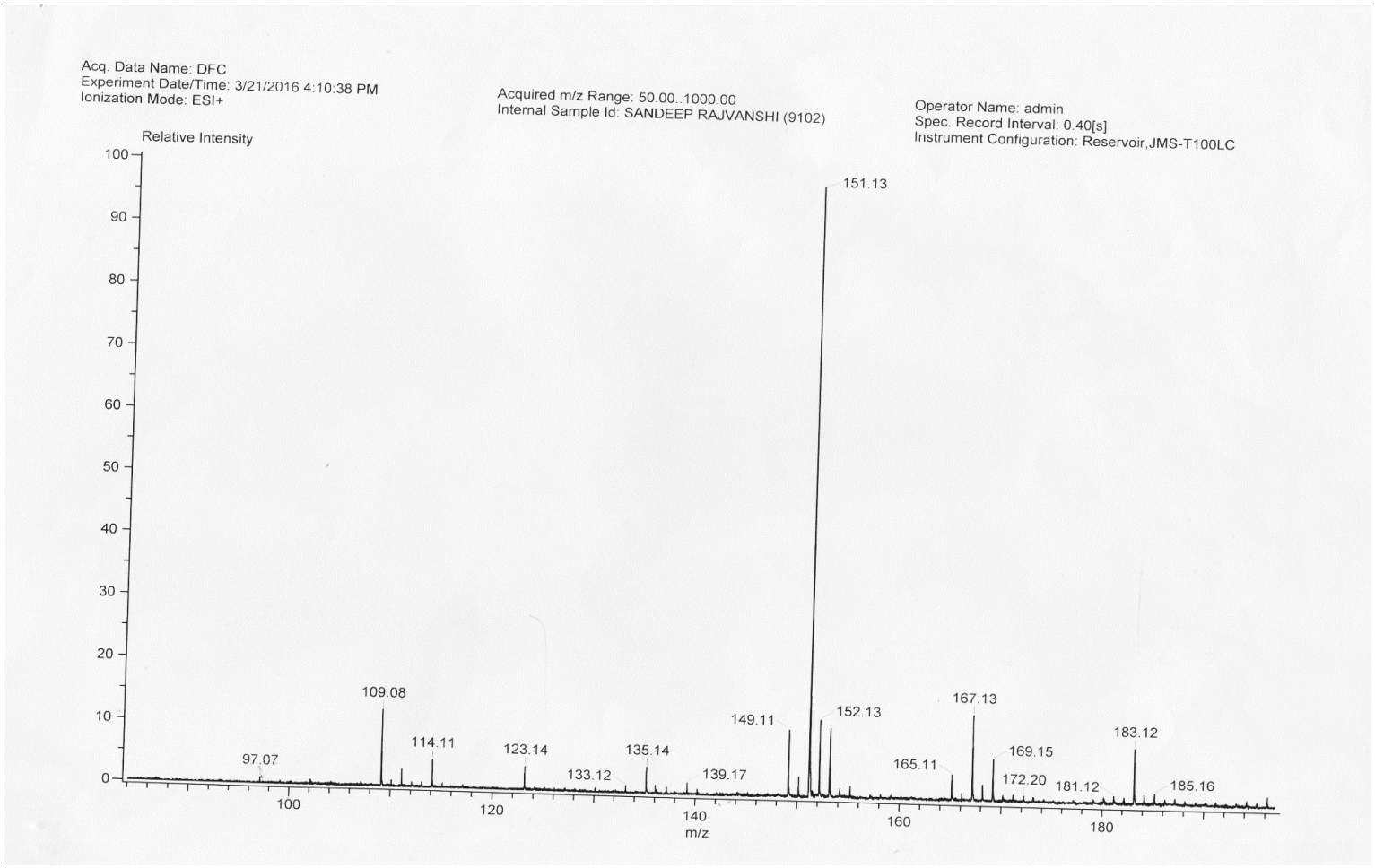


Fig. 4.11: DART-MS spectrum of hydro-distilled essential oil from dry flowers of African marigold (*Tagetes erecta* L.).

Table 4.8: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in hydro-distilled essential oil from fresh flower of African marigold (*Tagetes erecta* L.) subjected to DART – MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	114	114.11	$C_7H_{14}O$	2.5	n-heptanal, 2heptanone
2.	136	136.04	$C_{10}H_{16}$	0.7	terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene
3.	151	151.14	$C_{10}H_{18}0$	2	tagetone
4.	167	167.13	$C_{10}H_{16}O_2$	0.5	trans dihydrocarvone epoxide, a-campholenic acid
6.	207	207.18	$C_{13}H_{20}O_2$	0.6	trans and cis carvyl propionate
7.	218	218.24	$C_{13}H_{14}O_3$	2	hydroxytremetone
8.	246	246.28	$C_{17}H_{26}O$	2	avocadynofuran, amberone
9.	262	262.26	$C_{17}H_{26}O_2$	1.5	4bacetoxygymnomitr3(15)-ene
10.	274	274.31	$C_{17}H_{22}O_3$	12	2-acetoxyfuranoelemene
11.	279	279.27	$C_{18}H_{30}O_2$	11	6b-acetoxyeudesm-4(15)-en-7bol
12.	296	296.31	$C_{19}H_{36}O_2$	3	n-heneicosane (c21), methyl oleate
13.	297	297.31	$C_{19}H_{38}O_2$	3	methyl stearate
14.	318	318.35	$C_{15}H_{10}$	7	quercetagetin
15.	319	319.34	$C_{19}H_{28}O_4$	0.3	4b,5b-diacetoxygymnomitr-3(15)-ene
16.	346	346.40	$C_{22}H_{34}O_3$	0.6	11-b-hydroxykauren-15-a-yl-acetate
17	362	362.38	$C_{15}H_{22}O_{10}$	1.5	catalpol
18	391	391.34	$C_{24}H_{25}NO_4$	98	flavoxate
19	392	392.35	$C_{25}H_{28}O_4$	25	glabrol

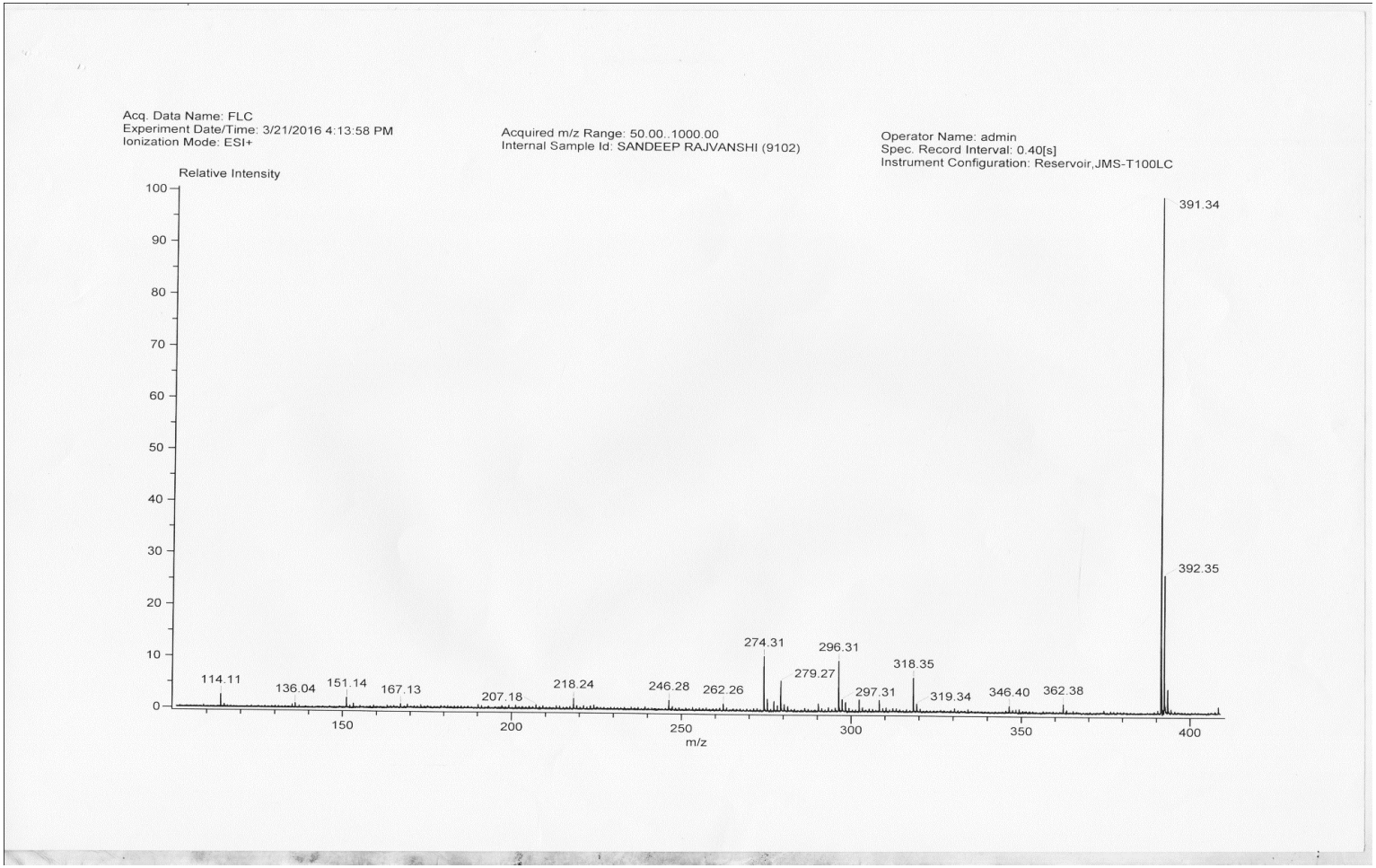


Fig. 4.12: DART-MS spectrum of hydro-distilled essential oil from fresh leaves of African marigold (*Tagetes erecta* L.).

Table 4.9: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in hydro-distilled essential oil from dry flower of African marigold (*Tagetes erecta* L.) subjected to DART – MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	218	218.24	$C_{13}H_{14}O_3$	3.9	hydroxytremetone
2.	274	274.32	$C_{17}H_{22}O_3$	9.9	2-acetoxyfuranoelemene
3.	296	296.31	$C_{19}H_{36}O_2$	19.8	n-heneicosane (c21), methyl oleate
4.	318	318.34	$C_{15}H_{10}$	7.2	quercetagetin
6.	391	391.34	$C_{24}H_{25}NO_4$	31.1	flavoxate
7.	392	392.35	$C_{25}H_{28}O_4$	26	glabrol
8.	393	393.34	$C_{24}H_{40}O_4$	5	chenodeoxycholic acid
9.	504	504.44	$C_{24}H4_0N_8O_4$	5	dipyridamole
10.	664	-	$C_{41}H_{76}O_6$	4	-
11.	798	-	$C_{51}H_{90}O_6$	4	-

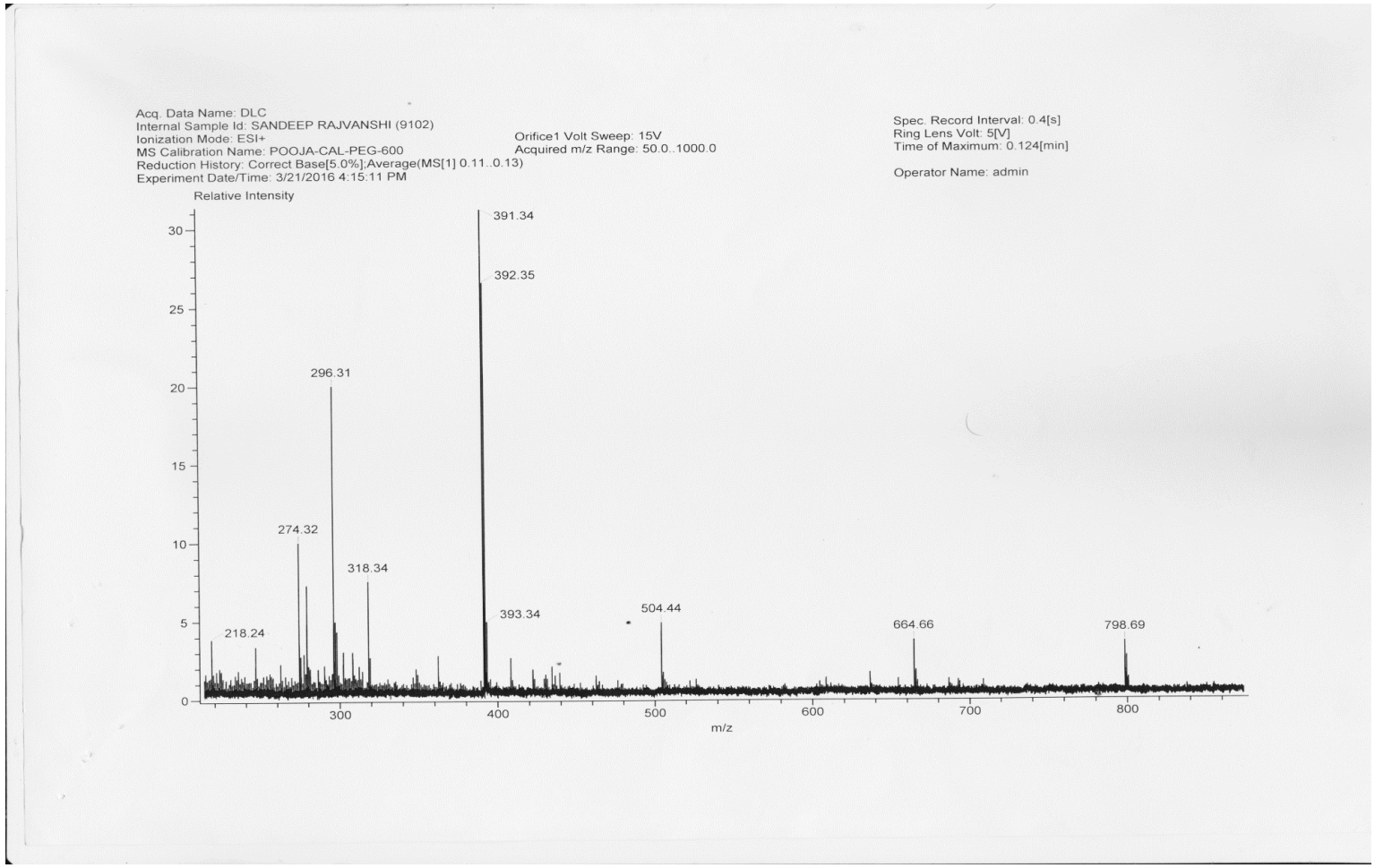


Fig. 4.13: DART-MS spectrum of hydro-distilled essential oil from dry leaves of African marigold (*Tagetes erecta* L.).

4.3.2. Quantitative estimation of hydro-distilled essential oil from dry flowers of marigold

The phytometabolites component present in hydro-distilled essential oil from dry flowers of African marigold was subjected to DART-MS and correlate with the results obtained by FT-IR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of hydro-distilled essential oil from dry flowers of African marigold is given in Table 4.7 and Fig 4.11. The constituents were identified by matching their mass spectra with those recorded in literature. In dry flowers essential oil, extracted by hydro-distillation method from African marigold, the peak at *m/z* 114.11 could be due to n-Heptanal or 2-Heptanone having relative intensity (R.I % 5), the peak at *m/z* 123.14 best match with the compound p-Methylanisol, or 4-Ethylphenol (R.I % 4). The peak at *m/z* 135.14 could be due to terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene, limonene (R.I % 4). However, they have the same molecular formula; a distinction could not be easily made. Besides this, the peak of other terpenes was observed at *m/z*, 139.17, 149.11, and 151.13 corresponding to trans-pinane, cis pinane (R.I % 0.5), piperitenone, ocimenone, umbellulone, verbenone (R.I % 11) and tagetone (R.I % 91) respectively. The peak at *m/z*, 152.13 could be due to piperitone, camphor having relative intensity (R.I % 12). The peak at *m/z* 167.13 best match with the compound Trans dihydrocarvone epoxide, or a-campholenic acid (R.I % 16). The phenolic compound galic acid was present at the peak *m/z*, 169.15 (R.I % 5). The peak at *m/z* 172.20, 181.12 and 183.12 could be due to trans-linalooloxide (R.I % 1), geranyl formate (R.I % 0.5) and mannitol (R.I % 10), respectively. The peak at *m/z* 185.16 best match with the compound 3,4-Dihydroxy-5-methoxybenzoic acid (R.I % 2).

4.3.3. Quantitative estimation of hydro-distilled essential oil from fresh leaves of marigold

The phytometabolites component present in hydro-distilled essential oil from fresh leaves of African marigold was subjected to DART-MS and correlates the results obtained by FTIR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of hydro-distilled essential oil from fresh leaves of African marigold were given in Table 4.8 and Fig 4.12. The constituents were identified by matching their mass spectra with those recorded in literature. In fresh leaves essential oil, extracted by hydro-distillation method from African marigold, the peak at *m/z* 114.11 could be due to n-Heptanal or 2Heptanone having relative intensity (R.I % 2.5), the peak at *m/z* 136.04 best match with the compound terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene (R.I % 0.7). The peak at *m/z* 151.14 could be due to tagetone (R.I % 2). However, they have the same molecular formula; a distinction could not be easily made. Besides this, the peak observed at *m/z*, 167.13, 207.1 and 218.24 corresponding to trans dihydrocarvone epoxide, a-campholenic acid (R.I % 0.5), trans and cis carvyl propionate (R.I % 0.6) and hydroxytremetone (R.I % 2) respectively. The peak at *m/z*, 246.28 could be due to Avocadynofuran or Amberone having relative intenity (R.I % 2). The peak at *m/z* 262.26 best match with the compound 4b-Acetoxygymnomitr3(15)-ene (R.I % 1.5). The peak at *m/z* 274.31,279.27 and 296.31 could be due to 2-Acetoxyfuranoelemene (R.I % 12), 6b-Acetoxyeudesm-4(15)-en-7bol (R.I % 11) and n-Heneicosane (C21), Methyl oleate (R.I % 3), respectively. The peak at *m/z* 297.31 best match with the compound Methyl stearate (R.I % 3). Besides this, the peak observed at *m/z*, 318.35, 319.34 and 346.40 corresponding to Quercetagetin (R.I %

7), 4b,5b-Diacetoxygymnomitr-3(15)-ene (R.I % 0.3) and 11-b-Hydroxykauren-15-a-yl-acetate (R.I % 0.6) respectively. In DART-MS spectrum the peak observed at *m/z*, 362.38, 391.34, and 392.35 could be due to Catalpol (R.I % 1.5), Flavoxate (R.I % 98) and Glabrol (R.I % 25) respectively.

4.3.4. Quantitative estimation of hydro-distilled essential oil from dry leaves of marigold

The phytometabolites component present in hydro-distilled essential oil from dry leaves of African marigold was subjected to DART-MS and correlates the results obtained by FT-IR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of hydro-distilled essential oil from dry leaves of African marigold were given in Table 4.9 and Fig 4.13. The constituents were identified by matching their mass spectra with those recorded in literature. In dry leaves essential oil, extracted by hydro-distillation method from African marigold, the peak at *m/z* 218.24 could be due to hydroxytremetone having relative intensity (R.I % 3.9), the peak at *m/z* 274.32 best match with the compound 2-Acetoxyfuranoelemene (R.I % 9.9). The peak at *m/z* 296.31 could be due to n-heneicosane (C21), methyl oleate (R.I % 19.8). However, they have the same molecular formula; a distinction could not be easily made. Besides this, the peak observed at *m/z*, 318.34 corresponding Quercetagetin (R.I % 7.2. The peak at *m/z*, 391.34 could be due to flavoxate having relative intensity (R.I % 31.1). The peak at *m/z* 392.35 best match with the compound glabrol (R.I % 26). The peak at *m/z* 393.34 and 504.44 could be due to chenodeoxycholic acid (R.I % 5) and dipyridamole (R.I % 5).

4.3.5. Quantitative estimation of solvent extracted essential oil from fresh flowers of marigold

The phytometabolites component present in solvent extracted essential oil from fresh flowers of African marigold was subjected to DART-MS and correlates the results obtained by FT-IR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of solvent extracted essential oil from fresh flowers of African marigold were given in Table 4.10 and Fig 4.14. The constituents were identified by matching their mass spectra with those recorded in literature. In fresh flowers essential oil, extracted by solvent extraction method from African marigold, the peak at *m/z* 114.11 could be due to n-Heptanal or 2Heptanone having relative intensity (R.I % 1.8), the peak at *m/z* 123.14 best match with the compound p-Methylanisol, 4-Ethylphenol (R.I % 1.8).The peak at *m/z* 135.14 could be due to Terpinolene, Thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene (R.I % 2.3). However, they have the same molecular formula; a distinction could not be easily made.). Besides this, the peak of other terpenes was observed at *m/z*, 139.17, 149.11, and 151.13 corresponding to Tran-pinane, Cis pinane (R.I % 1.6), Piperitenone, Ocimenone, umbellulone, verbenone (R.I % 6.2) and tagetone (R.I % 40.8) respectively. The peak at *m/z*, 153.19 could be due to Linalool, fenchol, Terpinen-4-ol (R.I % 7.4). The peak at *m/z* 165.11 best match with the compound Furomyrcenol (R.I % 2.5). The peak at *m/z* 167.12 could be due to Trans Dihydrocarvone epoxide, a-Campholenic acid (R.I % 6.1). Besides this, the peak of observed at *m/z*, 169.15, 173.18, and 180.20 corresponding to galic acid (R.I % 2.5), Trans Linalooloxide (furanoid), 3, 7-Dimethyl-3,7-dihydroxyoct-1-ene (R.I % 0.4) and TransPinocarvyl formate (R.I % 0.3) respectively. The peak at *m/z*, 183.12 could be due

to annitol (R.I % 4). The peak at *m/z* 185.14 best match with the compound 3,4-Dihydroxy-5-methoxybenzoic acid (R.I % 0.3) and the peak at *m/z* 201.18 best match with the compound Aromadendra1- (10),4(15)-diene (R.I % 2.0).

4.3.6. Quantitative estimation of solvent extracted essential oil from dry flowers of marigold

The phytometabolites component present in solvent extracted essential oil from dry flowers of African marigold was subjected to DART-MS and correlates the results obtained by FT-IR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of solvent extraction essential oil from dry flowers of African marigold were given in Table 4.11 and Fig 4.15. The constituents were identified by matching their mass spectra with those recorded in literature. In dry flowers essential oil, extracted by solvent extracted method from African marigold, the peak at *m/z* 114.11 could be due to n-Heptanal or 2Heptanone having relative intensity (R.I % 5.8), the peak at *m/z* 123.14 best match with the compound p-Methylanisol, 4-Ethylphenol (R.I % 3.7). The peak at *m/z* 135.14 could be due to terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene, limonene (R.I % 3.8). Besides this, the peak observed at *m/z*, 139.17 , 149.12, and 151.13 corresponding to tran-pinane, cis pinane (R.I % 2.2), piperitenone, ocimenone, umbellulone, verbenone (R.I % 7.9) and tagetone (R.I % 52) respectively. The peak at *m/z*, 153.15 could be due to linalool, fenchol, terpinen-4-ol (R.I % 14). The peak at *m/z* 165.12 best match with the compound Furomyrcenol (R.I % 4.3). The peak at *m/z* 167.13 could be due to trans dihydrocarvone epoxide, a-campholenic acid (R.I % 12). Besides this, the peak of observed at *m/z*, 169.15 and 180.13 corresponding to Galic acid (R.I % 6), and trans pinocarvyl formate (R.I % 0.3) respectively. The peak at *m/z*, 183.12

could be due to annitol (R.I % 7.9). The peak at *m/z* 185.15 best match with the compound 3,4-Dihydroxy-5-methoxybenzoic acid (R.I % 0.3) .The peak at *m/z* 193.18 and201.19 was best match with the compound trans sabinyl acetate (R.I % 0.3) and 6-ethoxy-2,4-dimethylquinoline (RI % 2.4).

4.3.7. Quantitative estimation of solvent extracted essential oil from fresh leaves of marigold

The phytometabolites component present in solvent extracted essential oil from fresh leaves of African marigold was subjected to DART-MS and correlates the results obtained by FTIR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of solvent extracted essential oil from fresh leaves of African marigold were given in Table 4.12 and Fig 4.16. The constituents were identified by matching their mass spectra with those recorded in literature. In fresh leaves essential oil, extracted by solvent extraction method from African marigold, the major peaks with higher relative intensity at *m/z* 173 which could be due to Trans Linalooloxide (furanoid), having relative intensity (R.I % 3.5), the peak at *m/z* 218.25 best match with the compound Hydroxytremetone (R.I % 12.5). The peak at *m/z* 246.26 could be due to Avocadynofuran, Amberone (R.I % 11). Besides this, the peak observed at *m/z*, 262.28, 274.32 , and 302.36 corresponding to 4b-Acetoxygymnomitr3(15)-ene (R.I % 10), 2-Acetoxyfuranoelemene (R.I % 97.5) and (Z)-Nuciferyl 2-methylbutyrate (R.I % 32) respectively. The peak at *m/z*, 318.34 could be due to flavonoid compound Quercetagetin (R.I % 95). The peak at *m/z* 319.34 best match with the compound 4b,5b-Diacetoxygymnomitr-3(15)-ene (R.I % 20). The peak at *m/z* 346.37 could be due to 11-b-Hydroxykauren-15-a-yl- acetate (R.I % 30). Besides this, the peak of observed at *m/z*, 362.38 and 374.42 corresponding to Catalpol (R.I % 33.5), and Geniposidic acid (R.I %

14) respectively. The peak at *m/z*, 418.43 could be due to Kaempferol-3-O-α-L-arabinoside (R.I % 3.5). The peak at *m/z* 429.42 best match with the compound 37β-hydroxysitosterol R.I % 6.0).

4.3.8. Quantitative estimation of solvent extracted essential oil from dry leaves of marigold

The phytometabolites component present in solvent extracted essential oil from dry leaves of African marigold was subjected to DART-MS and correlates the results obtained by FTIR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of solvent extracted essential oil from dry leaves of African marigold were given in Table 4.13 and Fig 4.17. The constituents were identified by matching their mass spectra with those recorded in literature. In dry leaves essential oil, extracted by solvent extraction method from African marigold, the major peaks with higher relative intensity at *m/z* 114.11 which could be due to 2-Heptanone having relative intensity (R.I % 3.0), the peak at *m/z* 136.04 best match with the terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene (R.I % 8.0). The peak at *m/z* 143.12 could be due to 2, 3, 5-trimethylvalerolactone (R.I % 2.1). Besides this, the peak observed at *m/z*, 151.14, 157.11, and 169.16 corresponding to tagetone (R.I % 3.4), menthol, dihydrolinalool (R.I % 6.0) and galic acid (R.I % 3.4) respectively. The peak at *m/z*, 173.13 could be due to trans linalooloxide (furanoid), (R.I % 4.1). The peak at *m/z* 183.12 best match with the compound mannitol (R.I % 3.4). The peak at *m/z* 190.17 could be due to 8, 9-Dehydrothymol acetate (R.I % 8.5). Besides this, the peak of observed at *m/z*, 193.16 and 197.15 corresponding to Trans Sabinyl acetate (R.I % 2.5), and syringic acid (R.I % 4.5) respectively. The peak at *m/z*, 218.25 could be due to Lemnalone or Taylorione (R.I % 15.0). The peak at *m/z* 225.18 best match with the compound g-tetradecanolide (R.I % 3.4).

Table 4.10: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in soxhlet extracted essential oil from fresh flower of African marigold (*Tagetes erecta* L.) subjected to DART – MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	109	109.08	-	6.3	-
2.	114	114.11	$C_7H_{14}O$	1.8	n-heptanal, 2heptanone
3.	123	123.14	$C_8H_{10}O$	1.8	p-methylanisol, 4-ethylphenol
4.	135	135.14	$C_{10}H_{16}$	2.3	terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene
6.	139	139.17	$C_{10}H_{18}$	1.6	tran-pinane, cis pinane
7.	149	149.11	$C_{10}H_{14}O$	6.2	piperitenone, ocimenone, umbellulone, verbenone
8.	151	151.13	$C_{10}H_{84}0$	40.8	tagetone
9.	153	153.19	$C_{10}H_{18}O$	7.4	linalool, fenchol, terpinen-4-ol
10.	165	165.11	$C_{10}H_{14}O_2$	2.5	furomyrcenol
11.	167	167.12	$C_{10}H_{16}O_2$	6.1	trans dihydrocarvone epoxide, a-campholenic acid
12.	169	169.15	$C_7H_6O_5$	2.5	galic acid
13.	173	173.18	$C_{10}H_{20}O_2$	0.4	trans linalooloxide (furanoid), 3,7-dimethyl-3,7-dihydroxyoct-1-ene
14.	180	180.20	$C_{11}H_{16}O_2$	0.3	transpinocarvyl formate
15.	183	183.12	$C_6H_{14}O_6$	4.0	Mannitol
16.	185	185.14	$C_8H_8O_5$	0.3	3,4-dihydroxy-5-methoxybenzoic acid
17	201	201.18	$C_{15}H_{22}$	2.0	aromadendra1- (10),4(15)-diene

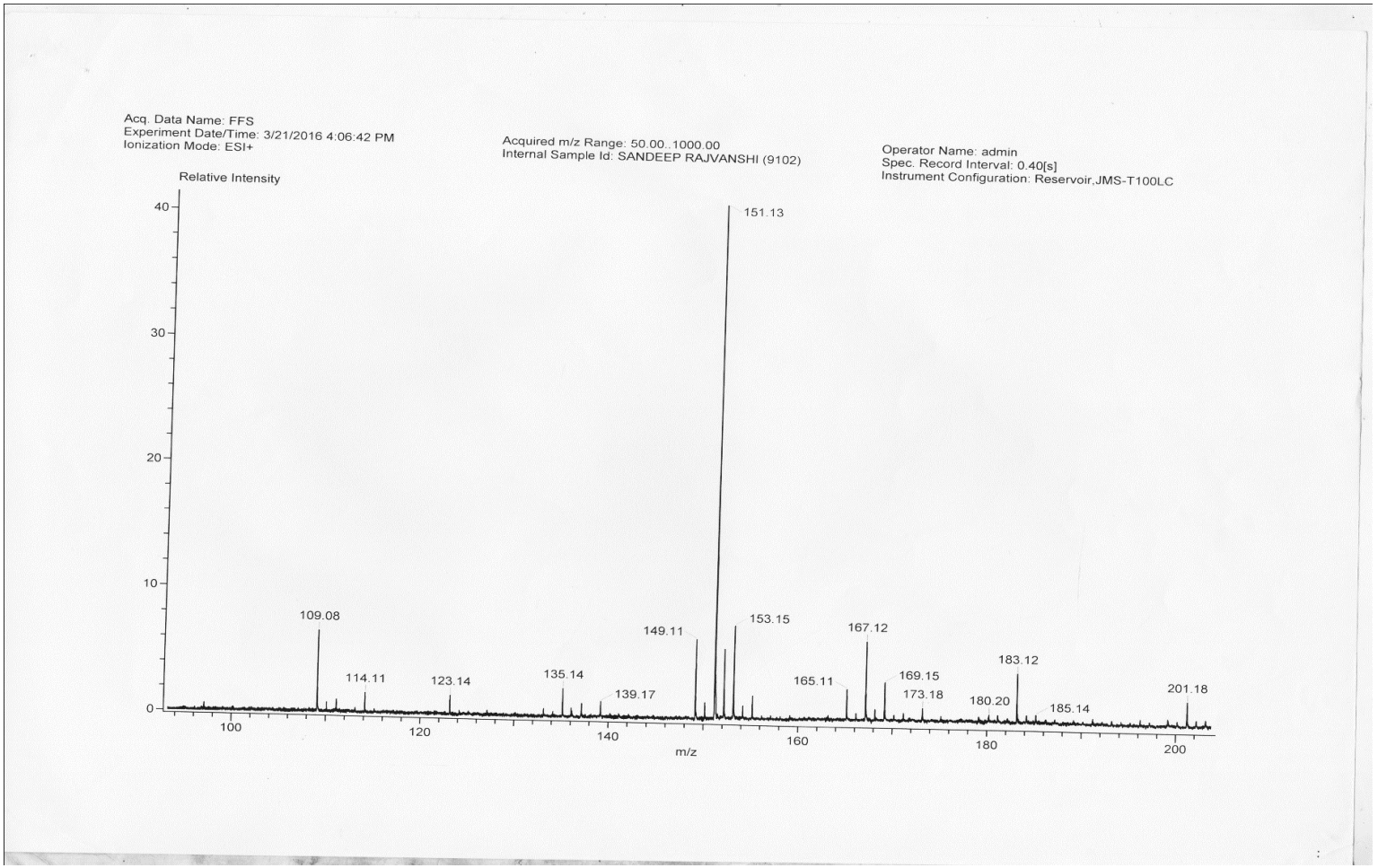


Fig. 4.14: DART-MS spectrum of essential oil extracted by solvent extraction from fresh flowers of African marigold (*Tagetes erecta* L.).

Table 4.11: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in soxhlet extracted essential oil from dry flower of African marigold (*Tagetes erecta* L.) subjected to DART – MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	97	97.08	-	0.5	-
2.	109	109.08	-	11.8	-
3.	114	114.11	$C_7H_{14}O$	5.8	2-heptanone
4.	123	123.14	$C_8H_{10}O$	3.7	p-methylanisol, 4-ethylphenol
6.	133	133.11	-	0.3	-
7.	135	135.14	$C_{10}H_{16}$	3.8	terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene
8.	139	139.17	$C_{10}H_{18}$	2.2	tran-pinane, cis pinane
9.	149	149.12	$C_{10}H_{14}O$	7.9	piperitenone, ocimenone, umbellulone, verbenone
10.	151	151.13	$C_{10}H_{18}0$	52.0	Tagetone
11.	153	153.15	$C_{10}H_{18}O$	14.0	linalool, fenchol, terpinen-4-ol
12.	159	159.14	$C_{10}H_{22}O$	0.3	1-decanol
13.	165	165.12	$C_{10}H_{14}O_2$	4.3	Furomyrcenol
14.	167	167.13	$C_{10}H_{16}O_2$	12.0	trans dihydrocarvone epoxide, a-campholenic acid
15.	169	169.15	$C_7H_6O_5$	6.0	galic acid
16.	171	171.17	-	0.2	-
17.	180	180.13	$C_{11}H_{16}O_2$	0.3	transpinocarvyl formate
18.	183	183.13	$C_6H_{14}O_6$	7.9	mannitol
19.	185	185.15	$C_8H_8O_5$	0.3	3,4-dihydroxy-5-methoxybenzoic acid
20.	193	193.18	$C_{12}H_{16}O_2$	0.3	trans sabinyl acetate
21.	201	201.19	$C_{13}H_{15}NO$	2.4	6-ethoxy-2,4-dimethylquinoline

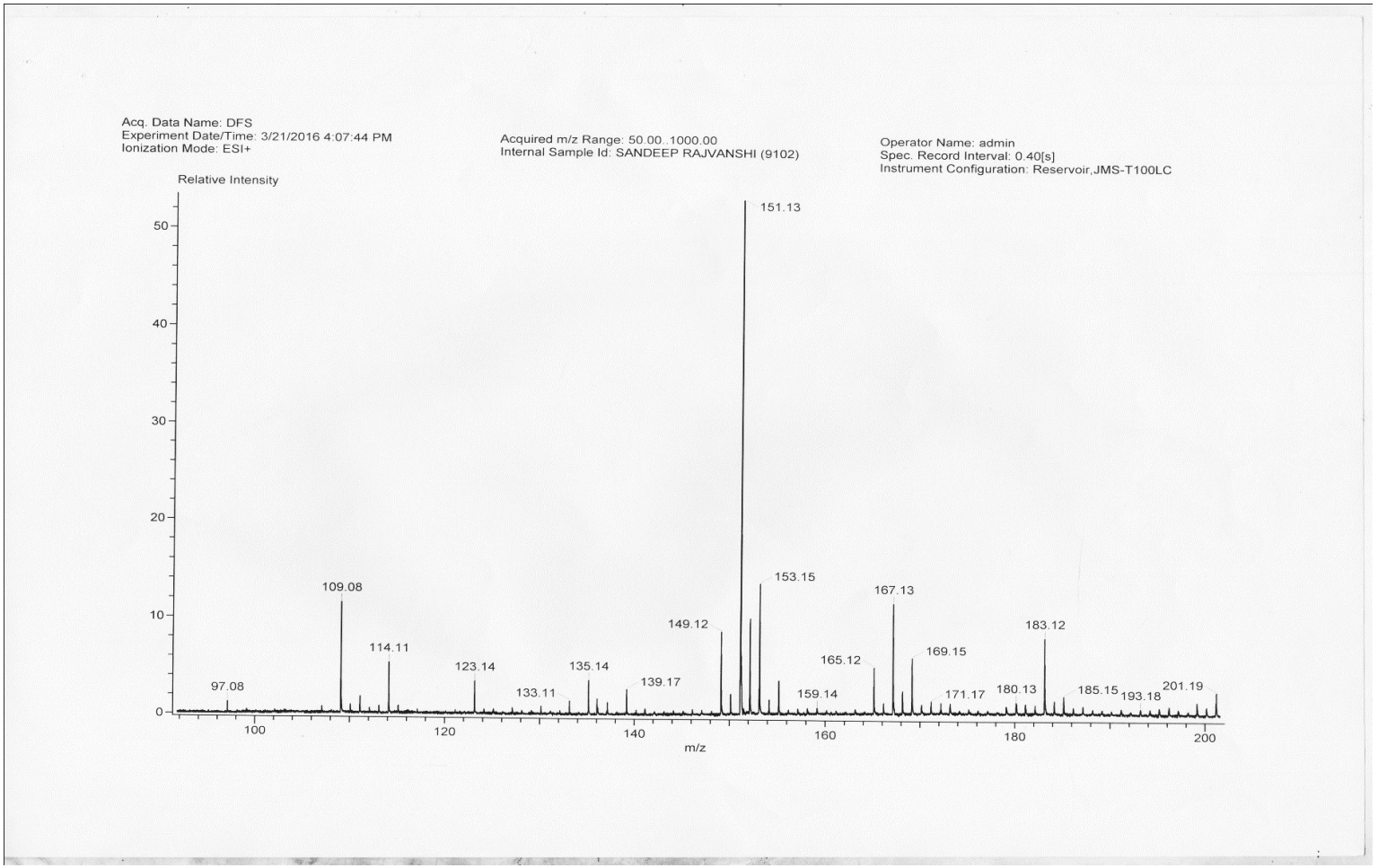


Fig. 4.15: DART-MS spectrum of essential oil extracted by solvent extraction from dry flowers of African marigold (*Tagetes erecta* L.).

Table 4.12: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in soxhlet extracted essential oil from fresh leaves of African marigold (*Tagetes erecta* L.) subjected to DART – MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	101	101.08	$C_6H_{12}O$	1.0	hex-5-en-1-ol, hex-5-en-3-ol, (z)-hex-3-en-1-ol
2.	120	120.08	C_8H_8O	1.0	phenylacetaldehyde
3.	137	137.09	$C_8H_{10}O_2$	1.0	2-methyl5propionylfuran, 1,3dimethoxybenzene
4.	157	157.15	$C_{10}H_{20}O$	3.5	dihydrolinalool
6.	173	173.14	$C_{10}H_{20}O_2$	3.5	trans linalooloxide (furanoid), 3,7-dimethyl-3,7-dihydroxyoct-1-ene
7.	187	187.14	$C_{11}H_{22}O_2$	2.0	methyl decanoate. n-heptyl butanoate
8.	207	207.19	$C_{13}H_{20}O_2$	3.0	trans and cis carvyl propionate
9.	218	218.25	$C_{15}H_{24}O_3$	12.5	hydroxytremetone
10.	219	219.24	$C_{15}H_{24}O$	1.0	caryophyllene oxide, spathulenol
11.	246	246.26	$C_{17}H_{26}O$	11	avocadynofuran, amberone
12.	262	262.28	$C_{17}H_{26}O_2$	10	4b-acetoxygymnomitr3(15)-ene
13.	274	274.32	$C_{17}H_{22}O_3$	97.5	2-acetoxyfuranoelemene
14.	302	302.36	$C_{20}H_{30}O_2$	32	(z)-nuciferyl 2-methylbutyrate
15.	318	318.34	$C_{15}H_{10}$	95	quercetagetin
16.	319	319.34	$C_{19}H_{28}O_4$	20	4b,5b-diacetoxygymnomitr-3(15)-ene
17.	346	346.37	$C_{22}H_{34}O_3$	30	11-b-hydroxykauren-15-a-yl- acetate
18.	362	362.38	$C_{15}H_{22}O_{10}$	33.5	catalpol
19.	374	374.42	$C_{16}H_{22}O_{10}$	14	geniposidic acid
20.	390	390.41	$C_{17}H_{26}O_{10}$	13	Loganin
21.	391	391.33	$C_{24}H_{25}NO_4$	5.0	flavoxate
22.	418	418.43	$C_{20}H_{18}$	3.5	kaempferol-3-o-α-l-arabinoside
23.	429	429.42	$C_{29}H_{50}O_2$	6.0	7β-hydroxysitosterol

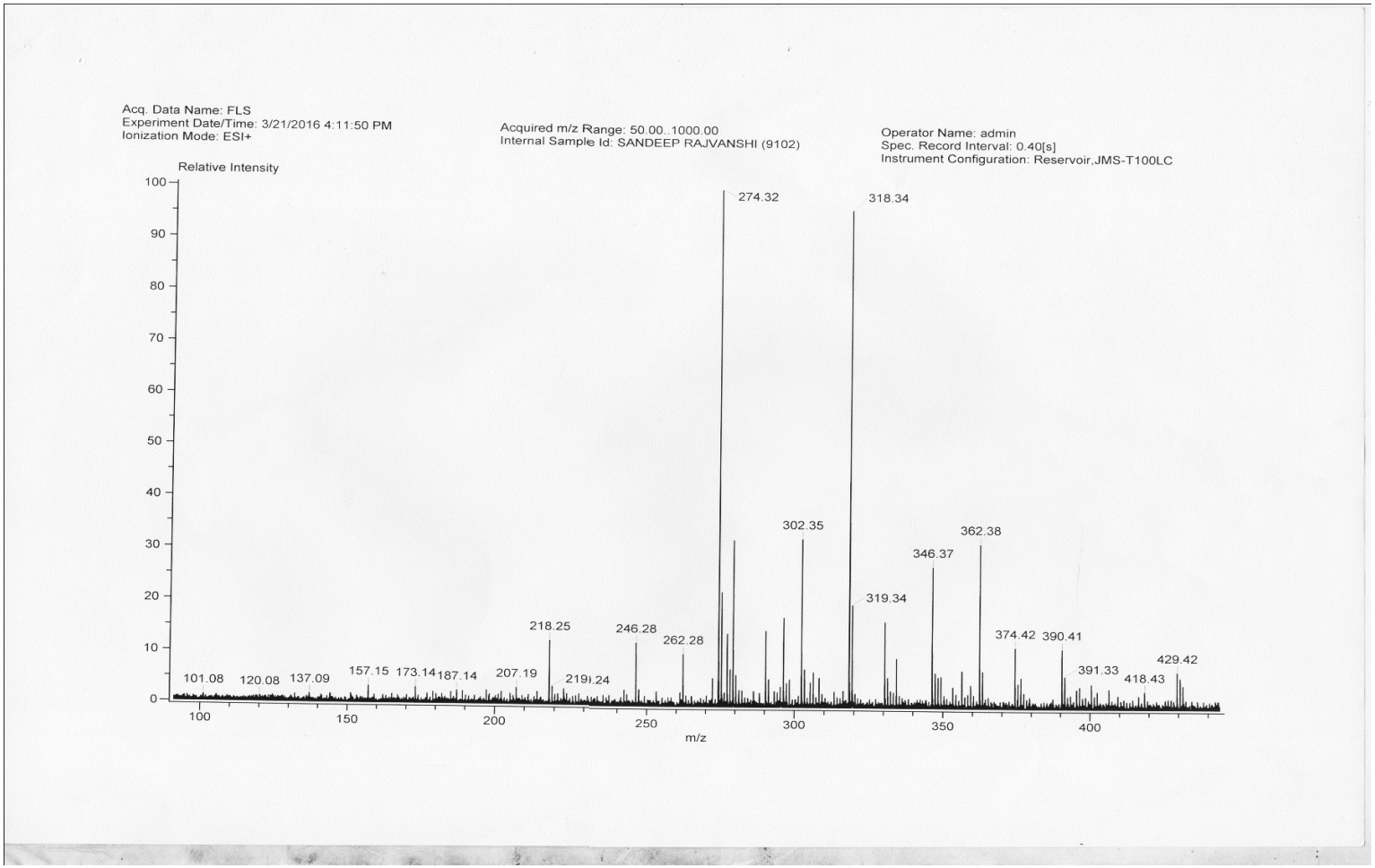


Fig. 4.16: DART-MS spectrum of essential oil extracted by solvent extraction from fresh leaves of African marigold (*Tagetes erecta* L.).

Table 4.13: Exact Mass data and relative intensity (R.I.%) in component bioactive phytometabolites in soxhlet extracted essential oil from dry leaves of African marigold (*Tagetes erecta* L.) subjected to DART – MS.

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	109	109.08	C_7H8_O	18	o-cresol
2.	114	114.11	$C_7H_{14}O$	3.0	2-heptanone
3.	115	115.10	$C_7H_{16}O$	3.2	2-heptanol, 3-heptanol
4.	118	118.09	C_8H_7N	2.2	Indole
6.	132	132.12	$C_6H_{12}O_3$	2.4	methyl 2-hydroxyisopentanoate
7.	136	136.04	$C_{10}H_{16}$	8.0	terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene,limonene
8.	143	143.12	$C_8H_{14}O_2$	2.1	2,3,5-trimethylvalerolactone
9.	146	146.09	$C_{11}H_{14}$	3.0	desmarestene
10.	151	151.14	$C_{10}H_{14}0$	3.4	piperonal, verbenone, thymol, carvone, carvacrol
11.	157	157.11	$C_{10}H_{20}O$	6.0	menthol, dihydrolinalool
12.	169	169.16	$C_7H_6O_5$	3.4	galic acid
13.	173	173.13	$C_{10}H_{20}O_2$	4.1	trans linalooloxide (furanoid), 3,7-dimethyl-3,7-dihydroxyoct-1-ene
14.	183	183.12	$C_6H_{14}O_6$	3.4	Mannitol
15.	190	190.17	$C_{12}H_{14}O_2$	8.5	8,9-dehydrothymol acetate
16.	193	193.16	$C_{12}H_{16}O_2$	2.5	trans sabinyl acetate
17.	197	197.15	$C_{12}H_{20}O_5$	4.5	lavandulyl acetate
18.	201	201.17	$C_{13}H_{15}NO$	4.0	6-ethoxy-2,4-dimethylquinoline
19.	211	211.17	$C_{12}H_{20}O_3$	4.0	5-acetoxylinalool,
20.	218	218.25	$C_{15}H_{22}O$	15.0	lemnalone, taylorione
21.	225	225.18	$C_{14}H_{26}O_2$	3.4	g-tetradecanolide

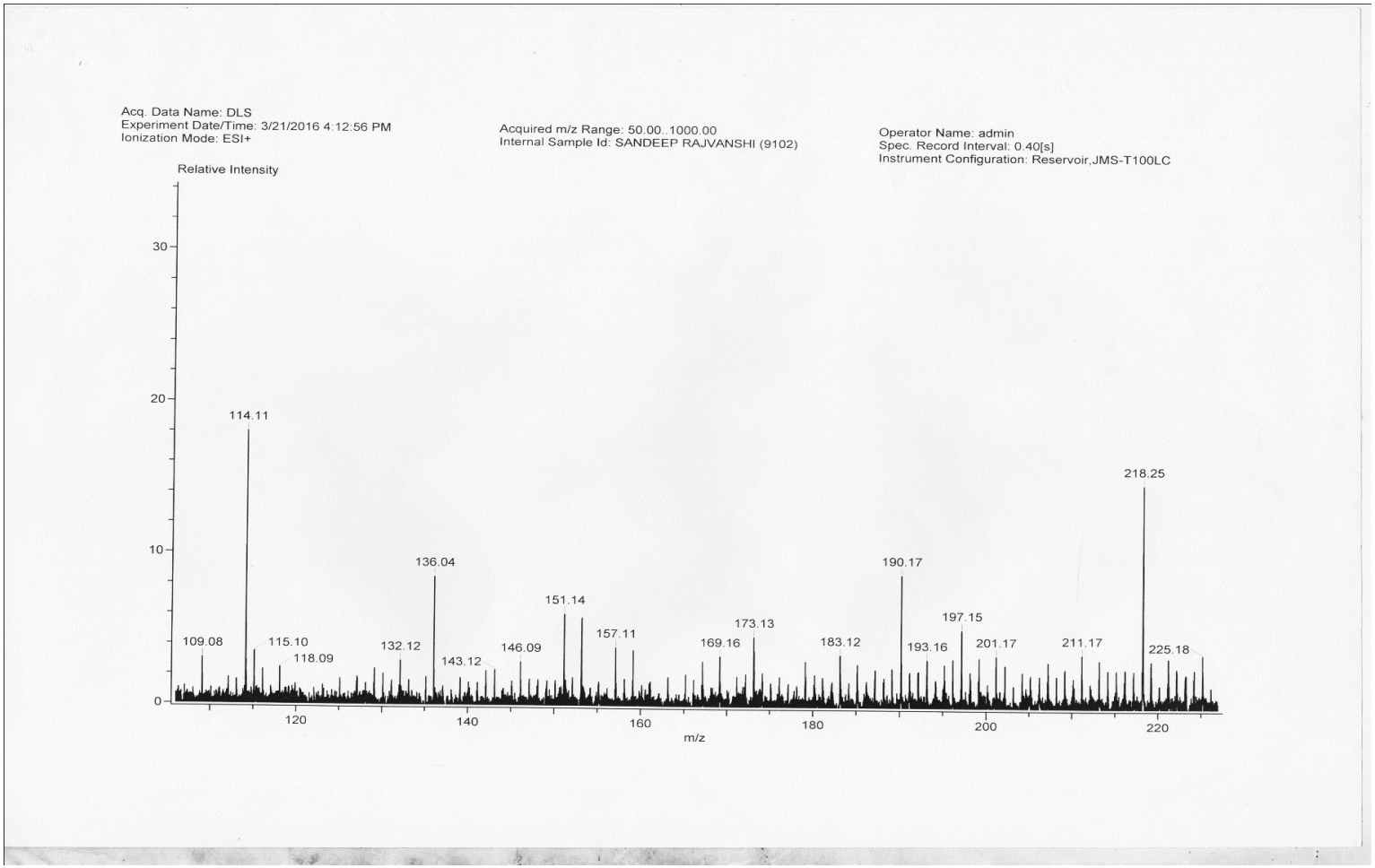


Fig. 4.17: DART-MS spectrum of essential oil extracted by solvent extraction from dry leaves of African marigold (*Tagetes erecta* L.).

4.4. Experiment Number 4

Comparative study of the yield of the essential oil from different flowering seasons

The Comparative study of the yield of the essential oil from different flowering seasons from African marigold (*Tagetes erecta* L.) plant parts was done using the methods described in the previous chapter.

To explore the most suited season for extraction of the essential oil from African marigold (*Tagetes erecta* L.) plant parts, a comparative study was designed with winter and summer season with both hydro-distillation and solvent extraction methods. The Table 4.14 and Fig 4.18 elucidated the variation of the yield extracted by both seasons by hydro-distillation and solvent extraction methods from various samples of African marigold plant parts.

The highest yield of essential oil from flowers was obtained from winter crop from the sample DFSE (8.33%) through solvent extraction method by using n-hexane as a solvent followed by summer crop from DFSE (7.0 %). The minimum recovery of essential oil from flowers was recorded in the summer crop from the sample FFSE (0.36%).

The highest yield of essential oil from leaves was obtained from winter crop from the sample DLSE (6.33%) through solvent extraction method by using a non-polar solvent n-hexane as a solvent followed by summer crop from DLSE (5.33 %). The minimum recovery of essential oil from leaves was recorded in the summer crop from the sample FLSE (0.30%).

Thus, on the basis of the above observations, it was concluded that the winter crop is more suitable for the extraction of essential oil from African marigold (*Tagetes erecta* L.). The hydro-distillation method was the cheap method for oil extraction but the oil recovery was record highest in the dry samples of marigold, using n-hexane as solvent by solvent extraction method.

Table 4.14: Comparative study of Percentage yield of essential oil extracted from African marigold (*Tagetes erecta* L.) in winter and summer seasons.

Winter season oil yield (%)								**Summer season oil yield (%)**							
Hydro-distillation				**Solvent extraction**				**Hydro-distillation**				**Solvent extraction**			
FFCE	DFCE	FLCE	DLCE	FFSE	DFSE	FLSE	DLSE	FFCE	DFCE	FLCE	DLCE	FFSE	DFSE	FLSE	DLSE
0.45%	0.42%	0.39%	0.41%	0.46%	8.33%	0.40%	6.33%	0.40%	0.38%	0.36%	0.39%	0.36%	7.00%	0.30%	5.33%

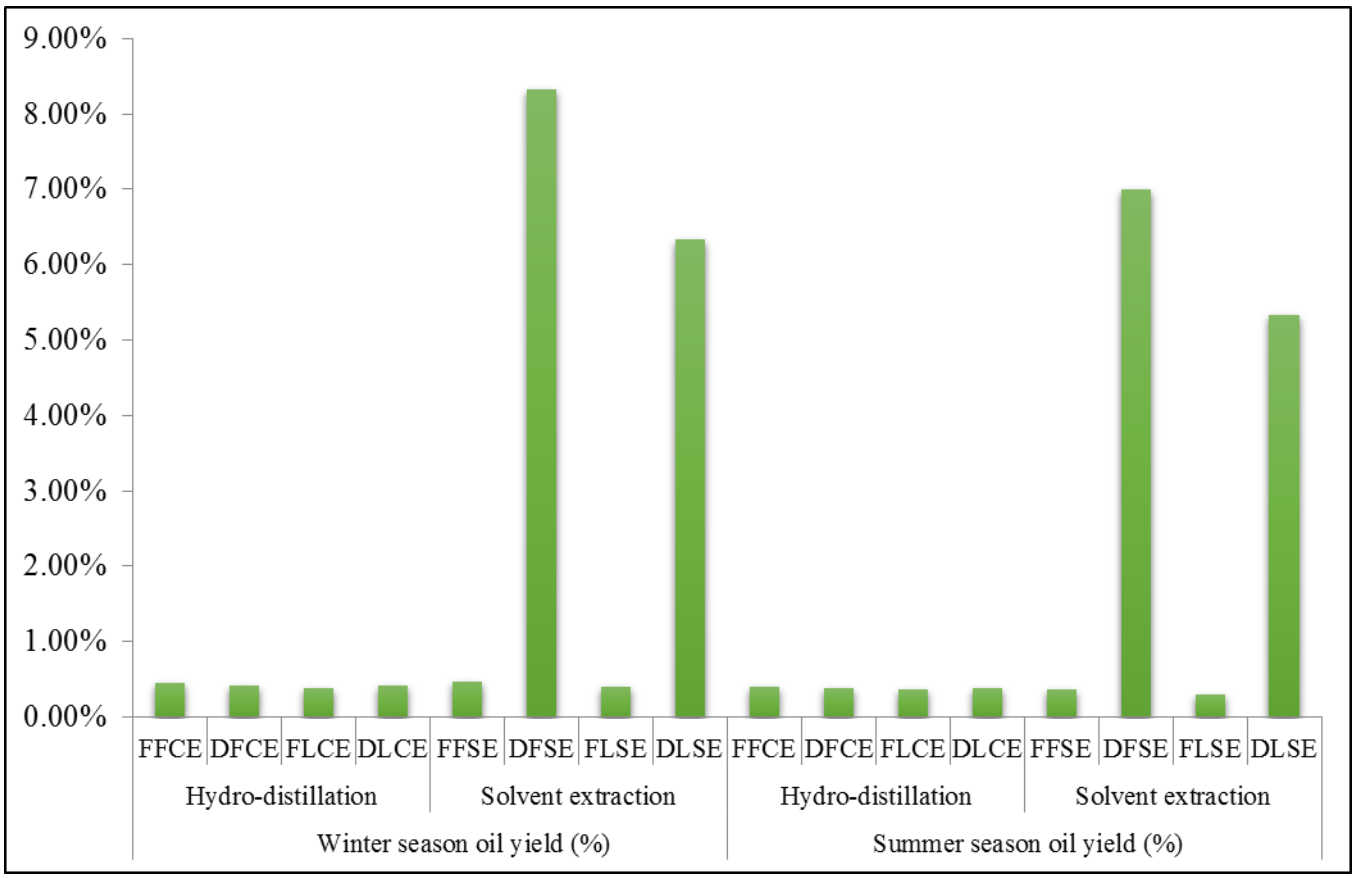


Fig. 4.18: Comparative study of Percentage yield of essential oil extracted from African (*Tagetes erecta* L.) in different growing season.

4.5. Antioxidant Activity (DPPH radical scavenging activity)

	Fresh Flowers (FF)	**Dry Flowers (DF)**	**Fresh Leaves (FL)**	**Dry Leaves (DL)**
DPPH (AAE/ mg extract)	10.64±0.47	5.56 ± 0.35	32.71± 0.35	16.48±0.35

AAE= Ascorbic acid equivalent

Table 4.19. Antioxidant activity of *Tagetes erecta* L. essential oils by DPPH assays

The free radical scavenging ability of Fresh flowers (FF), Dry flowers (DF), Fresh leaves (FL) and Dry leaves (DL) essential oil from T*agetes. erecta* L. was determined from reduction absorbance of DPPH radical at 517 nm. The capacity of natural products

to donate electrons can be measured by DPPH radical colour change from purple to form yellow DPPH. In this assay, the colour change was observed indicating reduction from purple DPPH colour to yellow DPPH signifying that the tested essential oils have antioxidant activity. Table 4.19 shows that FL (IC50=32.71±0.35) showed highest radical scavenging ability as compared to the other essential oils in the order: FL> DL> FF>DF. The antioxidant activity may be due to the presence of hydroxyl groups that might come from oxygenated terpenes from essential oil.

Chapter V

DICUSSION

Medicinal plants are natural factories of bioactive phytochemical which have the protective curative effect in any living system. Approximately 50% of today's prescribed drugs are derived either from a plant source or manmade imitations of plant compounds. The herbs have continuously been used to cure a number of illnesses from ancient times. Today, research continues to prove that most of our present medicines are the product of research on medicinal plants. Medicinal plants are under investigations all over the world, yet a significant portion is still unexplored. Asteraceae is the largest family of Angiosperm consisting of more than 1600 genera and 23000 species worldwide (Barkley *et al.,* 2006; Panero and Funk, 2008). Many of which have immense medicinal values.

In tropics, especially in Brazil and adjoining regions it is grown for essential oil production (Lawrence, 1985), which has numerous applications for the benefit of human society. The oils are good insect repellents and are used in the treatment of certain illnesses, such as smallpox, earache, and colds and to reduce fevers. In addition, it has been recognized to possess hypotensive, spasmolytic, antimicrobial, antifungal and nematicidal properties (Tereschuk et *al.,* 1997 and Mangena and Muyima, 1999). *Tagetes* 13 oil, which can be extracted with n-hexane (Wiese *et al.,* 1992), is an established product of flavor and a raw material for perfume production (Soule, 1993).

Major compounds reported to be present in *Tagetes* are essential oil and flavonoids. Majority of the studies regarding chemical composition of *Tagetes* focused on the yield and distribution of essential oils (Singh *et al.,* 1992; Chalchat *et al.,* 1995; Bansal *et al.,* 1999 and Gil *et al.,* 2000). The main constituents of *Tagetes* oil are ocimene (monoterpenes), limonene, dihydrotagetone and tagetenone (Kaul *et al.,* 2005 and Upadhyaya *et al.,* 2010).

The carotenoids are also important constituent of marigold flowers especially from *T. patula* and *T. erecta* (Vargas *et al.,* 2000). The yellow carotenoid pigments are used as source for food colouring and as a source of gum emulsification (Henken, 1992). Lutein as carotenoids may help to reduce the risk of some age-related eye disorders such as cataracts, photosensitivity disorders and cancer (Vargas and Lopez, 1996). Keeping this in view, the present experiment entitled "A Study on the Extraction of Essential Oil from Marigold (*Tagetes erecta* L.) and its Characterization" was planned for the execution of present experiment during the years 2013 to 2016. The preliminary qualitative screening of phytochemicals in the essential oil of different samples of various parts of marigold was done at the Laboratory of Department of Applied Plant Science (Horticulture). The FT-IR Spectroscopy was performed at University Scientific Instrumentation Centre (USIC), Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rae Bareli Road Lucknow (U.P.) India and the Direct Analysis in Real Time (DART) Mass Spectrometry was done at Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute (CSIR-CDRI), Lucknow, (U.P.) India. Experimental findings described in previous chapter are discussed here in with probable cases and supporting evidences on the subject which could be made available to the author.

Experiment I. Standardize the method for extraction of essential oil from marigold plant parts

The results from the study revealed that highest yield of essential oil was obtained from solvent extraction method by using n-hexane as a solvent, was recorded 8.33 % in the dry flowers soxhlet extract (DFSE) followed by the dry leaves soxhlet extracts (DLSE) which showed 6.33 % oil yield and the highest yield of essential oil from hydro-distillation method from various samples of African marigold plant parts was recorded in the Fresh flower clevenger extract (FFCE) sample 0.45 % followed by the Dry flower clevenger extract (DFCE) which showed 0.42% oil yield.. In addition to processing procedure of plant materials and method of extraction employed, yields of essential oils *T. minuta* have been found to vary considerably based on other factors such as the parts of the plant from, which the essential oils are extracted, harvesting season and plant growth stage Similar observations have also been noted by Senatore *et al*., 2004; Chamorro *et al*., 2008; Caburian and Osi 2010; Wanzala, 2009; Wanzala and Ogoma 2013; Makang'a, 2012; Karimian *et al*. 2014; Okoh *et al*., 2008; Sefidkon and Fathi, 2012; Boutekedjiret *et al*., 2003; Tandon, 2008; Tandon, 2008; Moradalizadeh *et al*. 2013; Singh *et al*. 2006).

The overall yield of essential oil from fresh and dry, flowers and leaves through hydro distillation was lower as compared to solvent extraction. Many authors have reported that hydro distillation produces lower extraction yields (Stashenko *et al*. 1997; Tuan and Iiangantileke 1997; Simandi *et al*. 1999). The reason could be that marigold possessed highly volatile aromatics that cannot survive the process of steam or hydro distillation. We observed that the solvent *n*-hexane had an ability to dissolve all the

odoriferous principles of the flower and possessed a low boiling point (69°C), allowing it to be easily removed (distilled off), without resorting to higher temperature. The odorous principles present in marigold flowers can be isolated more efficiently by extraction with volatile solvents than by hydro distillation. Our results are in agreement with studies of (Wang, 2000).

The difference in oil content of the tested species in turn indicates the positive response for selection for oil contents, i.e. an understanding of the variation in quality and quantity of oil will be helpful for the selection of species for oil extraction to promote farmers to adopt it as a commercial crop It is well known that the chemical composition of volatile oils isolated from aromatic plants depends strongly on the extraction method, among other variables (Muzika *et al*. 1990 and Stashenko *et al*. 1996). The method of extraction with volatile solvents was first applied to flowers in 1835 by Robiquest.

Therefore essential oil is scathed by the thermal conditions of hydro distillation (Clifford 1999). Semen and Hiziroglu (2005) examined that the yield of oil from *Juniperus virginiana* was higher when extracted through solvent extraction method than those of others obtained by hydro distillation (3.5%) which confirm with the results of the present study.

Experiment II. Qualitative estimation of essential oil from marigold plant parts

The result of the preliminary qualitative phytochemical screening revealed that phytochemicals present in the *Tagetes* oil was available in the samples. Total eleven phytochemical tests were performed. Out of eleven test performed, four phytochemical which is flavonoid, terpenoids, alkaloid and Coumarins were identified in all the samples.

The phytochemical tests for different samples of African marigold *Tagetes erecta* L. may prove to be a rich source of compounds with possible pharmacological values. as reported by many researchers, (Grover and Rao, 1978; Garg and Dengre, 1986; Nanir and Kadu, 1987; Penna *et al*., 1994; Srivastava and Srivastava, 1998; Mishra *et al.*, 1995; Arora *et al.*, 1984; Broussalis *et al.*, 1999; Chantraine *et al.*, 1998; Sindhu and Kuttan, 2007; Villar *et al.,* 1997; Khan and Evans, 1996; Rajvanshi and Dwivedi, 2017a).

Our results from the FT-IR analysis exhibits the vibrational frequencies in the range of functional group possess the secondary phytometabolites. confirmed the presence of (O-H stretch) alcohol and phenol at peak 3480 to 3400 cm^{-1} (C-H) aromatic, 890 to 720 cm^{-1} (C-N) aliphatic amines, 1200 to 900 cm^{-1} (C-H rock) alkane, 2900 to 2850 cm^{-1} (N-H stretch) amides, 3350 to 3300 cm^{-1} and 1650 to 1600 cm^{-1} (H-C=O: C-H Stretch) aldehydes, 2690 to 2350 cm^{-1} (N-O stretch) nitro compounds, 1550 to 1500 cm^{-1} (C=O) α, β- unsaturated aldehydes, ketones, 1750 to 1700 cm^{-1} and (C-O stretch) carboxylic acid at peak 1300 to 1200 cm^{-1} in all the samples i.e. fresh flowers clevenger extract (FFCE), dry flowers clevenger extract (DFCE), fresh leaves clevenger extract (FLCE) and dry leaves clevenger extract (DLCE) fresh flowers soxhlet extract (FFSE), dry flowers soxhlet extract (DFSE), fresh leaves soxhlet extract (FLSE) and dry leaves soxhlet extract (DLSE).

The Fourier Transform Infrared (FT-IR) spectrometer has proven to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts. Moreover, FT-IR spectroscopy is an established time saving method to characterize and identify functional groups. Similar work was also reported by (Ragupathi, 2011; Grube *et al.,* 2008; Chen *et al.,* 2001; Xie *et*

al., 2001; Bellamy, 1975; Dukor, 2001; Sohrabi *et al.*, 2005; Manoj and Ragothaman, 1999; Schrader, 1995: Rajvanshi and Dwivedi, 2017b; Rajvanshi and Dwivedi, 2017c).

Experiment III. Quantitative estimation of essential oil from marigold plant parts

The current study identified and quantifies a mixture of compounds in the various samples of essential oil extracted from flowers and leaves of African marigold (*Tagetes erecta* L.). The compound of the oil was identified by comparison of their *m/z* value with those of a computer library and with data published in the literature. We observed that the fresh flower clevenger extract (FFCE) sample exhibited the major compounds like tagetone, *m/z* 151.13 (Relative intensity 90%), linalool, fenchol, terpinen-4-ol, *m/z* 153.15 ((Relative intensity 13%), piperitenone, ocimenone, umbellulone, verbenone, *m/z* 149.12 (Relative intensity 10%). The dry flower clevenger extract (DFCE) sample showed presence of many peaks which could be best match with the compounds like tagetone, *m/z* 151.13 (Relative intensity 91%), trans dihydrocarvone epoxide, a-campholenic acid, *m/z* 167.13 (Relative intensity 16%), phenolic compound galic acid, *m/z* 169.15 (Relative intensity 7%). Quercetagetin, *m/z* 318.35 (Relative intensity 7%), were the compound which could be best match with the peak obtained in the fresh leaves clevenger extract (FLCE). Quercetagetin (Relative intensity 7.2%), n-Heneicosane (C21), Methyl oleate (Relative intensity 19.8%) were obtained in the dry leaves clevenger extract (DLCE) sample was best match with the *m/z* values of sample. The peaks obtained in fresh flower soxhlet extract (FFSE) were best matched with the compounds tagetone, *m/z* 151.13 (Relative intensity 40.8%), terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-

ocimene, limonene, *m/z* 135.14 (Relative intensity 2.3%). In dry flower soxhlet extract (DFSE) the peak obtained were could be due to tagetone, *m/z* 151.13 (Relative intensity 52%), linalool, fenchol, terpinen-4-ol, *m/z* 153.15 (Relative intensity 14%). In quantitative screening of fresh leave soxhlet extract (FLSE), the peaks were obtained were best matched with the Quercetagetin, *m/z* 318.4 (Relative intensity 95%). 2-Acetoxyfuranoelemene, *m/z* 274.32 (Relative intensity 97.5%). Kaempferol-3-O-α-L-arabinoside, *m/z* 418.43 (Relative intensity 3.5%). Terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene, limonene, *m/z* 136.04 (Relative intensity 8%). lemnalone, taylorione, *m/z* 218.25 (Relative intensity 15%).8,9-Dehydrothymol acetate, *m/z* 190.17 (Relative intensity 8.5%) were the compounds which could be best match with the peak obtained in the dry leaves soxhlet extract (FLCE). Among the compounds identified in EOs in the current study, tagetone was the most abundant constituent accounting in most of the samples.

The findings of the study were in agreement with earlier reports of (Baslas *et al*., 1981; Baslas and Singh, 1980; Machado *et al*., 1994; Dhingra and Dhingra, 1956; Ghosh, 2004; Tygadlo *et al*., 1994; Sharma *et al*., 1961; Xio *et al.*, 2006; Marotti *et al*., 2004; Pichette *et al*., 2005; De Feo *et al*., 2005 and Gupta *et al*., 2009).

Experiment IV. Comparative study of the yield of the essential oil from different flowering seasons

Our result revealed that the highest yield of essential oil from flowers was obtained from winter crop from the sample DFSE (8.33%) through solvent extraction by using n-hexane as a solvent followed by summer crop from DFSE (7.0 %). The minimum

recovery of essential oil from flowers was recorded in the summer crop from the sample FFSE (0.36%).

The highest yield of essential oil from leaves was obtained from winter crop from the sample DLSE (6.33%) through solvent extraction by using a non-polar solvent n-hexane as a solvent followed by summer crop from DLSE (5.33 %). The minimum recovery of essential oil from leaves was recorded in the summer crop from the sample FLSE (0.30%).

Temperature plays an important role in plant growth and essential oil yield as a whole Plants respond to temperature changes in most of their metabolic activities such as photosynthesis, respiration and transpiration (Murtagh, 1996). Water is one of the most important environmental factors required for plant growth and development (Yaniv & Palevitch, 1982 and Murtagh, 1996).

Photosynthetic activity is known to be reduced in crops subjected to low light levels and water deficit conditions. Letchamo & Xu (1995) hypothesized that dry matter formation and accumulation of essential oil in thyme was closely related to photosynthesis, and limitations in the net CO2 assimilation rates had a direct or indirect effect on shoot growth and production of the volatile oil.

Regions with sharply defined wet and dry seasons were able to produce good crop yield, provided there were no extended periods of water logging. For example, low herbage and high oil yield were recorded in geranium after three dry months as compared to three wet months (in Kenya (Weiss, 1997).

The season or month of harvesting was observed to exert remarkable influences on the oil yield of essential oil crops. Weiss (1997) reported that the highest oil content of

geranium was observed in July (rainy/monsoon) and lowest in February (spring) in southern India. Doimo, *et al., (*1999) reported that not only the seasons and months of harvest affected oil yield, but that the geographic area where these crops were grown also influenced yield.

Antioxidant Activity (DPPH radical scavenging activity)

Fresh leaves (IC50=32.71±0.35) showed highest radical scavenging ability as compared to the other essential oils in the order: FL> DL> FF>DF. The antioxidant activity may be due to the presence of hydroxyl groups that might come from oxygenated terpenes from essential oil. The findings of the study were in agreement with earlier reports of (Basavaraj *et al.,* 2011**;** Tripathi *et al.,* 2012; Popovic *et al.,* 1999; and Sindhu and Kuttan, 2007).

Chapter VI

SUMMARY AND CONCLUSION

The present investigation entitled "A Study on the Extraction of Essential Oil from Marigold (*Tagetes erecta* L.) and its Characterization" was carried out at the Horticulture Research Farm and Laboratory of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya-Vihar, Rae Bareli Road, Lucknow, Uttar Pradesh. The results pertaining to the study are summarized below-

- The highest yield of essential oil was obtained from solvent extraction method by using n-hexane as a solvent, was recorded (8.33 %) in the Dry Flower Soxhlet Extract (DFSE) followed by the Dry Leaf Soxhlet Extracts (DLSE) which showed (6.33 %) oil yield.
- The highest yield of essential oil from Hydro-distillation method from various samples of African marigold plant parts was recorded in the Fresh Flower Clevenger Extract (FFCE) sample (0.45 %) followed by the Dry Flower Clevenger Extracts (DFCE) which showed (0.42%) oil yield.
- For standardization of extraction procedure, it can be concluded that solvent extraction exhibited higher extraction efficiency than the hydro distillation method.
- In the preliminary qualitative phytochemical screening, total eleven phytochemical tests were performed. Out of which 4 phytochemicals which is flavonoid, terpenoids, alkaloid and Coumarins were identified in all the samples.
- Steroids, glycosides and saponins were absent in all the samples.

- Phenols were present in fresh and dry flowers oil extracted by both methods.
- Triterpenoids were present only in the dry flower sample, extracted by soxhlet.
- Tannins present in fresh and dry flowers oil extracted by hydro-distillation and dry flowers oil sample extracted by soxhlet apparatus.
- Quinones were absent in fresh leaves sample extracted by both methods
- Qualitative screening of Fresh Flower Clevenger Extract (FFCE) using FT-IR Spectroscopy showed the presence of total 16 peaks. The main functional groups found were amide, alkane, aldehydes, carboxylic acid and aliphatic amines.
- In Dry Flower Clevenger Extract (DFCE) sample, total 15 peaks were obtained with alcohols, phenols, alkanes and aromatics.
- The screening of Fresh Leaves Clevenger Extract (FLCE) showed the presence of 14 peaks. The main functional groups were 1* 2* amines, amides, alkanes and carboxylic acid.
- Dry Leaves Clevenger Extract (DLCE) showed the existence of 17 peaks and the main functional groups found were alkanes, nitro compounds, aliphatic amines and aromatics.
- The observations recorded from the qualitative screening of Fresh Flower Soxhlet Extract (FFSE) showed the presence of minimum peaks i.e. eleven (11) from the frequency ranges from 400 to 4000cm^{-1}. The characteristic functional group found were alcohols, phenols and alkanes.
- Dry Flower Soxhlet Extract (DFSE) showed the existence of 16 peaks. The main functional groups found were alkanes and aliphatic amines.

- The maximum number of peaks i.e. 22 was found in the Fresh leaves Soxhlet Extract (FLSE). Presence of more peaks clearly indicates the presence of more phytochemicals in the sample. Alkanes, aliphatic amines, phenols, alcohols,
- The observations recorded from the qualitative screening of Dry Leaves Soxhlet Extract (DLSE) showed the presence of 14 peaks from the frequency ranges from 400 to 4000cm^{-1}. The characteristic functional group found were alcohols, aromatics, aliphatic amines.
- The quantitative screening of Fresh Flower Clevenger Extract (FFCE) by DART-MS showed the existence of major compounds at *m/z* values from 50 to 1000. The sample exhibited the major compounds like tagetone (Relative intensity 90%), linalool, fenchol, terpinen-4-ol ((Relative intensity 13%), piperitenone, ocimenone, umbellulone, verbenone (Relative intensity 10%).
- The Dry Flower Clevenger Extract (DFCE) showed the presence of many peaks which could be best match with the compounds like tagetone (Relative intensity 91%), trans dihydrocarvone epoxide, a-campholenic acid (Relative intensity 16%), phenolic compound galic acid (Relative intensity 7%).
- Quercetagetin (Relative intensity 7%), 6b-Acetoxyeudesm-4 (15)-en-7bol (Relative intensity 11%), 2-Acetoxyfuranoelemene (Relative intensity 12%), were the compounds which could be best match with the peak obtained in the Fresh Leaves Clevenger Extract (FLCE).
- Quercetagetin (Relative intensity 7.2%), n-Heneicosane (C21), Methyl oleate (Relative intensity 19.8%) were obtained in the Dry Leaves Clevenger Extract (DLCE) sample was best match with the *m/z* values of sample.

- The peaks obtained in Fresh Flower Soxhlet Extract (FFSE) were best matched with the compounds tagetone (Relative intensity 40.8%), linalool, fenchol, tterpinen-4-ol (Relative intensity 7.4%), terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene, limonene (Relative intensity 2.3%).
- In Dry Flower Soxhlet Extract (DFSE) the peak obtained were could be due to tagetone (Relative intensity 52%), linalool, fenchol, terpinen-4-ol (Relative intensity 14%), mannitol (Relative intensity 7.9%).
- In quantitative screening of Fresh Leave Soxhlet Extract (FLSE), the peaks were obtained were best matched with the Quercetagetin (Relative intensity 95%). 2-Acetoxyfuranoelemene (Relative intensity 97.5%). Kaempferol-3-O-α-L-arabinoside (Relative intensity 3.5%).
- Terpinolene, thujene, sabinene, α-terpinolene, α pinene, β-ocimene, limonene (Relative intensity 8%). Lemnalone, Taylorione (Relative intensity 15%).8, 9-Dehydrothymol acetate (Relative intensity 8.5%) were the compounds which could be best match with the peak obtained in the Dry Leaves Soxhlet Extract (FLCE).
- The highest yield of essential oil from flowers was obtained from winter crop from the sample DFSE (8.33%) through solvent extraction method by using n-hexane as a solvent followed by summer crop from DFSE (7.0 %). The minimum recovery of essential oil from flowers was recorded in the summer crop from the sample FFSE (0.36%).
- The highest yield of essential oil from leaves was obtained from winter crop from the sample DLSE (6.33%) through solvent extraction method by using a non-polar solvent n-hexane as a solvent followed by summer crop from DLSE (5.33 %). The

minimum recovery of essential oil from leaves was recorded in the summer crop from the sample FLSE (0.30%).

- It was concluded from season wise yield of essential oil, that the winter crop is more suitable for the extraction of essential oil from African marigold (*Tagetes erecta* L.). The solvent extraction method exhibited higher extraction efficiency than the hydro distillation method using n-hexane as solvent by solvent extraction method, recorded maximum essential oil yield in both the winter and summer.
- The Fresh leaf (FL) sample (IC50=32.71±0.35) showed highest radical scavenging ability as compared to the other essential oils in the order: Fresh Leaf> Dry Leaf> Fresh Flower>Dry Flower.

Table: 6.1 Summary of FT-IR analysis of essential oil extracted from African marigold (*Tagetes erecta* L.).

S.NO.	FREQUENCY RANGE (cm^{-1})	ESSENTIAL OIL	TOTAL NO. OF PEAKS	MAIN FUNCTIONALS GROUPS
1.	**400-4000 (cm^{-1})**	Fresh flower clevenger extract	16	Amides, alkanes, aldehydes, carboxylic acid, Aliphatic amines
2.	**400-4000 (cm^{-1})**	Dry flower clevenger extract	15	Alcohols, phenols, alkanes, aromatic
3.	**400-4000 (cm^{-1})**	Fresh leaves clevenger extract	14	1*, 2* amines, amides, alkanes, arboxylic acid,
4.	**400-4000 (cm^{-1})**	Dry leaves clevenger extract	17	Alkanes, Carbonyls (general), Nitro compounds, Aliphatic amines, aromatic
5.	**400-4000 (cm^{-1})**	Fresh flowers soxhlet extract	11	Alchohols, phenols, , alkanes,
6.	**400-4000 (cm^{-1})**	Dry flowers soxhlet extract	16	Alkanes, aliphatics amines.
7.	**400-4000 (cm^{-1})**	Fresh leaves soxhlet extract	22	Alkanes, phenols, aliphatic amines. Carboxylic acid
8.	**400-4000 (cm^{-1})**	Dry leaves soxhlet extract	14	Aromatic, alkanes, Aliphatic amines

Table: 6.2 Summary of DART-MS analysis of essential oil extracted from African marigold (*Tagetes erecta* L.).

S.NO.	ESSENTIAL OILS	NO. OF COMPOUND IDENTIFIED	MAIN COMPONENTS PRESENT
1.	FFCE	17	Piperonal, verbenone, thymol, carvone, carvacrol (R.I 90.0%), Piperitenone, Ocimenone, umbellulone (R.I 10.0 %), Trans Dihydrocarvone epoxide (R.I 10%), Linalyl acetate, (R.I 1.0 %)
2.	DFCE	16	Piperonal, verbenone, thymol, carvone, carvacrol (R.I 91.0%), Piperitone, Camphor (R.I 12.0 %), Piperitenone, Ocimenone, umbellulone (R.I 11.0 %),
3.	FLCE	19	Flavoxate (R.I 98.0%), Quercetagetin (R.I 7.0%), Hydroxytremetone (R.I 2.0%), 2-Acetoxyfuranoelemene (R.I 12.0%),
4.	DLCE	11	n-Heneicosane (R.I 19.8%), Quercetagetin (R.I 9.9%), 2-Acetoxyfuranoelemene (R.I 7.2%),
5.	FFSE	17	Piperonal, verbenone, (R.I 40.8%), Trans Dihydrocarvone epoxide, (R.I 6.1%), Piperitenone, Ocimenone, (R.I 6.2%), Mannitol (R.I 4.0%),
6.	DFSE	21	Piperonal, verbenone, thymol, (R.I 52.0 %), 3,4-dihydrobenzoicacid (R.I 14.0 %), Trans Dihydrocarvone epoxide, a-Campholenic acid (R.I 12.0 %), Galic acid (R.I 6.0 %), β-ocimene,limonene (R3.8 %)
7.	FLSE	23	2-Acetoxyfuranoelemene (R.I 97.5%), 4b,5b-Diacetoxygymnomitr-3(15)-ene(R.I 20.0 %), 7β-hydroxysitosterol (R.I 6.0 %), Dihydrolinalool (R.I 3.5 %)
8.	DLSE	21	Terpinolene, Thujene, sabinene, α-terpinolene, α pinene, β-ocimene, limonene (RI 8.0 %), Menthol, Dihydrolinalool (RI 6.0 %), 8,9-Dehydrothymol acetate (RI 8.5 %)

Conclusion

The present investigation entitled "**A Study on the Extraction of Essential Oil from Marigold (*Tagetes erecta* L.) and its Characterization**" has concluded that the amount of oil obtained from the fresh part of the plant is very small as compared to the dry part of the plant. The solvent extraction exhibited higher extraction efficiency than the hydro distillation method.

The qualitative screening of essential oil extracted from *Tagetes* plant parts shows the presence of flavonoid, terpenoids, alkaloid, phenols and Coumarins in the plant which shows that *T. erecta* is an important source of many pharmacologically and medicinally important phyto-constituents. There is huge scope for research; the plant could be further exploited in future as a source of useful phytochemical compound for the pharma industry.

The quantitative screening of Extracts by DART-MS showed the existence of major compounds like tagetone, linalool, fenchol, terpinen-4-ol, piperitenone, ocimenone, umbellulone, verbenone.

The antioxidant activities obtained in marigold seem to be in good accordance with the medicinal uses of African marigold.

On the basis of results obtained during study, we can say that the fresh leaf soxhlet extracts showed better results than other samples.

Many Indian herbs are being used in traditional practices to cure various human ailments. *Tagetes erecta* has an important place among such anti-oxidants, anti-inflammatory medicinal plants; it can also be used in treating wound, liver disorder and diabetes. Furthermore, in future study, the isolated principles from *Tagetes erecta* needs to be evaluated in scientific manner using various innovative experimental models and clinical trials to understand its mechanism of action, in search of other active constituents, so that its other therapeutic uses can be widely explored.

BIBLIOGRAPHY

Agarwal, M., Kumar, A., Gupta, R. and Upadhyaya, S. (2012). Extraction of polyphenol, flavonoid from Embica Officinalis, Citrus limon, Cucumis sativus and evaluation of their antioxidant activity. *Orient Journal of Chemistry*, 28(2): 993-998.

Ahito, (2015). *Tagetes erecta* L. *Philippine Medicinal Plants*, Staurt exchange. Available at: http://www.stuartxchange.org/ Ahito.html (Accessed 11 March 2015).

Antonio, G. G., Braulio, M. F and Pedro, G. (1981). 13C NMR spectra of olean-18–ene derivatives. *Phytochemistry*, 20(8): 1919 – 21.

Areias, F., Valentao, P., Andrade, P. B., Ferreres, F. and Seabra, R. M. (2000). Flavonoids and phenolic acids of sage: influence of some agricultural factors. *Journal of Agriculture Food Chemistry*, 48: 60-81.

Arora, R. and Pandey, G.N. (1984). Effect of essential oils on citrus decay pathogens. *Biol. Mem.*, 9: 69-72.

Babu, G. D and Kaul, V. K. (2007). Variations in quantitative and qualitative characteristics of wild marigold (*Tagetes minuta* L.) oils distilled under vacuum and at NTP. *Industrial Crops and Products*, 26(3): 241–251.

Bajpai, V., Pandeya, R., Pal, M., Singh, N., Kumar, N and Kumar, B. (2013). DART MS Based Chemical Profiling for Therapeutic Potential of *Piper betle* Landraces. *Natural Product Communications*, 7(12): 62-79.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils – areview. *Food Chem Toxicol.*, 46: 446–75.

Bansal, R. P., Bahl, J. R., Garg, S. N., Naqvi, A. A., Sharma, S., Ram, M. and Kumar, S. (1999). Variation in quality of essential oil distilled from vegetative and reproductive stages of Tagetes minuta crop grown in North Indian plains. *J. Essent. Oil Res,* 11: 747-752.

Barkley, T. M., Brouillet, L. and Strother, J. L. (2006). Flora of North America, Asteraceae, part 1. Oxford University Press, New York.

Baslas, R. K. and Singh, A. K. (1980). Chromatographic separation of essential oil of *Tagetes erecta*. *Indian Perfume,* 24: 163-165.

Baslas, R. K. and Singh, A. K. (1981). Chemical examination of essential oil from the leaves of *Tagetes erecta* Linn. *J. Indian Chem. Soc,* 58: 104.

Baser, K. H. (2010). Handbook of Essential Oils: Science, Technology, and Applications */ K. Hüsnü Can Baser, Gerhard Buchbauer.* ISBN 978-1-4200-6315-8. Universitat Wien, Austria.

Basavaraj, C. V., Karnakumar, B. V., Rajabhau, S. S. and Kamshetty, M. V. (2011). *In vitro* antioxidant activity studies of the flowers of *Tagetes erecta L.* (Compositae). *International Journal of Pharma and Bio Sciences*, 2(3): 223-229.

Batish, D. R., Arora, K., Singh, H. P. and Kohli, R. K. (2007). Potential utilization of dried powder of *Tagetes minuta* as a natural herbicide for managing rice weeds. *Crop Protection,* 26: 566–571.

Bellamy, L. J. (1975). Infrared Spectra of Complex Molecules.Chapman Hall, London.

Bernhoft, A., Siem, H., Bjertness., Meltzer, M., Flaten. T. and Holmsen, E. (2010). Bioactive Compounds in Plants—Benefits and Risks for Man and

Animals, The Norwegian Academy of Science and Letters, Oslo, Norway, 11-17.

Boutekedjiret, C., Bentahar, F., Belabbes, R. and Bessiere, J. M. (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. *Flavour and Fragrance Journal*, 18(6): 481-484.

Brigida, D., Marina, D., Antonio, F. and Pietro, M. (2004). Structure elucidation and phytotoxicity of C13 nor-isoprenoids from *Cestrum parqui*. *Phytochemistry*, 65(4): 497-505.

Broussalis, A. M., Ferraro, G. E., Martino, V. S., Pinzon, R., Coussio, J. D. and Alvarez, J. C. (1999). Argentine plants as potential source of insecticidal compounds. *J. Ethnopharmacol.*, 67: 219-223.

Burt, S. (2004). Essential oils: their antimicrobial properties and potential application in foods:a review. *International Journal of Food Microbiology*, 94(3): 223-253.

Caburian, A. B. and Osi, M. O. (2010). Characterization and evaluation of antimicrobial activity of the essential oil from the leaves of *Piper betle* L. *International Scientific Research Journal* 2(1): 2-13.

Chalchat, J. C., Garry, R. P. and Muhayimana, A. (1995). Essential oil of *Tagetes minuta* from Rwanda and France: Chemical composition according to harvesting, location, growth stage and part of plant extracted. *J. Essent. Oil Res*, 7: pp. 375-386.

Chamorro, E. R., Ballerini, G., Sequeira, A. F., Velasco, G. A. and Zalazar, M. F (2008). Chemical composition of essential oil from *Tagetes minuta* L. leaves and flowers. *Journal of the Argentine Chemical Society*, 96(1-2): 80-86.

Chantraine, J. M., Laurent, D., Ballivian, C., Ibanez, R. and Vilaseca, L. A. (1998). Insecticidal activity of essential oils on *Aedes aegypti* larvae, *Phytother. Res*, 12: pp. 350-354.

Chatterjee, S. (2011). Comparative efficacy of *Tagetes erecta* and *Centella asiatica* extracts on wound healing in albino rats. *Scien Res*, 2: 138-42.

Chen, R. J., Xu, G. Y., Wang, H. Y., Xu, H. Z. and Liu, Y. M. (2001). Study on isolation and structure of polysaccharide XC-1 from *Angelica sinesis* (oliv) diels. *Chemistry Bulletin / Huaxue Tongbao*, 64 (6): 372-374.

Costaa, Da. C., Whitmarshb, S., Lynchb, T. and Creasera, S. C. (2016). The qualitative and quantitative analysis of lubricant oil additives by direct analysis in real time mass spectrometry. *International Journal of Mass Spectrometry,* vol 405 : pp. 24–31

Centeno, S., Calvo, M. A., Adelantado, C. and Figueroa S. (2010). Antifungal activity of *Rosmarinus officinalis* and *Thymus vulgaris* against *Aspergillus flavus* and *A. ochraceus*. *Pakistan Journal of Biological Sciences,* 13(9): 452–455.

Chi, P. T. (2013). Chemical composition, antioxidant and antimicrobial activities of essential oils extracted from citrus varieties in Vietnam. MSc Thesis, Vietnam National University, Vietnam.

Clifford, A.A. (1999). Extraction of natural products with superheated water. In: *Proceedings of the GVC-Fachausschss High Pressure Chemical Engineering*, Mar. 3-5, 1999, Karlsruhe, Germany.

Cody, R. B., Laramée, J. A. and Durst, H. D. (2005). Versatile new ion source for the analysis of materials in open air under ambient conditions. *Analytical Chemistry*, **77**(8): 2297–2302.

De, F. V., Soria, E. U., Soria, R. U. and Pizza, C. (2005). Composition and *in vitro* toxicity of the essential oil of *Tagetes terniflora* HBK (Asteraceae), *Flav. Fragr. J*, 20: pp. 89-92.

Coogan, C. K. and Horn, D. H. S. (1965). Nuclear magnetic resonance studies. IV. The analysis of the spectra of two thiophene derivatives. *Australian Journal of Chemistry*, 18(5): 723- 729.

Devika, R. And Justin, K. (2014). Screening and evaluation of bioactive components of *Tagetes erecta* L, by GCMS analysis. *Asian Journal of Pharmaceutical and Clinical Research*,7(2): 58 – 60.

Dhingra, S. N. and Dhingra, D. R. (1956). Essential oil of *Tagetes patula*. *Perf. Essent. Oil Rec.*, 47: pp. 391-394.

Doimo, L., Mackay, D. C., RintouL, G. B., Aray, B. R. D. and Fletcher, R. J. (1999). Citronellol: geraniol ratios and temperature in geranium (*Pelargonium hybrid*). *Journal of Horticultural Sciences & Biotechnology,* 74 (4): 528-530.

Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y and Perry, N. B. (2007). Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures. *Food Chem*, 101: 1417–24.

Dukor, R. K. (2002). Vibrational spectroscopy in the detection of cancer, in Handbook of Vibrational Spectroscopy, John Wiley and Sons, Chichester, 3335-3360.

Dutta, R., Sarkar, U. and Mukherjee, A. (2014). Extraction of oil from *Crotalaria Juncea* seeds in a modified Soxhlet apparatus: Physical and chemical characterization of a prospective bio-fuel, *Fuel*, 116: 794-802.

Farjana, N., Rowshanul, H. M., Zahangir, A. S., Rezaul, M. K., Apurba, K. R. and Shahriar, Z. (2009). Toxicological evaluation of chloroform fraction of flower of *Tagetes erecta* Linn. on rats. *International Journal of Drug Development and Research,* 1(1): 161-165.

Faizi, S. and Naz, A. (2002). Jafrine, a novel and labile β-carboline alkaloid from the flowers of *Tagetes patula*. *Tetrahedron,* 58(31): 6185- 6197.

Gallagher, R. T. and Stahr, H. M. (1980). Identification of a potential interference in the analysis of aflatoxin, 2,4-dimethyl-6-ethoxyquinoline, a reaction product of ethyoxyquin. *J. Agric. Food. Chem.,* 28(1): 133-135.

Garg, S. C. and Dengre, S. L. (1986). Antibacterial activity of essential oil of *Tagetes erecta* Linn., *Hind. Antibiot. Bull.*, 28: 27- 29.

Garg, S. N. and Mehta, V. K. (1998). Acyclic monoterpenes from the essential oil of *Tagetes minuta* flowers, *Phytochem.*, 48: pp. 395-396.

Gavahian, M., Farahnaky, A., Javidnia, K. and Majzoobi, M. (2012). Comparison of Ohmic-assisted hydrodistillation with traditional hydrodistillation for the extraction of essential oils from *Thymus vulgaris* L. *Innov Food Sci Emerg Technol,* 14**:** 85–91.

Gazim, C. Z., Claudia, M. R., Sandra, R. F., Benedito, P. D. F., Celso, V. N., Diógenes, A. and Garcia, C. (2008). Analysis of the essential oils from Calendula officinalis growing in Brazil using three different extraction procedures. *Brazilian Journal of Pharmaceutical Sciences.* vol. 44: n. 3, jul./set..

Ghosh, T., Bose, A., Dash, G. K. and Maity, T. K. (2004). Wound Healing Activity of *Tagetes erecta* Linn Leaves. URL http://www. *Pharmainfo.net/exclusive/reviews.*

Gil, A., Ghersa, C. M. and Leicach, S. (2000). Essential oil yield and composition of *Tagetes minuta* accessions from Argentina. *Biochem. Syst. Ecol,* 28: 261-274.

Golfakhrabadi, F., Khanavi, M., Ostad, S. N., Saeidnia, S., Vatandoost, H., Abai, M. R., Hafizi, M., Yousefbeyk, F., Rad, Y. R., Baghenegadian, A. and Ardekani. M. R. (2015). Biological activities and composition of *Ferulago carduchorum* essential oil. *Journal of Arthropod-Borne Diseases*, 9(1): 104-115.

Govender, H. A. (2010). Comparative Study of Solvent Extraction, Soxhlet Extraction, Steam Distillation, Headspace Analysis and Headspace Solid Phase Microextraction for the Extraction of Volatile Terpenoid Compounds in the Curry Leaf Plant. *B. Sc. Thesis*. University of KwaZulu-Natal, Durban.

Greca, M. D., Monaco, P. and Previta, L. (1990). Stigmasterol from Typha latifolia. *J. Nat. Prod.*, 53:1430-5.

Grover, G. S. and Rao, J. T. (1978). *In vitro* antimicrobial studies of the essential oil of *Tagetes erecta*. *Perf. Flav. Int.*, 3: 28.

Grube, M. O., Muter, S., Strikauska, M. G. and Limane, B. (2008). Application of FT IR spectroscopy for control of the medium composition during the biodegradation of nitro aromatic compounds. .*Journal of Indian Microbiology and Biotechnology*, 35: 1545 1549.

Gupta, Y. N. and Bhandari, K. S. (1975). Essential oil from the leaves of *Tagetes minuta*, *Indian Perfum.*, 19: pp. 29-32.

Gupta, P., Vasudeva, N. and Sharma, S. K. (2009). Pharmacognostical study and preliminary phytochemical screening of the roots of *Tagetes erecta* Linn. *Hamdard Med.*, 52: 153-160.

Gupta, D. N., Ranjan, S., Saha, P., Jain, R., Malhotra, S. and Saleh, M. A. (2012). Antibacterial Activity of Leaf Extract of Mexican Marigold (*Tagetes erecta*) Against Different Gram Positive and Gram Negative Bacterial Strains. *Journal of Pharmacy Research*, 5(8): 4201-4203.

Gupta, P. and Vasudeva, N. (2010). *In vitro* antiplasmodial and antimicrobial potential of *Tagetes erecta* roots, *Pharm. Biol*., (In Press).

Hadjiakhoondi, A., Vatandoost, H., Abousaber, M., Khanavi, M. and Abdi, L. (2008). Chemical composition of the essential oil of *Tagetes minuta* L. and its effects on Anopheles stephensi larvae in Iran. *J. Med. Plants*, 7: 33-39.

Hai, L. and Yue, W. G. (2008). Three New Thiophene Acetylenes from *Rhaponticum uniflorum* (L.). *Helvetica Chimica Acta*., 91(1): 130- 135.

Handa, K. L., Chopra, M. M. and Nigam, M. C. (1963). The Essential oil of *Tagetes minuta* L.. *Perf. Essent. Oil Rec.*, 54: 372-374.

Hassanpouraghdam, B. M., Shekari F., Emarat J. P. and Safi, S. M. (2011). Sesquiterpene rich volatile seed oil of Tagetes patula L. from northwest Iran. *Journal of Central European Agriculture*, 12(2): 304-311.

Henken, H. (1992). Chemical and physiological behavior of feed carotenoids and their effect on pigmentation. *Poultry Sci,* 71: 711-717.

Hethelyi, E., Danos, B., Tetenyi, P. and Juhasz, G. (1987). Phytochemical studies on *Tagetes* species, intraspecific differences of the essential oil in *Tagetes minuta* and *Tagetes tenuifolia*. *Herba Hung.*, 26:145-158.

Hethelyi, E., Tetenyi, P., Kaposi, P., Danos, B., Kernoczi, Z. S., Buki, G. Y. and Koczka, I. (1988). GC/MS investigation of antimicrobial and repellent

compounds, *Herba Hung.*, 27: 89-105.**Huang, S. (2006).** *Tagetes erecta*. *Nat. Prod. Res. Dev.*, 18: 57.

Huang, S. (2007). Bioactive compounds studies on the flowers of *Tagetes erecta* L. *Master's thesis Abstr.* Southwest Jiaotong University.

Huang, S., Zhou, X. L. and Wang, H. Y. (2007). Chemical constituents from the flowers of *Tagetes erecta* L. *West China J Pharm Sci.*, 22: 370-373.

Hussain, K., Ismail, Z., Sadikun, A. and Ibrahim, P. (2009). Evalution of Metabollie changes in Fruit of *Piper armentosum* in various Seasons by Metabolomic using Forier Transform Infrared (FTIR) Spectroscopy. *International Journal of Pharmaceutical and Clinical Research*, 1(2): 68-71.

Hussain, A. I., Anwar, F., Sherazi, S. T. and Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, 108(3): 986-995.

Mostafavi, H. and Pezhhanfar, S. (2015). Qualitative phytochemical analysis of ajwain (*Trachyspermum ammi*) from north-west Iran. *International Research Journal of Pharmacy*, 6(9): 610-615.

Murtagh, G.J. and Smith, G.R. (1996). Month of harvest and yield components of tea tree II: Oil concentration, composition and yield. *Australian Journal of Agricultural Research*, 47: 817-827.

Husan, B. (2002). Chemical constituents of *Tagetes patula* l. *Pak J Pharma Sci.*, 15 (2): 1-12.

Iauk, L., Lo-Bue, A. M., Milazzo, I., Rapisarda, A. and Blandino, G. (2003). Antibacterial Activity of Medicinal Plant Extracts Against Periodontopathic Bacteria. *Phytother Res.*, 17: 599-604.

Janakiram, T. and Rao, T. M. (1996). Marigold. *Tagetes* spp. *Indian Horticulture,* 41: cover.

Joy, P. P., Thomas, J., Mathew, S. and Skaria, B. P. (2001). Ed. Medicinal Plants. *Tropical Horticulture*, Naya Prokash, Calcutta publisher, Vol. 2: 449-632.

Kabura, J. T. (2009). Steam extraction of essential oils; investigation of the process parameters. MSc Thesis, University of Johannesburg, South Africa.

Karimian, P., Kavoosi, G. and Amirghofran, Z. (2014). Anti-oxidative and anti-inflammatory effects of *Tagetes minuta* essential oil in activated macrophages. *Asian Pacific Journal of Tropical Biomedicine,* 4(3): 219-227.

Kaul, P. N., Bhattacharya, A. K., Rao, B. R. R., Syamasundar, K. V. and Ramesh, S. (2005). Essential oil composition of *Tagetes minuta L.* fruits. *J. Essent. Oil Res,* 17: 184-185.

Khan, M. T. and Evans, F. J., (1996). Clinical Evaluation of *Tagetes erecta* in the treatment of parakeratosis. *Phytother. Res.,* 10: S186-S188.

Khulbe, A. (2015). A review on *Tagetes Erecta* .*World J Pharm Sci.,* 3(3): 645-649.

Kim, J., Marshall, M. R. and Wei, C. I. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry,* 43(11): 2839-2845.

Kim, H. J. and Jang, P. Y. (2011). Identification of ambiguous cubeb fruit by DART MS based fingerprinting combined with principal component analysis. *Food Chem*., *129*(2): 1019–1024.

Kiranmai, M. and Ibrahim, M. (2012). Anti bacterial potential of different extracts of *Tagetes erecta* Linn. Int J Pharm., 2(1): 90-96.

Kirtikar, K. R. and Basu, B. D. (2009). *Indian Medicinal Plants*, Vol. II: pp. 1385-1386.

Kishore, G. K., Pande, S. and Harish, S. (2007). Evaluation of essential oils and their components for broad-spectrum antifungal activity and control of late leaf spot and crown rot diseases in peanut. *Plant Diseases*, 91(4): 375-379.

Kojima, H., Sato, N., Hatano, A. and Ogura, H. (1990). Sterol glycosides from *Prunella vulgaris. Phytochemistry*, 29: 2351- 2355.

Kosar, M., Dorman, H. J. D. and Hiltunen, R. (2005). Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. *Food Chem.*, 91: 525–533.

Krishna, A., Kumar, S., Mallavarapu, G. R. and Ramesh, S. (2004). Composition of the essential oils of the leaves and flowers of *Tagetes erecta* L.. *J. Essent. Oil Res.*, 16: pp. 520-522.

Kumar, K. S. (2010) *Extraction of essential oil using steam distillation.* Retrieved September 5, 2015, from, http://ethesis.nitrkl.ac.in/1949/.

Lambert, R. J., Skandamis, P. N., Coote, P. J. and Nychas, G. J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, 91(3): 453-462.

Lawrence, B. M. (1985). A review of the world production of essential oils - 1984. *Perfum. Flavorist.* 10: 1-16.

Leigh, H. W., Ruth, H. W. and Luis, W. L. (1999). Carotenoid composition of marigold (*Tagetes erecta*) flower extract used as nutritional supplement. *J. agri. Food Chemistry*, 47(10): 4189 – 4197.

Letchamo, W. and XU, H.L. (1995). Variations in photosynthesis and essential oil in thyme. *Journal of Plant Physiology*, 147: 29-37.

Liu, R. H. and Kong, L. G. (2005). *Tagetes* Species. *Nat. Prod. Res. Dev.*, 17: 437.

Li, X. M., Tian, S. L., Pang, Z. C., Shi, J. Y., Feng, Z. S. and Zhang, Y. M. (2009). Extraction of *Cuminumcyminum* essential oil by combination technology of organic solvent with low boiling point and steam distillation. *Food Chem*istry, 115: 1114–1119.

Machado, M. I. L., Silva, M. G. V., Matos, F. J. A., Craveiro, A. A. and Alencar J. W. (1994). The Presence of indole as minor constituents of *Tagetes erecta* leaf oil, *J. Essent. Oil Res.*, 6: 203-205.

Makang'a, O. B. (2012). Composition and repellency of essential oils of *Tagetes minuta* from different zones in Kenya against brown ear tick (*Rhipicephalus appendiculatus*). MSc. Thesis, Kenyatta University, Kenya.

Mangena, T. and Muyima, N. Y. (1999). Comparative evaluation of the antimicrobial activities of essential oils of Artemisia afra, Pteronia incana and Rosmarinus officinalis on selected bacteria and yeast strains. *Lett. Appl. Microbiol*, 28: 291- 296.

Manoj, K. and Ragothaman, G. (1999). Effect of mercury, copper and cadmium on the red blood cells of *Boleophthamus duosumieri* (Cuv.*). Pollution Research*, 18(2): 149-152.

Marotti, M., Piccaglia, R., Biavati, B. and Marotti, L. (2004). Characterization and Yield evaluation of essential oils from different *Tagetes* species,. *J. Essent. Oil Res.*, 16: 440-444.

Martijena, I. D., Garcia, D. A., Marin, R. H., Perillo, M. A. and Zygadlo, J. P. (1998). Anxiogenic-like and antidepressant like effects of the essential oil from *Tagetes minuta*. *Fitoterapia*, 69: 155-160.

Masango, P. (2005). Cleaner production of essential oils by steam distillation. *J Cleaner Prod.*, 13: 833–9.

Maurer, B. and Hauser, A. (1984). 5-Isobutyl-3-methyl-2-furancarbaldehyde, a new monoterperiod from the essential oil of *Tagetes glandulifera* Schrank, *Tetrahedron Lett.*, 25: 1061-1062.

Mehran, M., Moein, M., Maryam, S. and Masoud, N. (2013). Chemical constituents of the essential oils from the leaves, flowers and seeds of *Tagets minuta* L. by GC-MS. *International journal of Advanced Biological and Biomedical Research*. Vol. 1(9): 1124-1128.

Mishra, D., Chaturvedi, R. V. and Tripathi, S. C. (1995). The fungitoxic effect of the essential oil of the herb *Nardostachys jatamansi* DC. *Trop. Agri.*, 72: 48-52.

Mohamed, A. A., El-Emary, G. A. and Ali, H. F. (2010). Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in *Ehrlich ascites* Carcinoma cells. *J Am Sci* ., 6: 820–826.

Moradalizadeh, M., Mehrabpanah, M., Salajeghe, M. and Nayebli, M. (2013). Chemical constituents of the essential oils from the leaves, flowers and seeds of *Tagetes Minuta* L. by GC/MS. *International Journal of Advanced Biological and Biomedical Research*, 1(9): 1124-1128.

Muzika, R. M., Campbell, C. L., Hanover, J. W. and Smith, A. L. (1990). Comparison of techniques for extracting volatile compounds from conifer needles. *Journal of Chemical Ecology*, 16: 2713-2722.

Nair, R., Gunasekaran, R. and Sujatha, K. (1995). Flavonol glycosides from leaves of *Eupatorium glandulosum*. *Phytochemistry*, 40(1): 283 – 285.

Nanir, S. P. and Kadu, B. B. (1987). Effect of some medicinal plant extracts on some fungi, *Acta Bot. Ind.*, 15: 170-175.

Okoh, O. O. (2010). Chemical transformations and phytochemical studies of bioactive components from extracts of *Rosmarinus officinalis* L. PhD Thesis, University of Fort Hare, South Africa.

Okoh, O. O., Sadimenko, A. P., Asekun, O. T. and Afolayan, A. J. (2008). The effects of drying on the chemical components of essential oils of *Calendula officinalis* L.. *African Journal of Biotechnology*, 7 (10): pp. 1500-1502.

Panero, J. L. and Funk, V. A. (2008). The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. *Mol. Phylogene Evol*. 47, 757-782.

Patel, B. V. (2001). A report of the seminar on Herbal Drugs: Present Status and future prospects Perd centre. Ahmedabad.

Pavia, L. D., Lampman, M. G., Kriz, S. G. and Engel, G. R. (2005). Introduction to organic laboratory techniques a small scale approach (2nd ed). Australia: Thomsone Brooks Cole Publication,101-104.

Pavithra, K. and Vadivukkarasi, S. (2012). GC-MS evaluation of Chemical constituents from methanolic leaf extract of *Kedrostis foetidissima* (Jacq.) Cogn. *Asian J of Pharma and Clin Res.*,5 (4): 77-81.

Penna, C. A., Radice, M., Gutkind, G. O., VanBaren, C., Broussalis, A., Muschietti, L., Martino, V. and Ferraro, G., (1994). Antibacterial and antifungal activities of some Argentinean plants. *Fitoterapia*, 65: 172-174.

Pérez, G. R. M., Luha, H. H. and Garrido, S. H. (2006). Antioxidant activity of *Tagetes erecta* essential oil. *Journal of Chilean Chemical Society*, 51 (2): 883-886.

Pichette, A., Garneau, F. X., Collin, G., Jean, F. I., Gagnon, H. and Arze, J. B. L. (2005). Essential oil from Bolivia, IV, compositae: *Tagetes aff. maxima* kuntze and *Tagetes multiflora* H.B.K. *J. Essent. Oil Res.*, 17: 27-28.

Pizzale, L., Bortolomeazzi, R., Vichi, S., Beregger, E. and Conte, L. S. (2002). Antioxidant activity of sage (*Salvia officinalis and S fruticosa*) and oregano (*Origanumonites and O indercedens*) extracts related to their phenolic compound content. *J Sci Food Agric.*, 82:1645–1651.

Pooja, A., Arun, N. and Maninder, K. (2013). Screening of plant essential oils for antifungal activity against *Malassezia furfur*, *International Journal of Pharmacy and Pharmaceutical Sciences,* 5(2): 37-39.

Popovic, M., Kaurinovic, B., Mimica-Dukic, N., Vojinovic-Miloradov, M. and Cupic, V. (1999). Combined effects of plant extracts and xenobiotics on liposomal lipid peroxidation. Part 1. Marigold extract-ciprofloxacin/pyralene. *Oxidation Commum*. 22: 487-494.

Prabuseenivasan, S., Jayakumar, M. and Ignacimuthu, S. (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine,* 6:39.

Raaman, N. (2006). *Phytochemical techniques*. New India Publishing Agency: New Delhi, India.

Ragupathi, R. K., Rengasamy, A., Rajasekaran., A., Perumal. (2011). Fourier Transform Infrared Spectroscopy Analysis of Sea grass Polyphenols. *Current Bioactive Compounds,* 7(2): 118-125.

Rai, M., Acharya, D. and Wadegaonkar, A. (2010). Plant derived antimycotics: potential of Asteraceous plants. In Plant-derived antimycotics: Current Trends and Future prospects, Haworth press, N-York, London, Oxford, 165-185.

Rajvanshi, S. K. and Dwivedi, D. H. (2017a). Phytochemical screening studies of bioactive compounds of African marigold (*Tagetes erecta* L.). *Journal of Pharmacognosy and Phytochemistry,* 6(4): 524-527.

Rajvanshi, S. K. and Dwivedi, D. H. (2017b). Secondary Phytometabolite Profiling of Hydrodistilled Essential Oil of Dry Flower from African marigold (*Tagetes erecta* L.) by FTIR and DART Mass Spectrometry. *Int. J. Pure App. Biosci.,* 5(1): 729-734.

Rajvanshi, S. K. and Dwivedi, D. H. (2017c). Studies on bioactive phytochemicals in hydrodistilled essential oil of African marigold (*Tagetes erecta* L.). *The Bioscan***,** 12(1): 633-636.

Rao, V. P. and Pandey, D. P. (2007). *Extraction of essential oil and its application.* Retrieved September 13, 2015 from http://ethesis. nitrkl.ac.in/4292/1/Extraction_of_Essential.pdf.

Ray, D., Prasad, D. and Singh, R. P. (2000). Chemical examination and antinemic activity of marigold (*Tagetes erecta* L.) flower. *Ann. Pl. Protec. Sci.*, 8: 212-217.

Razdan, T. K. (1984). Colorimetric analysis of the constituents of essential oils, *Parfum Kosmet.,* 65: 190-195.

Raghava, S. P. S. (1998). Pusa Narangi Gainda and Pusa Basanti Gainda: New Marigolds, *Indian Horticult.,* 43: 31.

Rasoanaivo, P., Petitjean, A. M., Ratsimamanga, U. S. and Rakoto-Ratsimamanga, A. (1992). Medicinal plants used to treat malaria in Madagascar, *J. Ethnopharmacol.,* 37: 117-127.

Rhama, S. and Madhavan, S. (2011). Antibacterial Activity of the Flavonoidpatulitrin isolated from the flowers of *Tagetes erecta* L.

International Journal of Pharmaceutical Technology and Research, 3(3): 1407- 1409.

Romagnoli, C., Mares, D., Fasulo, M. P. and Bruni, A. (1994). Antifungal effects of a-terthienyl from *Tagetes patula* on five dermatophytes, *Phytother. Res.*, 8: pp. 332-336.

Ruberto, G. amd Baratta, M. T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.,* 69: 167–74.

Saha, S., Walia, S., Kundu, A., Kumar, B. and Joshi, D. (2012). Antifungal acetylinic thiophenes from *Tagetes minuta*: potential biopesticide. *Journal of Applied Botany and Food Quality,* 85(2): 207 - 211.

Saxena, R. C., Dixit, O. P. and Sukumaran, P. (1992). Laboratory assessment of indigenous plant extracts for anti-juvenile hormone activity in *culex quinquefasciatus, Indian J. Med. Res.*, 95: 204-206.

Schmidt, E. (2010). Production of essential oils. In: K.H. Başer and *G.* Buchbauer G, (Eds.), *Handbook of essential oils. Science, technology, and applications*. CRC Press: Boca Raton, Florida, United States.

Schrader, B. (ed.). (1995). Infrared and Raman spectroscopy, Methods and Applications, VCH Weinheim, New York. 203–210.

Sefidkon, F. and Fathi, E. (2012). Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Eucalyptus sargentii. Journal of Agricultural Science and Technology,* 14(5): 1035-1042.

Sefidkon, F., Salehyar, S., Mirza, M. and Dabiri, M. (2004). The essential oil of *Tagetes erecta* L. occurring in Iran. *Flavour Fragr. J.* 19: 579–581.

Semen, E. and Hiziroglu, S, (2005), Production, yield and derivatives of volatile oils from eastern redcedar (*Juniperus virginiana* L.). *American Journal of Environmental Sciences,* 1: 133-138.

Senatore, F., Napolitano, F., Mohamed, M., Harris, P. J., Mnkeni, P. N. and Henderson, J. (2004). Antibacterial activity of *Tagetes minuta* L. (Asteraceae) essential oil with different chemical composition. *Flavour and Fragrance Journal,* 19(6): 574-578.

Siddiqui, A. M. and Alam, M. M. (1988). Toxicity of different plant parts of *Tagetes lucida* to plant parasitic nemotodes, *Indian J. Nematol.,* 18: 181-185.

Silva, F. C., Chalfoun, C. M., Siqueira, V. M., Botelho, D. M., Lima, N. and Batista, L. R. (2012). Evaluation of antifungal activity of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. *Brazilian Journal of Pharmacognosy,* 22(5): 1002-1010.

Simandi, B., Deak, A., Ronyai, E., Yanxiang, G., Veress, T., Lemberkovics, E., Then, M., Sasskiss, A. and Vamos, F.Z. (1999). Supercritical carbon dioxide extraction and fractionation of fennel oil. *Journal of Agriculture and Food Chemistry*, 47:1635-1640.

Sindhu, E. R. and Kuttan, R. (2007). Antioxidant and anti-inflammatory activity of lutein and its ester, *Amala Res. Bull.*, 27: 261- 270.

Singh, A., Khanuja, S. P., Arya, S. J., Singh, S. and Yadaw, A. (2006). Essential oil quality and yield with respect to harvest index in *Tagetes minuta* cultivated in sub-tropical plains of North India. *Journal of Essential Oil Research,* 18(4): 362-365.

Singh, B., Sood, R. P. and Singh, V. (1992). Chemical composition of *Tagetes minuta L.* oil from Himalachal Pradesh (India). *J. Essent. Oil Res,* 4: 525-526.

Singh, V., Gupta, K. A., Singh, S. P. and Kumar, A. (2012). Direct Analysis in Real Time by Mass Spectrometric Technique for Determining the Variation in Metabolite Profiles of Cinnamomum tamala Nees and Eberm Genotypes. *ScientificWorldJournal*, Published online Jun 4.

Shaheen, F. and Aneela, N.(2004). Palmitoleate (= (9Z)- Hexadeca-9-enoate) Esters of Oleanane Triterpenoids from the Golden Flowers of *Tagetes erecta*: Isolation and Autoxidation Products. *Helvetica Chimica Acta*, 87(1):46- 56.

Sharma, M. L., Nigam, M. C., Handa, K. L. and Chopra, I. C. (1961). Essential oil of *Tagetes erecta, Perf. Essent. Oil Rec.*, 52,: pp. 561-562.

Shiva, M. P., Lehri, A. and Shiva, A. (2002). *Aromatic and Medicinal Plants*, 219-222.

Sohrabi, M. R., Davallo, M., Tadayyon, F., Nabipoor, F. and Khamneifar A. (2005). Simultaneous Determination of Acetyl Salicylic Acid and Acetaminophen in A.C.A Tablets by FT/IR/ATR Spectrometry with Multivariate Calibration Data Treatment. *Asian Journal of Chemistry*, 17(1): 541-547.

Soule, J. A. (1993). *Tagetes minuta:* Potential New Herbs from South America. In: *New Crops J. Janick and J,E. Simon*, eds. Wiley, New York., 649-654.

Srivastava, A. and Srivastava, M. (1998). Fungitoxic effect of some medicinal plants (on some fruit pathogens), *Philippine J. Sc.*, 127: 181-187.

Stashenko, E., Prada, N.Q. and Martinez, J. R. (1996). Effects of extraction techniques on the chemical composition and antioxidant activity of *Eucalyptus camaldulensis* var. *brevirostris* leaf oils. *High Resolution Chromatography*, 19: 353- 358.

Steinmetz, K. A. and Potter, J. D. (1991). Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control, 2(6): 427-428.

Takahashi, K. and Takani, M. (1976). Studies on Constituents of Medicinal Plants. XVII. Constituents of Schizandra nigra MAX. and Their Carbon-13 Nuclear Magnetic Resonance Spectra. *Chem.pharm.bull.*, 24: 2000.

Tandon, S. (2008). Distillation technology for essential oils. In: Handa, S. S., Khanuja, S. P., Longo, G, Rakesh, D. D. (Eds). Extraction technologies for medicinal and aromatic plants. ICS UNIDO, Trieste, 115–143.

Tereschuk, M. L., Riera, M. V., Castro, G. R. and Abdala, L. R. (1997). Antimicrobial activity of flavonoids from leaves of *Tagetes minuta. J. Ethnopharm*, 56: 227- 232.

Thomas, P. and Devasagayam, A. (2006). Radiation Biology and Health Sciences Division, Bhabha Atomic Research centre. Mumbai.

Tripathi, A. K., Khan, S. A., Vaishnava, M. M. and Gupta, K. R. (1992). Compounds of taxonomic significance in *Tagetes*, *Fitoterapia*, 63: 85.

Tripathy, A. K. and Gupta, K. R. (1991). Plant Phenolics of *Tagetes erecta*, *Fitoterapia*, 62: 91-92.

Tripathi, B., Bhatia, R., Walia, S. and Kumar, B. (2012). Chemical Composition and Evaluation of *Tagetes erecta* (Var. Pusa Narangi Genda) Essential Oil for its Antioxidant and Antimicrobial Activity. *Biopestic. Int.*, 8(2): 138-146.

Tsumotu, K., Hideki, S. and Tsuyoshi, T. (1986). Diacetyl Formation and Degradation by Streptococcus lactis subsp. diacetylactis 3022. *Agriculture and Biological Chemistry*, 50(10): 263.

Tuan, D. Q. and Iiangantileke, S. G. (1997). Liquid CO2 extraction of essential oil from star anise fruits (*Illicium verum* H.). *Journal of Food Engineering*, 31:

47-57. **Tygadlo, J. A., Maestri, D. M. and Ariza, E. l. (1993).** The volatile oil of *Tagtes argentina Cabrera. Journal of Essential Oil Research*, 5: 185-186.

Upadhyaya, K., Chanotiya, C. S., Padalia, R., Bhatt, Z. A. and Bahuguna, Y. M. (2010). Comparative phytochemistry and pharmacological evaluation of *Tagetes minuta.* J. *Pharm. Res,* 3: 1434-1437.

Vargas, D. F. and Lopez, P. O. (1996). Correlation of HPLC and AOAC methods to assess the all-trans-lutein content in marigold flowers. *J. Sci. Food Agric,* 72: 283-290.

Vargas, D. F., Jimenez, A. R. and Lopez, P. O. (2000). Natural pigments: carotenoids, anthocyanins and betalains. Characteristics, biosynthesis, processing and stability. *Crit. Rev. Food Sci. Nutr,* 40: 173-289.

Vilegas, W., Nehme, C. J. and Dokkedal, A. L. (1999). Quercetagetin 7-methyl ether glycosides from *Paepalanthus vellozioides* and *Paepalanthus latipes*. *Phytochemistry,* 51(3): 403- 409.

Villar, R., Calleja, J. M., Morates, C. and Caceres, A. (1997). Screening of 17 Guatemalan medicinal plants for platelet antiaggregant activity. *Phytother. Res.*, 11: 441-445.

Wang, S. (2000). Analysis of composition of volatile flower oil of *Rosa*. *Yaubian University of Natural Science,* 25: 262-265.

Wang, X. Y. (2007). Investigation the Chemical Constituents and Bioactivity of *Saussurea Cauloptera*. Masters dissertation on organic chemistry, Lanzhou University.

Wanzala, W. and Ogoma, S. B. (2013). Chemical composition and mosquito repellency of essential oil of *Tagetes minuta* from the Southern slopes of Mount Elgon in Western Kenya. *Journal of Essential Oil Bearing Plants*, 16(2): 216-232.

Wanzala, W. (2009). Ethnobotanicals for management of the brown ear tick *Rhipicephalus appendiculatus* in western Kenya. PhD Thesis, Wageningen University, The Netherlands.

Wiese, B., Quiroga, O. E., Vigo, M. S. and Nolasco, S. M. (1992). Seeds of *Tagetes minuta L.* chemical composition of seed oil and residual seed meal. [Semilla de 103 *Tagetes minuta* L. composicion quimica del aceite seminal y de la harina residual, 2: 135-137.

Worwood, V. A. (1990). The fragrant Pharmacy a complete guide to Aromatherapy and Essential oils , Cox &Wyman Ltd, Great Britain, U.K.

Wei, H. X., Gao, W. Y., Tian, Y. K. and Guan, Y. K. (1997). New Eudesmane Sesquiterpene and Thiophene Derivatives from the Roots of *Rhaponticum uniflorum*. *Pharmazie,* 52: 245 -247.

Weiss, E.A. (1997). Essential oil crops. *Centre for Agriculture and Biosciences* (CAB) International, New York and UK.

Werner, H. and Kinzo, W. (1983). Sesquiterpene alcohols and triterpinoids from *Liatris microcephala*. Phytochemistry, 22 (6): 1457- 1459.

Wong, Y. C., Ahmad-Mudzaqqir, M. Y. and Wan-Nurdiyana, W.A. (2014). Extraction of Essential Oil from Cinnamon (*Cinnamomum zeylanicum*). *Oriental journal of chemistry,* 30 (1): 37-47.

Wu, S. H., Wu, D. G. and Chen, Y. W. (2005). Chemical constituents and bioactivities of plants from the genus Paeonia. *Trad. Herb. Drug,* 36: 648.

Xie, J. X., Chang, J. B. and Wang, X. M. (2001). Infrared Spectrum, Science Press, Beijing, 77: 212-309.

Xio, Z. P., Wu, H. K., Wu, T., Hang, B. and Aisha, H. A. (2006). Kaempferol and quercetin flavonoids from *Rosa rugosa*. *Chemistry of natural compounds,* 42(6): 736 – 737.

Xue, M. A., Duo-long, D. and Yan, P. S. (2008). Triterpenoids and steroids from *Ixeridium gracile*. *Chemistry of natural compounds,* 44(3): 399 – 401.

Yang, N. Y., Duan, J. A., Qian, S. H. and Tian, L. J. (2003). Studies on the chemical constituents of *Tagetes erecta* L. *J Shenyang Pharm. Uni*., 20: 258-259.

Yavin, Z. and Palevitch, D. (1982). Effect of drought on the secondary metabolites of medicinal and aromatic plants - A review In: Regional Research Laboratory Jammu- Tawi (India). *In*. Yavin, Z. & Palevitch, D, (ed.) 1-12.

Zang, Y. and Zhang, T. T. (2010). Studies on the chemical constituents from the stem and leaves of *Tagetes erecta*. *Journal of Chinese medicinal materials,* 2010; 33(9):1412 -4.

Zellner, B. A., Dugo, P., Dugo, G. and Mondello, L. (2010). Analysis of essential oils. In: K.H. Başer and G. Buchbauer G, (Eds.), *Handbook of essential oils. Science, technology, and applications*. CRC Press: Boca Raton, Florida, United States.

Zhou, D. Y., Yang, X. S. and Yang, B. (2007). Isolation from *Tagetes species*. *Nat. Prod. Res. Dev.*, 19: 807.