Marigold

A Potential Ornamental Plant Drug

Pankaj Gupta* and Neeru Vasudeva

Department of Pharmacognosy,
Guru Jambheshwar University of Science and Technology,
Hisar-125001 (Haryana),
India.

Marigold botanically identifies as *Tagetes* (Compositae) genus is an ethnobotanically known drug, used from ancient times in the Indian system of Medicine for the treatment of rheumatism, cold, bronchitis, eye diseases, ulcers etc. *Tagetes* species, commonly known as marigold, are grown as ornamental plants and thrive in varied agro-climates. The genus has been recognized as a potential source of very interesting biologically active products i.e. carotenoids that are used as food colorants, feed additives and possess anticancer and antiageing effects, essential oil known for antimicrobial and insecticidal properties, thiophenes with a marked biocidal activity and flavonoids having pharmacological properties. The *Tagetes* oil has been mainly used for the compounding of high-grade perfumes and also acts as antihaemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, diaphoretic and emmenagogue. This genus has been investigated for various biological activities like antimicrobial, antiplasmodial, antioxidant, insecticidal etc. The present review summarizes the biological activities and phytoconstituents of this genus.

Introduction

Tagetes (Compositae) is a genus of herbs, commonly known as marigold, native of Mexico and other warmer parts of America and naturalized elsewhere in the tropics and sub-tropics (Anonymous, 1976). In India, these were introduced by the Portuguese. Several species are grown in gardens for ornamental purpose. The name marigold is however indiscriminately applied to several genera of Compositae with golden or yellow capitula and there are about 33 species of the genus Tagetes, out of which, five species have been introduced into the Indian gardens viz. Tagetes erecta L. (Aztec or African Marigold), Tagetes minuta L. (Tagetes glandulifera Schrank), Tagetes patula L. (French Marigold), Tagetes lucida Cav. (Sweet-Scented Marigold), Tagetes tenuifolia Cav. (Striped Marigold) (Rydberg, 1915). The Marigolds spread quickly because of the ease in cultivation, longer blooming period and beautiful flowers with excellent shelf life. They are extensively used for making garlands, religious offerings and exhibitions and major work on their improvement has been done in the USA, Switzerland, France and West Germany (Raghava, 1998; Janakiram and Rao, 1996). The genus is held in great esteem in the indigenous systems of medicine.

Tagetes erecta L. has been used for the treatment of a wide variety of diseases and ailments. The infusion of the plant has been used against rheumatism, cold and bronchitis, juice of leaves for ear-ache, leaves and florets as emmenagogue and their infusion prescribed as a vermifuge, diuretic and carminative (Anonymous, 1976). Its florets have been used for the treatment of eye diseases and ulcers and an extract of the roots credited as laxative (Kirtikar and Basu, 1975). The decoctions of the leaves of Tagetes erecta and Tagetes patula have been traditionally used as antimalarial and as febrifuge (Rasoanaivo et al., 1992). Tagetes minuta has been traditionally used for repelling mosquitoes in Kenya (Ejobi, 1997) and also possesses strong larvicidal effect. Its juice causes irritation of the eyes and skin while the flowers are used as stomachic, aperient, diuretic and diaphoretic. The roots and seeds of Tagetes patula are used as purgative, its leaves contain certain skin-irritating principles and the flower heads possess stimulant and anthelmintic properties. The juice from Tagetes patula contains iodine and is used on cuts and wounds whereas the decoction of its flowers is used as a carminative. The Tagetes lucida plant is used in foods as an ingredient of soup in place of tarragon and its leaves and flower-heads are used to perfume bathing-water. Traditionally,

the shoots of *Tagetes lucida* and *Tagetes filifolia* have been used for the preparation of native teas in Mexico (Laferriere, 1991) whereby they act as popular beverages and medicinal preparations for colic and gastrointestinal disorders (Linares and Bye, 1987).

The *Tagetes* oil has been mainly used for the compounding of high-grade perfumes. *Tagetes minuta* is considered as the best source of valuable essential oil than other species of the genus and its oil has a very strong and penetrating odour that makes a good market in the perfumery world (Chopra *et al.*, 1963). Its essential oil possesses great aromatic potency in formulations, especially for the purpose of modifying and embellishing the fruit-like initial notes in colognes and perfumes (Johnson and Robertel, 1986). The essential oil from *Tagetes erecta* flowers has a fruity floral aroma (Igolen, 1936) and finds application in high class perfumery. Additionally, the essential oil also acts as antihaemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, diaphoretic and emmenagogue and is valuable in aromatherapy for its powerful skin healing properties (Shiva *et al.*, 2002).

According to the literature, many *Tagetes* species have been investigated and found to contain terpenoids, thiophenes, flavonoids, carotenoids, phenolic compounds etc. This genus has been investigated for various biological activities viz. antimicrobial, antiplasmodial, insecticidal etc. The review describes the presently available chemical and biological data of the genus *Tagetes* and suggests that terpenoids, flavonoids, carotenoids and thienyls are the main classes of phytoconstituents of interest to phytochemists and pharmacologists.

Chemistry

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).

Essential Oil from Aerial Parts

The essential oil of the leaves of *Tagetes minuta* was reported to contain d-limonene, ocimene, β -myrecene (1), aromadendrene (2), l-linalool, linalyl acetate, linalool monoxide, d-carvone, tagetone, 1:8 cineole (3) and salicylaldehyde (4) (Gupta and Bhandari, 1975). The essential oil of the leaves of *Tagetes erecta* showed the presence of d-limonene, α -pinene (5), β -pinene (6), dipentene, ocimene, β -phellandrene (7), linalool (8), gereniol (9), menthol (10), tagetone, nonanal and linalyl acetate (Baslas and Singh, 1981a). The essential oil of *Tagetes erecta* was further reported to contain camphene (11), sabinene (12), myrecene, (z)- β -ocimene, (E)- β -ocimene, γ -terpinene (13), terpinolene (14), p-mentha-1,3,8-triene, terpinen-4-ol (15), p-cymen-9-ol, piperitone (16), thymol (17), indole (18), carvacrol (19), piperitenone (20), geranyl acetate, β -elemene, cyperene, β -caryophyllene (21), (E)- β -farnesene (22), γ -muurolene, γ -elemene, and nerolidol (23) (Machado *et al.*, 1994). The leaf oil of *Tagetes erecta* was later reported to contain a total of forty four constituents with major constituents being limonene (24), terpinolene, (Z)-myroxide, piperitone and piperitenone (Krishna *et al.*, 2004).

The essential oil of the flowers of *Tagetes erecta* showed the presence of d-limonene, ocimene, 1-linalyl acetate, 1-linalool, tagetone and n-nonyl aldehyde (Sharma *et al.*, 1961). Further studies revealed the presence of aromadendrene, phenylethyl alcohol (**25**), salicylaldehyde, phenylacetaldehyde (**26**), 2-hexen-1-al, eudesmol (**27**), tagetone, ocimene, linalyl acetate and an unidentified carbonyl compound in the essential oil of *Tagetes erecta* (Handa *et al.*, 1963). Three acyclic monoterpene ketones *viz.* 3,7-dimethyloct-1-en-6-one (**28**), 3,7-dimethyl-5-hydroxyoct-1-en-6-one (**29**) and 3,7-dimethyloct-1,7-dien-6-one (**30**) were further reported in the essential oil of *Tagetes erecta* flowers (Garg and Mehta, 1998) in addition to myrecene, caryophyllene, p-cymene, d-carvone and eugenol (**31**) (Baslas and Singh, 1981b). A monoterpenoid, 5-isobutyl-3-methyl-2-furancarbaldehyde (**32**) was reported in the essential oil of the flowers of *Tagetes glandulifera* (Maurer and Hauser, 1984). Acetaldehyde, acetone, α -thujene, β -pinene, toluene, ethyl-2-methylbutyrate, camphene, β -pinene, 3-methylbutyl acetate, sabinene, α -phellandrene, *cis*- β -ocimene, *trans*- β -ocimene, α -thujone, β -thujone (**34**), *cis*- β -ocimene epoxide, *trans*-tagetone (**35**), *cis*-tagetone (**36**), linalool, *trans*- α -bergamotene, terpinen-4-ol, methyl carvacrol, menthol, *trans*- β -farnesene, methyl chavicol (**37**), α -humulene, *trans*-tagetenone, γ -elemene, β -cadinene, geraniol, thymolhydroquinone dimethyl-ether, isopiperitenone, piperitenone, nerolidol, methyl

eugenol (38), thymol, carvacrol, and α -cadinol were reported in the essential oil of the flowers of *Tagetes minuta* (Lawrence *et al.*, 1986) in addition to tagetone, tagetenones, 1-carvone, ocimene, phenylethylalcohol, aromadendrene, salicylaldehyde, β -myrcene, linalylaceate and β -phellandrene (Razdan *et al.*, 1986). The essential oil of the flowers of *Tagetes erecta* was finally reported to contain an overall of forty five constituents including limonene, terpinolene, (Z)-myroxide, piperitone, piperitenone, piperitenone oxide and b-caryophyllene (Krishna *et al.*, 2004).

The essential oil of the leaves, flowers and stems of *Tagetes patula* was reported to contain ocimene, limonene, linalool, linalyl acetate and tagetone (Dhingra and Dhingra, 1956). The terpenes, α-pinene, β-pinene, dipentene, menthol and geraniol were reported in the essential oil of the leaves and flowers of *Tagetes erecta* (Baslas and Singh, 1980). b-ocimene (39) was reported in the essential oil from the flowering shoots of Tagetes minuta and Tagetes tenuifolia. The essential oil of the flowering shoots of *Tagetes patula* was reported to contain Z-ocimene and E-ocimene besides limonene, caryophyllene, piperitone and piperitenone. The oil from the flowering shoots of *Tagetes erecta* contained piperitone and caryophyllene. The essential oil of flowering shoots of *Tagetes lucida* was reported to contain linalool, estragol and methyl eugenol (Hethelyi et al., 1987). The essential oil of the flowering shoots of Tagetes minuta was reported to contain β-ocimene, dihydrotagetone, tagetone and ocimenones. The essential oil of flowering shoots of Tagetes patula was reported to contain limonene, α-terpinolene, tagetone, ocimenone, piperitone, piperitenone and caryophyllene (Hethelyi et al., 1988). Geraniol was estimated in the essential oil of Tagetes minuta through colorimetric analysis (Razdan, 1984) while thirty seven constituents were later reported in the essential oil of the leaves and flowers of Tagetes minuta with (Z)-β-ocimene predominating in the oil from flowers and dihydrotagetone predominating in that from leaves (Chalchat et al., 1995). The essential oil of the flowering stems of Tagetes lemmonii Gray was reported to contain ethyl-2-methyl butyrate, α-phellandrene, (E)-β-ocimene, dihydrotagetone, allo-ocimene, (Z)-tagetone, (E)-tagetone, β-carhophyllene, (Z)-ocimenone (40), (E)-ocimenone (41) and germacrene D (Turker and Maciarello, 1996). Fifty three compounds were revealed in the essential oil of the flowering herbs of *Tagetes lucida* with major components being anethole (42), methyleugenol and estragole (Bicchi et al., 1997). Sixty one compounds were detected in the essential oil of the aerial parts of Tagetes mandonii Sch. Bip, the major ones being cis-ocimene, transocimene, ocimenones, tagetones, limonene, spathulenol and cis-anethole (Senatore and De Feo, 1992). Fifty seven compounds were identified in the essential oil of the aerial parts of Tagetes filifolia Lag. with cis-anethole and estragole as the major constituents (De Feo et al., 1998). Thirty five compounds were revealed 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 (Marotti et al., 2004). Eighteen components were reported from the essential oil of the aerial parts of Tagetes aff. maxima and Tagetes multiflora whereby (Z)-tagetone, dihydrotagetone and (E)-ocimenone were identified as major components in the oil of Tagetes aff. maxima while the oil of Tagetes multiflora contained (Z)-tagetone, (E)-ocimenone and (Z)-β-ocimene as major components (Pichette et al., 2005). Sixty five compounds were identified in the essential oil of the aerial parts of Tagetes terniflora HBK with the main components being tagetones, ocimenes, ocimenones, (E)-β-ocimene, transtagetone, limonene, isomenthone, spathulenol, cis-anethole and trans-anethole (De Feo et al., 2005). The essential oil of the dried plant of Tagetes minuta was reported to contain trans-ocimenone, cis-ocimene, dihydrotagetone and cis-tagetone whereas the major constituents of the essential oil of the fresh plant were cis-ocimene, cis-ocimenone, trans-ocimenone, limonene, allocimene and cis-tagetone (Hadjiakhoondi et al., 2008).

Essential Oil from Fruits

Twenty four constituents were revealed in the essential oil of the dried mature fruits containing seeds of *Tagetes minuta* with the major components being (Z)- β -ocimene, (Z)-tagetone, (Z)-tagetenone and (E)-tagetenone (Kaul *et al.*, 2005).

Thiophenes

Four thiophenes viz. 5-(4-hydroxy-1-butenyl)-2,2'-bithienyl (43), 5-(4-acetoxy-1-butenyl)-2,2'-bithienyl (44);

5-(3-buten-1-ynyl)-2,2'-bithienyl (45) and 2,2': 5'2"-terthienyl (46) were reported from the roots, shoots and flowers of *Tagetes patula* (Downum and Towers, 1983). Thiophenes have also been isolated from the 'hairy root' cultures of *Tagetes erecta* (Flores, 1992). 5-(but-1-ol-3-ynyl)-2,2'-bithienyl (47) was isolated from the whole dried plant of *Tagetes erecta* (Tripathi *et al.*, 1992) while the fresh roots of *Tagetes minuta* were reported to contain a-terthienyl and 5-(3-buten-1-ynyl)-2,2'-bithienyl (Horn and Lamberton, 1963). Furthermore, a new bithienyl derivative *viz*. 2-hydroxymethyl-non-3-ynoic acid 2-[2,2']-bithiophenyl-5-ethyl ester was isolated from the ethyl acetate fraction of the roots of *Tagetes erecta* (Vasudeva *et al.*, 2007).

Flavonoids

The flowers of *Tagetes erecta* revealed the presence of 6-hydroxykaempferol-7-O-β-D-alloside (48) (Das and Tripathi, 1997) whereas isorhamnetin-7-O-β-D-galactoside was reported from the 90% ethanolic extract of shaded dried flowers of Tagetes patula (Tripathi et al., 1991). Eleven flavonol glycosides viz. quercetin-3-(3", 6"-diacetyl galactoside), quercetin-3-(2",3",4"-triacetyl galactoside), quercetin-3-galactoside, isorhamnetin-3-rhamnosyl(1-6)glucoside, myricetin-3-glucoside, quercetin (49), isorhamnetin-3-galactoside, quercetin-3-(6"-galloyl galactoside), quercetin-3rhamnoside, quercetin-3-rhamnosyl¹⁻⁶ galactoside and rhamnetin were reported from the methanolic extract of *Tagetes* elliptica Sm. (D'Agostino et al., 1992). Another, eleven flavonoids were revealed from the methanolic extracts of the leaves and flowers of four Tagetes species viz. Tagetes Laxa, Tagetes argentina, Tagetes biflora and Tagetes perezi including myricetin-3-glucoside, myricetin-7-glucoside, quercetagetin-7-glucoside, patuletin-7-glucoside, quercetin-7-glucoside, quercetin-3-glucoside, quercetin-3-rhamnoside, quercetin-5-glucoside, quercetin-3-methyl ether, kaempferol-7-glucoside and kaempferol-3-glucoside (De Israilev et al., 1991). Several other flavonoids were identified from the aqueous methanolic extract of the defatted flower heads and leaves of *Tagetes erecta* including quercetagetin (50), quercetagetrin (51) and 6-hydroxykaempferol-7-O-glucoside (52) (El-Emary and Ali, 1983). Additionally, the extract of the leaves particularly yielded kaempferol (53), kaempferol-7-O-rhamnoside (54) and kaempferitrin (55) and later quercetin was also reported from the dried flowers of Tagetes erecta (Tripathy and Gupta, 1991). Two primary flavonoids viz. patulitrin and patuletin were recently reported from the water-ethanolic extract of Tagetes patula flowers and it was further revealed via analysis of component dynamics during the plant growth that these compounds were only found during and after flowering (Guinot et al., 2008).

Carotenoids

The extracts of *Tagetes erecta* were initially reported to contain epoxides such as lutein 5, 6-epoxide and other oxidation products of lutein (Alam *et al.*, 1968; Goodwin, 1980). Some preliminary studies during the same phase demonstrated the predominance of lutein (56) and zeaxanthin (57) (88-92%) in the plant in addition to the fifteen other separated pigments, of which less than 3% were reported to be epoxy pigments (Quackenbush and Miller, 1972). The carotenoid composition of marigold extract was later analyzed via high performance liquid chromatography (HPLC) whereby epoxides namely violaxanthin and neoxanthin were reported (McGeachin and Bailey, 1995). The saponified *Tagetes erecta* flower extract was reported for 93% utilizable pigments (detected at 450 nm), consisting of all *trans* and *cis*-isomers of zeaxanthin (5%), all *trans* and *cis*-isomers of lutein, and lutein esters (88%). Among the lutein isomers, *trans*-lutein was reported as the major component with several *cis*-lutein isomers as minor components from the commercial marigold flower extract (Hadden *et al.*, 1999). The yield of xanthophyll content in well preserved flowers of *Tagetes erecta* was reported to be 105.19 g/kg in contrast to the unpreserved flower sample (58.87 g/kg), thereby emphasizing the significance of flower preservation during the extraction of xanthophylls (Pratheesh *et al.*, 2009).

Phenolic Compound

The dried flowers of *Tagetes erecta* revealed the presence of phenolic compounds such as syringic acid (58) (Tripathy and Gupta, 1991) in addition to various other components such as ethyl gallate and methyl-3,5-dihydroxy-4-methoxy benzoate (Tripathi *et al.*, 1992).

Biological Activity

Tagetes genus has been investigated by many workers for its various pharmacological effects *viz.* antiplasmodial, antioxidant, antidepressant, antimicrobial etc.

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). 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, Bacillus mycoides, Bacillus pumilus, Bacillus subtilis, Salmonella paratyphi A, Salmonella paratyphi B, Salmonella paratyphi C, Salmonella typhi H, Salmonella enteritides, Salmonella flexneri, Salmonella typhimurium, Shigella sonnei, Shigella schimizii, Shigella shiga, Vibrio cholerae Inawa, Vibrio cholerae Ogawa, Vibrio cholerae Eltor and Xanthomonas campestris (Garg and Dengre, 1986). The alcoholic extract of Tagetes erecta revealed slight antifungal activity against Penicillium chrysogenum, Penicillium notatum, Aspergillus niger ISO-I, Aspergillus niger ISO-II and Mucor species (Nanir and Kadu, 1987). The extract of the aerial parts of Tagetes erecta revealed inhibitory effect against some Gram-positive microorganism's viz. Bacillus subtilis, Micrococcus luteus and Staphylococcus aureus (Penna et al., 1994) while the essential oil of Tagetes erecta exhibited less than 100% toxicity against Aspergillus flavus, Aspergillus niger and Fusarium oxysporum (Mishra et al., 1995). The leaf extract of Tagetes erecta was reported for its antifungal activity against Alternaria alternata, Drechslera halodes and Helminthosporium speciferum (Srivastava and Srivastava, 1998).

Two thiophenes viz. 5-(3-buten-1-ynyl)-2,2'-bithienyl and β-terthienyl from the roots of *Tagetes patula* revealed significant antifungal activity against Candida albicans (Chan et al., 1975). The ethanolic extract of the aerial parts of Tagetes patula exhibited slight antimicrobial activity against Bacillus subtilis, Pseudomonas aeruginosa and Proteus vulgaris. However the extract was ineffective against Staphylococcus aureus, Escherichia coli, Candida albicans and Candida pseudotropicalis (Jawad et al., 1988). B-terthienvl isolated from Tagetes patula revealed significant antifungal activity against five strains of dermatophytes viz. Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton violaceum, Epidermophyton floccosum and Microsporum cookie (Romagnoli et al., 1994). The essential oil of the flowering sprouts of *Tagetes minuta* and *Tagetes patula* exhibited antimicrobial activity against Pseudomonas pyocyan, Escherichia coli K-12, Escherichia coli 6R, Escherichia coli poliresist, Shigella sonnei, Proteus vulgaris XI, Proteus vulgaris K, Staphylococcus aureus 209 P, Staphylococcus aureus Smith, Staphylococcus aureus 53, Bacillus subtilis ATCC 6633, Streptococcus faecilis, Escherichia coli R-15, Escherichia coli R-222, Escherichia coli R-671, Mycobacterium pellegrino, Candida albicans, Candida albicans Japan, Fusarium solani, Ophibolus graminis, Streptococcus heam. Robb, Pneumococcus heam, Streptococcus aegui heam, Streptococcus heam C-145, Streptococcus heam C-1 132/1, Streptococcus heam A-118, Streptococcus heam A-117, Haemophyllus influensae, Saccharomyces cerev FL-599, Saccharomices cerev FL-200, Agrobacterium tumefaciens, Escherichia coli DC-0, Escherichia coli DC-2, Corynebacterium fascians, Xanthomonas vesicolour and Pseudomonas pisi (Hethelyi et al., 1989).

The 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 Trichoderma viride but was found ineffective against Microsporum gypseum (Razdan et al., 1986). The total extract, ethyl acetate and aqueous fractions of the leaves of Tagetes minuta exhibited significant antimicrobial activity against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Stapyhlococcus epidermidis and Pseudomonas aeruginosa while no activity was observed against Lactobacillus rhamnosus, Lactobacillus plantarum, Saccharomyces cerevisiae and Zymomonas mobilis. However, the major component of the extract quercetagetin-7-arabinosyl-galactoside also showed significant antimicrobial activity against Bacillus subtilis, Escherichia coli, Staphylococcus aureus and

Staphylococcus epidermidis (Tereschuk et al., 1997). The essential oil of the leaves and flowers of Tagetes lucida, the tincture prepared from its leaves and flowers and its n-hexane, ethanol and aqueous extracts were tested for inhibitory effect against Vibrio cholerae whereby it was reported that the best vibriocidal activity was exhibited by the n-hexane extract while the essential oil did not show any effect (Caceres et al., 1993a). The ethanolic extract of Tagetes filifolia exhibited antibacterial activity against Staphylococcus aureus, Streptococcus pneumoniae and Streptococcus pyogenes while the ethanol, acetone and n-hexane extracts of Tagetes lucida did not revealed any antibacterial effect (Caceres et al., 1993b). A 0.2% concentration of marigold oil emulsion was reported for its significant fungicidal action against citrus fruit pathogens viz. Penicillium digitatum, Diplodia natalensis, Penicillium italicum and Alternaria tenuis (Arora et al., 1984). Recently, 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 (59) 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 between 12.5-100 μg/mL (Gupta and Vasudeva, 2010).

Insecticidal Activity

The aqueous leaf extract of *Tagetes erecta* exhibited significant nematicidal effect against *Meloidogyne incognita* and *Rotylenchulus reniformis* (Mahmood *et al.*, 1979) and the acetone extract of the whole plant was reported for the growth inhibitory and juvenile hormone mimicking activity against the larvae of *Culex quinquefasciatus* (Saxena *et al.*, 1992). The leaf extract of *Tagetes erecta* further exhibited fatal effects on the growth of blackgram inoculated with *Glomus faciculatum* and *Meloidogyne incognita* (Sankaranarayanan and Sundarababu, 1996) while the dichloromethane and methanolic extracts of the aerial parts of the plant from Argentine were reported for significant insecticidal activity against *Sitophilus oryzae* (Broussalis *et al.*, 1999). 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 petroleum ether extract of the roots of *Tagetes patula* exhibited toxicity against the third stage mosquito larvae of *Culex fatigens* (Singh and Kataria, 1985) while the aqueous and methanolic extracts of leaves, stems and buds of *Tagetes patula* were reported for insecticidal activity against the second stage larvae of *Tylenchulus semipenetrans* and *Anguina tritici* (Kumari *et al.*, 1986). The aqueous extracts of 30 and 60 days old marigold plant (*Tagetes patula*) were reported for inhibiting hatching of *Meloidogne javanica* juveniles (Walia and Gupta, 1997). A new compound (5E)-ocimenone from the fresh leaves and flowers of *Tagetes minuta* exhibited significant larvicidal activity against the third instar mosquito larvae of *Aedes aegypti* (Maradufu *et al.*, 1978) while the aqueous extract exhibited significant nematicidal effect against the root-knot nematode *Meloidogyne incognita* (Singh and Singh, 2002). The essential oils from the fresh and dried plants of *Tagetes minuta* were reported to be highly effective against the larvae of *Anopheles stephensi* with the LC50 values of 1.0532 and 1.0315 mg/L (Hadjiakhoondi *et al.*, 2008).

The essential oils of *Tagetes pusilla* seeds and *Tagetes minuta* aerial parts exhibited significant insecticidal activity against *Aedes aegypti* larvae (Chantraine *et al.*, 1998). The aqueous extracts of flowers, seeds, roots and leaves of *Tagetes lucida* revealed significant nematicidal effect against *Meloidogyne incognita, Rotylenchulus reniformis, 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 (Siddiqui and Alam, 1988). The essential oil of aerial parts of *Tagetes terniflora* showed considerable cytotoxicity against *Artemia salina* with ED50 value of 3.16 mg/mL (De Feo *et al.*, 2005).

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

[14]

[13]

[12]

`OH

[16]

[15]

[56]

15 15' OH

[57]

ester from the roots of the plant exhibited significant schizonticidal activity against chloroquine sensitive and resistant strains of *Plasmodium falciparum* (Gupta and Vasudeva, 2010).

Antioxidant Activity

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).

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).

Miscellaneous

Fifty percent ethanolic extract of the entire plant of *Tagetes minuta* revealed antiviral activity against the Ranikhet disease virus (Bhakuni *et al.*, 1969) while the essential oil of fresh *Tagetes minuta* plant was reported to exhibit significant tranquilizing, hypotensive, bronchodilatory, spasmolytic and anti-inflammatory activities (Chandhoke and Ghatak, 1969). The aqueous extract of the leaves and flowers of *Tagetes lucida* exhibited significant platelet antiaggregant activity (Villar *et al.*, 1997) whereas the marigold paste, tincture and oil from the fresh leaves and flowers of *Tagetes erecta* were found effective in the treatment of hyperkeratotic plantar lesions (Khan *et al.*, 1996). The ethanolic extract of *Tagetes erecta* yellow color petals and leaves, in combination with the protective pad, was found to be effective in the treatment of parakeratosis. The extract also exhibited significant anti-inflammatory activity against carrageenan and dextran-induced acute paw oedema in mice (Khan and Evans, 1996).

Conclusion

Genus *Tagetes* may prove to be a rich source of compounds with possible pharmacological values. Most of the species of *Tagetes viz. Tagetes tenuifolia*, *Tagetes patula* have still remained untouched by the phytochemists and pharmacologists. The ornamental plants are being used as traditional medicine in one or other parts of the world, therefore more attention is required to thoroughly screen this genus for its phytoconstituents and biological activities.

REFERENCES

- 1. Anonymous, (1976). The Wealth of India Raw Materials, Vol. X, pp. 109-112.
- 2. Rydberg, P.A., (1915). Tagetes, North Am. Flora, 34, pp. 148-159.
- 3. Raghava, S.P.S., (1998). Pusa Narangi Gainda and Pusa Basanti Gainda: New Marigolds, *Indian Horticult.*, 43, p. 31.
- 4. Janakiram, T. and Rao, T.M., (1996). Marigold: Tagetes spp., Indian Horticult., 41, cover.
- 5. Kirtikar, K.R. and Basu, B.D., (1975). Indian Medicinal Plants, Vol. II, pp. 1385-1386.
- 6. Rasoanaivo, P., Petitjean, A.M., Ratsimamanga-Urverg, S. and Rakoto-Ratsimamanga, A., (1992). Medicinal plants used to treat malaria in Madagascar, *J. Ethnopharmacol.*, 37, pp. 117-127.
- 7. Ejobi, F., (1997). Local knowledge repels mosquitoes in Kenya, Appropriate Tech., 23, pp. 32-33.
- 8. Laferriere, J.E., Weber, C.W. and Kohlhepp, E.A., (1991). Mineral composition of some traditional Mexican teas, *Plant Foods Human Nut.*, 41, pp. 277-282.
- 9. Linares, E. and Bye, R.A., (1987). A study of four medicinal plant complexes of Mexico and adjacent United States, *J. Ethnopharmacol.*, 19, pp. 153-183.
- 10. Chopra, I.C., Nigam, M.C., Kapoor, L.D. and Handa, K.L., (1963). Indian Tagetes oil, Soap Perfum. Kosmet., 36, pp. 686-689.

11. Johnson, P. and Robertel, P., (1986). Creative contribution of natural substances in present day perfumery, *Perf. Flav.*, 11, pp. 1-8.

- 12. Igolen, G., (1936). Tagetes, Parfums, 14, pp. 6-11.
- 13. Shiva, M.P., Lehri, A. and Shiva, A., (2002). Aromatic and Medicinal Plants, pp. 219-222.
- 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, pp. 153-160.
- 15. Gupta, Y.N. and Bhandari, K.S., (1975). Essential oil from the leaves of Tagetes minuta, Indian Perfum., 19, pp. 29-32.
- 16. Baslas, R.K. and Singh, A.K., (1981a). Chemical examination of essential oil from the leaves of *Tagetes erecta Linn., J. Indian Chem. Soc.*, 58, pp. 104.
- 17. 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, pp. 203-205.
- 18. 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.
- 19. 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
- 20. Handa, K.L., Chopra, M.M. and Nigam, M.C., (1963). The Essential oil of *Tagetes minuta L., Perf. Essent. Oil Rec.*, 54, pp. 372-374.
- 21. Garg, S.N. and Mehta, V.K., (1998). Acyclic monoterpenes from the essential oil of *Tagetes minuta* flowers, *Phytochem.*, 48, pp. 395-396.
- 22. Baslas, R.K. and Singh, A.K., (1981b). Chemical examination of essential oil of *Tagetes minuta Linn.*, *J. Indian Chem. Soc.*, 58, pp. 422-423.
- 23. 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, pp. 1061-1062.
- 24. Lawrence, B.M., Powell, R.H., Smit, T.W. and Kramer, S., (1986). Chemical composition of *Tagetes* oil, *Perfum Flav.*, 10, pp. 56-58
- 25. Razdan, T.K., Wandchoo, R.K. and Dhar, K.L., (1986). Chemical composition and antimicrobial activity of the oil of *Tagetes minuta* Linn., *Parfum. Kosmet.*, 67, pp. 52-55.
- 26. Dhingra, S.N. and Dhingra, D.R., (1956). Essential oil of Tagetes patula, Perf. Essent. Oil Rec., 47, pp. 391-394.
- 27. Baslas, R.K. and Singh, A.K., (1980). Chromatographic separation of essential oil of Tagetes erecta, Indian Perfum., 24, pp. 163-165.
- 28. 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, pp. 145-158.
- 29. 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, pp. 89-105.
- 30. Razdan, T.K., (1984). Colorimetric analysis of the constituents of essential oils, Parfum Kosmet., 65, 190-195.
- 31. 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.
- 32. Turker, A.O. and Maciarello, M.J., (1996). Volatile leaf oil of Tagetes lemonii gray, J. Essent. Oil Res., 8, pp. 417-418.
- 33. Bicchi, C., Fresia, M. and Rubiolo, P., (1997). Constituents of *Tagetes lucida* Cav ssp. *lucida* essential oil, *Flav. Frag.*, 12, pp. 47-52.
- 34. Senatore, F. and De Feo, V., (1999). Chemical composition of the essential oil from *Tagetes mandonii* Sch. Bip. (Asteraceae), *Flav. Fragr. J.*, 14, pp. 32-34.
- 35. De Feo, V., Porta, G.D., Soria, E.U., Soria, R.U. and Senatore, F., (1998). Composition of the essential oil of *Tagetes filifolia*, *Lag.*, *Flav. Fragr. J.*, 13, pp. 145-147.
- 36. 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, pp. 440-444.
- 37. 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, pp. 27-28.
- 38. De Feo, 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.
- 39. 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, pp. 33-39.
- 40. Kaul, P.N., Bhattacharya, A.K., Rao, B.R.R., Syamsundar, K.V. and Ramesh S. (2005). Essential oil composition of *Tagetes minuta* L. fruit, *J. Essent. Oil Res.*, 17, pp. 184-185.
- 41. Downum, K.R. and Towers, G.H.N., (1983). Analysis of thiophenes in the *Tageteae* (asteraceae) by HPLC, *J. Nat. Prod.*, 46, pp. 98-103.
- 42. Flores, H.E., (1992). Plant roots as chemical factories, Chem. Ind., 10, pp. 374-377.
- 43. Tripathi, A.K., Khan, S.A., Vaishnava, M.M. and Gupta, K.R., (1992). Compounds of taxonomic significance in *Tagetes, Fitoterapia*, 63, p. 85.
- 44. Horn, D.H.S. and Lamberton, J.A., (1963). The nematicidal principles of Tagetes roots, Australian J. Chem., 16, pp. 475-479.

45. Vasudeva, N., Gupta, P. and Sharma, S.K., (2007). A new bithienyl constituent from *Tagetes erecta* Linn. Roots, *Indian J. Heterocy. Ch.*, 16, pp. 303-304.

- 46. Das, K.C. and Tripathi, A.K., (1997). 6-hydroxy-kaempferol-7-O-β-D-alloside from Tagetes erecta, Fitoterapia, 68, pp. 477.
- 47. Tripathi, A.K., Paliwal, M.K. and Singh, K.J., (1991). Isorhamnetin-7-O-β-D- galactoside from *Tagetes patula*, *J. Indian Chem. Soc.*, 68, pp. 674.
- 48. D'Agostino, M., De Simone, F., Zhov, Z.L. and Pizza, C., (1992). Flavonol glycosides from *Tagetes elliptica*, *Phytochem.*, 31, pp. 4387-4388.
- 49. De Israilerv, L.R.A., Martinez, A.M.D.P.D. and Seeligmann, P., (1991). Myricetin in *Tagetes*: Chemosystematic significance, *Phytochem.*, 30, pp. 4037-4038.
- 50. El-Emary, N.A. and Ali, A.A., (1983). Revised Phytochemical study of Tagetes erecta, Fitoterapia, 54, pp. 9-12.
- 51. Tripathy, A.K. and Gupta, K.R., (1991). Plant Phenolics of Tagetes erecta, Fitoterapia, 62, pp. 91-92.
- 52. Guinot, P., Gargadennec, A., Valette, G., Fruchier, A. and Andary, C., (2008). Primary flavonoids in marigold dye: extraction, structure and involvement in the dyeing process, *Phytochem. Anal.*, 19, pp. 46-51.
- 53. Alam, A.U., Couch, J.R. and Creger, C.R., (1968). The carotenoids of the marigold, Tagetes erecta, Can. J. Bot., 46, pp. 1539-1541.
- 54. Goodwin, T.W., (1980). Carotenoids in higher plants, The Biochemistry of the Carotenoids, Chapman and Hall Edn., New York.
- 55. Quackenbush, F.W. and Miller, S.L., (1972). Composition and analysis of the carotenoids in the marigold petals, *J. Assoc. Off. Anal. Chem.*, 55, pp. 617-621.
- 56. McGeachin, R.B. and Bailey, C.A., (1995). Determination of carotenoid pigments, retinol and α-tocopherol in feeds, tissues and blood serum by normal phase high performance liquid chromatography, *Poult. Sci.*, 74, pp. 407-411.
- 57. Hadden, W.L., Watkins, R.H., Levy, L.W., Ragalado, E., Rivadeneira, D.M., Van Breemen, R.B. and Schwartz, S.J., (1999). Carotenoid composition of marigold (*Tagetes erecta*) flower extract used as nutritional supplement, *J. Agric. Food Chem.*, 47, pp. 4189-4194.
- 58. Pratheesh, V.B., Benny, N. and Sujatha, C.H., (2009). Isolation, stabilization and characterization of xanthophyll from marigold flower-Tagetes erecta L., Mod. App. Sci., 3, pp. 19-28.
- 59. Grover, G.S. and Rao, J.T., (1978). In vitro antimicrobial studies of the essential oil of Tagetes erecta, Perf. Flav. Int., 3, pp. 28.
- 60. Garg, S.C. and Dengre, S.L., (1986). Antibacterial activity of essential oil of *Tagetes erecta* Linn., *Hind. Antibiot. Bull.*, 28, pp. 27-29.
- 61. Nanir, S.P. and Kadu, B.B., (1987). Effect of some medicinal plant extracts on some fungi, Acta Bot. Ind., 15, pp. 170-175.
- 62. 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, pp. 172-174.
- 63. 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, pp. 48-52.
- 64. Srivastava, A. and Srivastava, M., (1998). Fungitoxic effect of some medicinal plants (on some fruit pathogens), *Philippine J. Sc.*, 127, pp. 181-187.
- 65. Chan, G.F.Q., Neil-Towers, G.H. and Mitchell, J.C., (1975). Ultraviolet-mediated antibiotic activity of thiophene compounds of *Tagetes, Phytochem.*, 14, pp. 2295-2296.
- 66. Jawad, A.M., Jaffer, H.J., Al-Naib, A., Saber, H. and Razzak, A.A.W., (1988). Antimicrobial activity of some Iraqi plants, *Fitoterapia*, 59, pp. 130-133.
- 67. 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.
- 68. Hethelyi, E., Koczka, I. and Tetenyi, P., (1989). Phytochemical and antimicrobial analysis of essential oils, *Herba Hung.*, 28, pp. 99-115.
- 69. Tereschuk, M.L., Riera, M.V.Q., Castro, G.R. and Abdala, L.R., (1997). Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*, *J. Ethnopharmacol.*, 56, pp. 227-232.
- 70. Caceres, A., Figueroa, L., Taracena, A.M. and Samayoa, B., (1993a). Plants used in Guatemala for the treatment of respiratory diseases. 2: Evaluation of activity of 16 plants against Gram-positive bacteria, *J. Ethnopharmacol.*, 39, pp. 77-82.
- 71. Caceres, A., Torres, M.F., Ortiz, S., Cano, F. and Jauregui, E., (1993b). Plants used in Guatemala for the treatment of gastrointestinal disorders. IV. Vibriocidal activity of five American plants used to treat infections, *J. Ethnopharmacol.*, 39, pp. 73-75.
- 72. Arora, R. and Pandey, G.N., (1984). Effect of essential oils on citrus decay pathogens, Biol. Mem., 9, pp. 69-72.
- 73. Gupta, P. and Vasudeva, N., (2010). In vitro antiplasmodial and antimicrobial potential of Tagetes erecta roots, Pharm. Biol., (In Press).
- 74. Mahmood, I., Masood, A., Saxena, S.K. and Husain, S.I., (1979). Effect of some plant extracts on the mortality of *Meloidogyne incognita* and *Rotylenchulus reniformis*, *Acta Bot. Ind.*, 7, pp. 129-132.
- 75. 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, pp. 204-206.
- 76. Sankaranarayanan, C. and Sundarababu, R., (1996). Effect of Leaf extracts on growth of blackgram inoculated with vesicular *Arbuscular mysorrhiza Glomus fasciculatum* and root-knot nematode *Meloidogyne incognita*, *Indian J. Nematol.*, 26, pp. 144-147.
- 77. 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, pp. 219-223.
- 78. 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, pp. 212-217.

79. Singh, R.P. and Kataria, P.K., (1985). Toxicity of some plant extracts to mosquito larvae, *Indian J. Entomol.*, 47, pp. 401-404.

- 80. Kumari, R., Verma, K.K., Dhindsa, K.S. and Bhatti, D.S., (1986). *Datura, Ipomea, Tagetes* and *Lawsonia* as control of *Tylenchulus semipenetrans* and *Anguina tritci. Indian J. Nematol.*, 16, pp. 236-240.
- 81. Walia, K.K. and Gupta, D.C., (1997). Management of root knot nematode, *Meloidogyne Javanica* on vegetable crops with *Tagetes* sp., *Indian J. Nematol.*, 27, pp. 18-23.
- 82. Maradufu, A., Lubeca, R. and Dorn, F., (1978). Isolation of (5E)-ocimenone, a mosquito larvicide form *Tagetes minuta*, *Lloydia*, 41, pp. 181-183.
- 83. Singh, S.P. and Singh, V., (2002). Evaluation of some plant extracts against root-knot nematode, *Meloidogyne incognita*, *Indian J. Nematol.*, 32, pp. 226-227.
- 84. Chantraine, J.M., Laurent, D., Ballivian, C., Saavedra, G., Ibanez, R. and Vilaseca, L.A., (1998). Insecticidal activity of essential oils on *Aedes aegypti* larvae, *Phytother. Res.*, 12, pp. 350-354.
- 85. Siddiqui, A.M. and Alam, M.M., (1988). Toxicity of different plant parts of *Tagetes lucida* to plant parasitic nemotodes, *Indian J. Nematol.*, 18, pp. 181-185.
- Sindhu, E.R. and Kuttan, R., (2007). Antioxidant and anti-inflammatory activity of lutein and its ester, Amala Res. Bull., 27, pp. 261-270.
- 87. 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, pp. 155-160.
- 88. Bhakuni, D.S., Dhar, M.L., Dhar, M.M., Dhawan, B.N. and Mehrotra, B.N., (1969). Screening of Indian plants for biological activity: Part II, *Indian J. Exp. Biol.*, 7, pp. 250-262.
- 89. Chandhoke, N. and Ghatak, B.J.R., (1969). Studies on *Tagetes minuta*: some pharmacological actions of the essential oil, *Indian J. Med. Res.*, 57, pp. 864-876.
- 90. Villar, R., Calleja, J.M., Morates, C. and Caceres, A., (1997). Screening of 17 Guatemalan medicinal plants for platelet antiaggregant activity, *Phytother. Res.*, 11, pp. 441-445.
- 91. Khan, M.T., Potter, M. and Birch, I. (1996). Podiatric treatment of hyperkeratotic plantar lesions with marigold *Tagetes erecta*, *Phytother. Res.*, 10, pp. 211-214.
- 92. Khan, M.T. and Evans, F.J., (1996). Clinical Evaluation of *Tagetes erecta* in the treatment of parakeratosis, *Phytother. Res.*, 10, pp. S186-S188.