Scientific Name

Tagetes patula L.

Synonyms

No synonyms recorded

Family

Asteraceae

Common/English Names

Dwarf French Marigold, Dwarf Marigold, French Marigold, Spreading Marigold, Stinkweed, Wild Marigold

Vernacular Names

Asturian: Claveles Turcos

Brazil: Cravo De Defunto, Cravo-Fétido,

Roso-Do-Bobo

Catalan: Clavell De Moro, Damasquina

Chinese: Kong Que Cao

Cook Islands: Merīkō, Merīkōro, Mērīkōro,

Mērīkoro (Maori)

Croatian: Garofal Žuti, Gromniščica, Fratrići,

Kadifica, Konavljanin, Žutjelj Mali

Czech: Aksamitník Rozkladitý

Danish: Fløjlsblomst, Udbredt Fløjlsblomst,

Udspærret Fløjlsblomst *Eastonian*: Madal Peiulill

Finnish: Ryhmäsamettikukka, Samettikukka

French: Oeillet D'inde

German: Gewöhnliche Samtblume, Studentenblume

Hungarian: Bársonyvirág

India: Genda (Bengali), Guljharo, Makhanala (Gujarati), Gainda, Gaindaa, Genda, Gultera, Taigeteej Petulaa, Taigetiz Petula, Sthulapushpa (Hindi), Chendumalli (Malayalam), Genda, Mentok, Tangla (Punjabi), Ganduga, Sandu, Sthulapushpa, (Sanskrit), Tulukka (Tamil), Bantichettu (Telugu)

Italian: Tagete Commune

Japanese: Koō-Sō *Korean*: Mansugug

Majorcan: Clavell De Moro, Clavell De Mort,

Clavaller De Moro, Pomposas Nepali: Barhamase Sayaptree Norwegian: Fløyelsblom

Polish: Aksamitka Rozpierzchła

Portuguese: Ballutets, Clavell De Moro, Cravo De Defunct, Cravo De Defunto, Cravo De Tunes

Russian: Barchatcy Otklonennye

Slovašcina: Rjavkasta Žametnica, Žametnica,

Žametnica Rjavkasta

Slovencina: Aksamietnica Rozložitá

Spanish: Amapola Amarilla, Canicuba, Clavel De La India, Clavel De Las Indias, Clavel De Muerto, Clavellina Plegada, Clavellina Rizada, Copetes, Copetillo, Copetito, Damasquina, Escopetón, Escopetones, Flores De Muerto

Swedish: Sammetsblomster, Sammetstagetes

Thai: Dao Ruang Lek *Tongaarevan*: Merikō *Turkey*: Kadife Çiçeği

Vietnamese: Cúc Cà Cuống, Vạn Thọ Nhỏ, Cúc

Vạn Thọ Lùn

Origin/Distribution

The species is native to the Americas—Central (Mexico and Guatemala) and southwestern United States (Arizona, New Mexico and southwest Texas). It is cultivated and has naturalized elsewhere.

Agroecology

In its native range, it is found from near sea level up to altitude of 1,350 m. It is frost tender and is quite adaptable to poor soils, heat, humidity and especially drought but not adaptable to shade or water-logged sites. It thrives best in full sun, on well-drained sandy or loamy soils.

Edible Plant Parts and Uses

Flowers and leaves are edible (Kunkel 1984; Facciola 1990). Flowers are used in refreshing drinks and the leaves are used for flavouring food. The dried flowers are used as an adulterant of saffron (*Crocus sativus*) and used for colouring foods yellow. The essential oil is used as a food flavouring, though it is inferior to the oil obtained from *T. minuta* (Bown 1995).

Botany

A small, bushy, erect, branched, glabrous, herbaceous annual, 25–100 cm high with a tap root. Leaves 4–7 cm long, deeply sinuate to the midrib with linear-lanceolate segments with serrated margins (Plates 1, 2, 3, 4 and 5). Capitulum solitary and terminal, 1.5–3 cm across, on 30–15 cm long peduncle. Single flower heads have widely spreading ray florets, but double-flowered head has mounding ray florets in the shape of globular,



Plate 1 Orangey red flowers and leaves



Plate 2 Variegated reddish-brown-yellow flowers and leaves



Plate 3 Yellow flowers and leaves

flabellate button flower heads. Ray florets 5–9 (25+), female, ligulate, flabellate to oval-quadrate, yellow, orange to red or variegated



Plate 4 Orangey flowers and leaves



Plate 5 Yekkow double-flowered globose flowers and leaves

blends of red-brown, yellow/red-brown (Plates 1, 2, 3, 4 and 5). Disc florets numerous, tubular, bisexual. Fruit 6–11 mm black achene, with scaly pappus.

Nutritive/Medicinal Properties

Flower Phytochemicals

Rop et al. (2012) reported that edible flowers of Tagetes patula had a dry matter content (%w/w) of 9.68 %, crude protein of 2.95 g/kg and the following elements (mg/kg fresh mass (FM)): P 478.25 mg, K 3808.72 mg, Ca 346.85 mg, Mg 205.19 mg, Na 114.32 mg, Fe 8.72 mg, Mn 7.86 mg, Cu 1.09 mg, Zn 13.29 mg and Mo 0.37 mg. The flowers had total antioxidant capacity of 6.70 g ascorbic acid equivalents/kg FM, total phenolic content of 4.58 g gallic acid/kg FM and total flavonoid content of 1.90 g rutin/kg FM. The flowers of *T. patula* cultivars were found to be rich in carotenoids (Kishimoto et al. 2007). Carotenoids in orange Tagetes patula flowers were lutein, antheraxanthin, zeaxanthin and violaxanthin. Total carotenoids in Tagetes patula Safari Tangerine (orange flowers) were 2019.6 µg/g FW, in Bonanza orangea (orange flowers) 1957.7 µg/g FW, in Safari Yellow (yellow flowers) 312.3 µg/g FW and in Bonanza yellow 270.3 μg/g FW (Kishimoto et al. 2007). The maximum amount of xanthophylls accumulated in the flowers with orange (or orange with claret spots) petals, where the content of carotenoids (recalculated for lutein) exceeded 5 mg/g of fresh petals against 1 mg/g for yellow and 0.2 mg/g for lemonyellow flowers (Deineka et al. 2007). The Marigold flowers of orange colour with claret spots were characterized by a high content of anthocyanins. The main component was cyanidin-3-glucoside; some samples also contained a significant amount (10–50 %) of another cyanidin derivative identical to that of cyanidin-3glucoside acylated with malonic acid. The main acids of isolated lutein diester were myristic and palmitic acids (accounting for 85–90 % of the total sum of acid radicals); smaller fractions represented stearic and lauric acid radicals.

Lutein (1) and eight lutein esters: (2) lutein monomyristate, (3) lutein monopalmitate, (4) lutein monostearate, (5) unknown, (6) lutein dimyristate, (7) lutein myristate-palmitate, (8) lutein dipalmitate, (9) lutein palmitate-stearate, (10) in two different parts (petals and calyces) of flower heads from different types of Marigold belonging to the species Tagetes patula and T. erecta were evaluated (Piccaglia et al. 1998). Relevant quantitative differences were found among the Marigold types which had a total content of pigments ranging from 17 to 570 mg/100 g in the petals and from 0.4 to 18.6 mg/100 g in the calyces. Marigold (T. patula) flowers were found to be a rich source of carotenoids mainly lutein (Bhattacharyya et al. 2008). Among the solvents methanol showed the highest extractability (52.51 %). Of three varieties (orange, yellow and red) the orange variety contained the maximum amount of lutein 154.96 mg/g of extract. The fatty acid composition of the ester fraction was determined, and saturated fatty acid content was maximum (about 75 %) and unsaturated fatty acid was about 25 %. The lutein ester was also reacted with capric acid (C₁₀) in presence of Mucor miehei immobilized lipase, and about 17.5 % C_{10} fatty acid was incorporated to produce modified lutein for application in various functional foods.

A new acyclic monoterpene glucoside, 2-methyl-6-methylen-2,7-octadiene-1-*O*-β-Dglucopyranoside, and known compounds, helenien, xanthophyll, patuletin and patuletrin, were isolated from *Tagetes patula* flowers (Garg et al. 1999a, b). One triterpene lupeol [lup-20-(2a)-en-3β-ol] and steroids, cholesterol, β-sitosterol $(24-R-stigmast-5-ene-3\beta-ol)$ and stigmasterol [24-(S)-stigmast-5,22E-dien-3 β -ol] were isolated from the flowers (Bano et al. 2002). A tetrahydroβ-carboline alkaloid, (+) jafrine, was isolated from the petroleum ether extract of flowers (Faizi and Naz 2002). The transformation of jafrine as well as 4-*N*-acetyl tetrahydroharmine into 2-acetyl tryptamine derivatives by autoxidation was observed.

The flavonol, patuletin, was isolated from *T. patula* flower petals (Rao and Seshadri 1941).

Flavonoids patulitrin (patuletin 7-*O*-glucoside), patuletin, quercetagetin, quercetagetin 7-*O*-glucoside, luteolin (Bhardwaj et al. 1980a), allopatuletin

(3,6,7,3',4'-pentahydroxy-5-methoxyflavone) a quercetagetin monomethyl ether (Bhardwaj et al. 1980a, b), and kaempferol) and quercetin-like structures (Ivancheva and Zdravoka 1993) (patulitrin (patuletin 7-O-glucoside), patuletin, quercetagetin 7-O-glucoside) and several unknown minor flavonoids (Guinot et al. 2008) (pateletin, patulitrin, patuletin cinnamate derivative, patuletin benzoate derivative (Faizi et al. 2008)) were isolated from the flowers. From the polar extract and fractions of yellow flowers of Tagetes patula, phenolic compounds (flavonoids and phenolic acids) were isolated (Faizi et al. 2011b). In the nonpolar extract, a few fatty acids, their methyl esters and thiophenes (including α -terthienyl) were detected. Patuletin, patulitrin and methyl protocatechuate were isolated from the flowers (Faizi et al. 2011a).

Thiophene derivatives were found in *T. patula* cv. Carmen flowers in solvent distillate (SE): 5-(3-buten-1-ynyl)-2,2′-bithienyl (BBT) (3.6 %); 5′-methyl-5-(3-buten-1-ynyl)-2,2′-bithienyl (MeBBT) (nd=not detected); 5-(1-pentynyl)-2,2′-bithienyl (PBT) (50 %); 5-(4-hydroxy-1-butynyl)-2,2′-bithienyl (BBTOH) (nd); 2.2′,5′,2″-terthienyl (α-T) (34.8 %); 5-(4-acetoxy-butynyl)-2,2′-bithienyl (BBTOAc) (11.6 %); 5-methylaceto-5′-(3-buten-1-ynyl)-2,2′-bithienyl (AcOCH2BBT) (nd); and 5-(3,4-diacetoxy-1-butynyl)-2,2′-bithienyl (BBT(OAc)₂) (nd) (Margl et al. 2002).

Of the 22 constituents identified in the hydrodistilled oil of T. patula capitula, grown in Lucknow, 9 were monoterpene hydrocarbons (49.4 %), 3 sesquiterpene hydrocarbons (8.5 %) and 10 oxygenated monoterpenes (32.0 %) (Garg et al. 1999a, b). The major constituents were limonene (24.5 %), terpinolene (12.1 %), (Z)- β ocimene (10.4 %) and (E)- and (Z)-tagetone (9.3 %). Krishna et al. (2002) found the main constituents of the hydrodistilled oil of the capitula were (Z)- β -ocimene (19.9 %), (Z)-tagetenone (12.4 %), (E)-tagetenone (10.4 %), piperitenone (5.8 %) and β -caryophyllene (15.1 %). In the essential oil of T. patula flowers grown in Venezuela, 21 compounds were identified, and α-terthienyl (43.1 %), pentatriacontane (23.9 %) and 2-ethyl-l-dodecanol (7.9 %) were the major constituents (Martínez et al. 2009).

Flowers had significantly less volatile oil and hairy root cultures produced significantly more volatile oil than intact roots (Szarka et al. 2006). The capitula oil was rich in sesquiterpene β-caryophyllene (53.5 %); other sesquiterpenes included caryophyllene oxide (4.1 β-cubebene (3.5 %), spathulenol (0.9 %), traces of (E)- β -farnesene and α -humulene; monoterpenes (z)-tagetenone (4.2 %), (Z)- β -ocimene (3.8 %), terpinolene (3.3 %), (E)-tagetenone (1.9 %), piperitenone (1.6 %), piperitone (0.7 %), (Z)-tagetone (0.6 %), (E)-tagetone (0.6 %), piperitenone oxide(0.3 %) and limonene trace; and thiophenes 5-(3-penten-1-ynyl)-2,2'-bithienyl (PBT) (5.1 %), α -terthienyl (2.4 %) and BBT (0.8 %) BBTOAc (trace). Thirty compounds were identified in the capitula essential oil, representing 89.1 % of the total detected (Romagnoli et al. 2005). The main components were piperitone (24.74 %), piperitenone (22.93 %), terpinolene (7.8 %), dihydrotagetone (4.91 %), cis-tagetone (4.62 %), limonene (4.52 %) and allo-ocimene (3.66 %). Other minor compounds included α-terpineol (2.46 %), caryophyllene (2.09 %), *cis*-ocimene (1.8 %), 4-terpineol (1.4 %), 2-phenyl ethyl acetate (1.29 %), transtagetone (1.13 %), linalool (0.7 %), caryophyllene oxide (0.67 %), trans-ocimene (0.61 %), sabinene (0.43 %), 3-hexen-1-ol (0.4 %), spathulenol (0.39 %), isoborneol (0.35 %), germacrene D (0.34 %), trans-nerolidol (0.32 %), δ -cadinene (0.31 %), bornyl acetate (0.22 %), p-cymene (0.22 %), β -bisabolene (0.2 %), α -pinene (0.18 %), α -phellandrene (0.15 %), β -mircene (0.13 %) and β -copaene (0.13 %). In another study, leaf oil were richer in monoterpenes (terpinolene (21.1 %), piperitone (11.2 %), (E)-tagetone (9.5 %), (Z)- β -ocimene (6.9 %), limonene (5.9 %), (E)-tagetenone (5.8 %), piperitone (4.6%), (Z)-tagetone (4.4%),(z)-tagetenone (4.1 %) and piperitenone oxide (2.3 %)), sesquiterpenes (β-caryophyllene (3.5 %), β-cubebene (1.5 %), spathulenol (0.8 %) and caryophyllene oxide (0.7 %)) and thiophenes (α -terthienyl (trace), BBT (trace) and BBTOAc (trace)) (Szarka et al. 2006). In a recent study, the major compounds identified in the essential oil from T. patula capitula were (Z)- β -ocimene, (E)- β ocimene, terpinolene, (Z)-ocimenone, (E)-ocimenone

and δ -elemene (Prakash et al. 2012). The SPME-GC-FID analyses of live capitula showed that the volatiles were rich in monoterpenoids in comparison to the plucked capitula. In contrast, the plucked capitula recorded significant increase in sesquiterpenoids in comparison to the living capitula. *T. patula* inflorescence oil was composed mainly of β -caryophyllene (23.7 %), terpinolene (15.6 %) and *cis*- β -ocimene (15.5 %) (Armas et al. 2012).

Fruit/Seed Phytochemicals

Thiophene derivatives found in *T. patula* cv. Carmen fruits (achenes) in solvent extract comprised traces of α -T and BBTOAc (Margl et al. 2002). Phytomelanin is found in the hard, black, resistant layer in the pericarp (Pandey 1998).

Forty constituents were identified in Tagetes patula seed oil, comprising 94 % of the total oil (Hassanpouraghdam et al. 2011). Sesquiterpene hydrocarbons (52.7 %) and oxygenated sesquiterpenes (15.8 %) were the main subclasses of volatile oil components followed by monoterpene hydrocarbons (12.6 %). The principal constituents were (E)-caryophyllene (44.6 %), caryophyllene oxide (14.8 %), germacrene D (3.8 %), (Z)- β -ocimene (3.8 %) and limonene (3.7 %). 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.

Leaf/Stem/Aerial Part/Seedling Phytochemicals

An enzyme with high substrate specificity and MW of 67,000, namely, 5-(4-acetoxy-1-butinyl)-2,2'-bithiophene, acetate esterase, was partly purified from the aerial parts of *Tagetes patula* (Sütfeld and Towers 1982); 3,4-diacetoxy-butinylbithiophene was also detected (Pensl and Sűtfeld 1985). Thiophene derivatives were found in *T. patula* cv. Carmen shoots: (3-buten-1-ynyl)-2,2-bithie-nyl (BBT), trace from steam distillate

(SD) and 7.9 % from solvent extract (SE)); 5'-methyl-5-(3-buten-1-ynyl)-2,2'-bithienyl (MeBBT), (nd SE, trace SD; 5-(1-pentynyl)-2,2'-bithienyl (PBT) (13.7 SE; 46.4 % SD); 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH) (nd SE; nd SD); 2,2',5,2"-terthienyl (alpha-T) (nd% SE, 31.8 % SD); 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc) (12 %SE, 11.6 % SD); 5-methylaceto-5'-(3-buten-1-ynyl)-2,2'-bithienyl (AcOCH₂BBT) (tr SE; nd SD); 5-(3,4-diacetoxy-1-butynyl)-2,2'-bithienyl (BBT(OAc)₂ (nd SE, nd SD) (Margl et al. 2002). A benzofuran, isoeuparin, was isolated from the seedling (Burke et al. 1986).

T. patula seedlings were found to contain four thiophene derivatives: bithienylbutinen, acetoxybutinylbithiophene, hydroxybutinylbithiophene and a-terthiophene in high concentrations (Bohlmann and Zdero 1979; Sütfeld and Towers 1982; Sütfeld 1982). In T. patula seedling, bithienylbutinen, the major thiophene compound in hypocotyls and roots, was found to accumulate earlier than the other thiophene derivatives; acetoxybutinylbithiophene was not found in cotyledons and its synthesis appeared to be light induced, while hydroxybutinylbithiophene was synthesized specifically in the roots, α-terthiophene in the cotyledons (Sütfeld 1982).

The essential oil of T. patula contained equal amounts of (Z)- and (E)-ocimenone and also contained limonene and β -caryophyllene (Héthélyi et al. 1986). The hydrodistilled oil of the leaves contained limonene (6.5%), terpinolene (16.2%), (Z)-tagetone (13.0%), (Z)-tagetenone (5.5%), (E)-tagetenone (8.2%), piperitone (10.2%) and piperitenone (12.5%) as the main constituents (Krishna et al. 2002). The oil of the shoots was found to have limonene (6.8%), (Z)-beta-ocimene (13.7%), terpinolene (12.0%), piperitone (5.8%) and beta-caryophyllene (10.5%) as the major constituents (Krishna et al. 2002).

The major constituents of the essential oil of the aerial parts were piperitone (33.77 %), *trans*- β -ocimene (14.83 %), terpinolene (13.87 %), β -caryophyllene (9.56 %) and limonene (7.78 %) (Rondon et al. 2006). Minor components included *cis*- β -ocimene (5.01 %), epoxy-ocimene (2.27 %), β -farnesene (1.48 %), germacrene D (1.45 %), bicyclogermacrene (1.52 %), linalool

(0.73 %) and myrcene (0.53 %). The leaf oil was found to have a high content of terpinolene (21.1 %) (Szarka et al. 2006). The following compounds were found in the essential oil of T. patula leaves and stems: limonene (37.05 %), terpinolene (32.6 %), piperitone (14.40 %), neophytadiene (5.91 %), sabinene (2.88 %), trans-ocimene (2.02)%), β-caryophyllene (1.98 %), farnesol (1.84 %) and α -pinene (1.30 %) (Restello et al. 2009). T. patula leaf oil showed terpinolene (20.9 %) and piperitenone (14.0 %) as main components (Armas et al. 2012).

Callus cultures of *T. patula* were found to contain thiophene-biocides, mainly 5-(but-3-en-1-ynyl)-2,2'-bithiophene (BBT)and5-(4-acetoxy-1-butynyl)-2,2'-bithiophene (BBTOAc) (Ketel 1988). Secondary calli contained about three times higher concentrations of thiophenes than tertiary calli. The occurrence of the major thiophenes (BBTOH, BBT, BBTOAc, α -T) in leaves and roots of *T. patula* (Breteler and Ketel 1993) corresponded with the data of Sütfeld (1982).

Root Phytochemicals

Two benzofurans, 4-hydroxydehydrotremetone and hydroxytremetone, were isolated from roots and hypocotyls of seedlings (Sütfeld et al. 1985). Bithiophenes 5-(4-acetoxy-1-butenyl)-2,2'bithiophene and 5-(buten-1-nyl)-2,2'-bithiophene and a benzofuran isoeuparin (5-acetyl-4-hydroxy-2-isopropenylbenzofuran) were isolated from root cultures (Parodi et al. 1988). The benzofurans, isoeuparin and (-)-4-hydroxytremetone were isolated from *T. patula* root cultures (Margl et al. 2005). The benzenoid ring and the acetoxy group were found to be predominantly (>98 %) derived from phenylalanine via 1-deoxy-Dxylulose 5-phosphate pathway. The data indicated that isoeuparin and (–)-4-hydroxytremetone were assembled from 4-hydroxyacetophenone and dimethylallyl diphosphate via prenyl-substituted 4-hydroxyacetophenone and dihydrobenzofurans as intermediates. Root oils were rich in thiophenes (BBT (44 %), α-terthienyl (21.8 %), PBT (0.3 %), BBTOAc (6.1 %)), sesquiterpenes $(\alpha$ -gurjunene (4.3%), (E)- β -farnesene (3.9%),

β-caryophyllene (0.7 %), β-cubebene (0.6 %)) and monoterpene (piperitenone (0.2 %)) (Szarka et al. 2006).

 α -Terthienyl was isolated from hairy roots induced by infection with *Agrobacterium rhizogenes* (Kyo et al. 1990). Depending on the hairy root line used, the level of α -terthienyl varied from 15 to 1,268 µg/g dry weight, a level that corresponded to 0.15–12.7-fold that in intact roots. Opines were also detected.

From hairy root cultures, four bithiophenes (5-(3-buten-1-ynyl)-2,2'-bithienyl 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH), 5-(4-acetoxy-butynyl)-2,2'-bithienyl (BBTOAc) and 5-(3,4-diacetoxy-1-butynyl)-2,2'-bithienyl (BBT(OAc)2); stigmasterol, β-farnesene) and 3 benzofurans (dehydrotremetone, 14-isobutyryloxyeuparin and 2,3,-dihydro-14-isobutyryloxyeuparin) isolated were (Menelaou et al. 1991). Thiophene production in hairy root cultures of *Tagetes patula* was found to increase with the addition of an elicitor derived from mycelial extracts of Fusarium conglutinans (Mukundan and Hjortso 1990). The major thiophenes produced were 5-(4-aceoxy-1-butenyl)-2,2'-bithiophene and 5-(buten-1-enyl)-2,2'bithiophene. Rajasekaran et al. (1999) found that treatment of cultured hairy roots with mycelial extract of Aspergillus niger (1.5 % v/v) elicited an increase in thiophene content by 1.6-folds over the control. Maximum production of thiophene was recorded at the end of the fourth week in culture with a content of 0.138 % (w/w on dry weight basis). α -Terthienyl was predominant.

Thiophene derivatives were found in *T. patula* cv. Carmen roots: BBT (65 % from steam distillate (SD) and 93.2 % from solvent extract (SE)); PBT (trace SE; nd SD); BBTOH (trace SE; nd SD); α-T (8.5 % SE, 5.2 % SD); BBTOAc (24.7 %SE, 1.6 % SD); AcOCH₂ BBT (tr SE; nd SD); and BBT(OAc)₂ (tr SE, nd SD) (Margl et al. 2002). Two thiophenes, 5'-hydroxymethyl-5-(3-butene-1-ynyl)-2,2'-bithiophene and methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2'-bithiophene(2), were isolated from the roots (Bano et al. 2002).

Roots of *Tagetes* species (*T. erecta*, *T. filifolia*, *T. lucida*, *T. minuta*, *T. patula* and *T. tenuifolia*) have the highest diversity and contents of thio-

phenes (from 64 to 100 % of the total thiophene amount), with 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) as the main component followed by 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc), 2,2':5',2"-terthienyl (alpha-T) and 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH) (Marotti et al. 2010). The SFE (supercritical fluid CO₂) extraction of intact roots and flowers yielded 717 and 480 $\mu g/g$ 2,2':5',2"-terthiophene (α -T), respectively, while the leaves did not contain considerable amounts of thiophenes (Szarka et al. 2010). Remarkable amounts of thiophene metabolites, 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) 5-(3,4-diacetoxy-1-butynyl)-2,2'-bithienyl and [BBT(OAc)2], were characteristic of the SFE of hairy root cultures. Other compounds identified in the roots included 5-(4-hydroxy-1-butynyl)-2,2'bithienyl (BBTOH), 2,2':5',2'-terthiophene (α -T), 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc), α-gurjunene (4.3 %), (E)-β-farnesene (3.9 %), β-caryophyllene, β-bisabolene, caryophyllene alcohol, methyl palmitate, palmitic acid and linoleic acid.

Four thiophenes (α -terthienyl (α -T), BBT, BBTOH, BBTOAc) and two benzofurans (euparin and 6-hydroxy-2-isopropenyl-5-acetyl, cumaranon (dihydro-euparin)) were identified in the root exudates collected from the undisturbed rhizosphere of Marigold (Tagetes patula) (Tang et al. 1987). Based on the total ion current chromatograms, the ratios of thiophenes in the rhizosphere were 20:25:12:1 for BBT, BBTOH, BBTOAc and α -T, respectively. These ratios were different from those obtained from the ethyl acetate extract of the roots, in which the ratios were 12:0.2:8:1. Dihydroeuparin had been isolated as a major benzofuran from T. patula by Bohlmann and Zdero (1979). Citric and malic acids, pyridine hydrochloride and 2-hydroxy, 5-hydroxymethyl furan, were isolated from the methanol root extract (Saleem et al. 2004).

The biosynthesis of 5-(3-buten-1-ynyl)-2,2′-bithiophene was studied in root cultures of *Tagetes patula* (Margl et al. 2001). Their data confirmed the biosynthetic route was via progressive desaturation of long-chain fatty acids precursors to C_{18} polyacetylenes and the incorporation of a suitable S-source into pentaynene (CI3) as proposed by Jente et al. (1981) who used

tritiated precursors given to soil-grown *Tagetes* patula. However, Margl et al. (2001) stated that a polyketide-like biosynthesis via a carbocyclic intermediate cannot be excluded. The monothiophene 2-(but-3-en-l-ynyl)-5-(penta-1,3-diynyl)thiophene, present in small amounts in *Tagetes* patula hairy roots, was found to be the precursor of all bithienyls that had been described for this species but not of α -terthienyl (Arroo et al. 1995). They found that 5'-methyl-5-(3-buten-lynyl)-2,2'-bithienyl was converted into (5'-but-3-en-l-ynyl-[2,2']bithiophenyl-5-yl)-methyl acetate, probably via (5'-but-3-en-l-ynyl-[2,2'] bithiophenyl-5-yl)-methanol. Substitution of the butenyl side chain of 5-(3-buten-1-ynyl)-2-2'bithienyl resulted in the formation 5-(3,4-dihydroxy-l-butynyl)-2,2'-bithienyl and 5-(4-hydroxy-l-butynyl)-2,2'-bithienyl, were subsequently converted into, respectively, 5-(3,4-diacetoxy-l-butynyl)-2,2'-bithienyl 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl. The end products of this biosynthetic pathway were all bithienyl-acetate esters.

Antioxidant Activity

Of three Indian Marigold (*Tagetes patula*) cultivars, the Marigold orange (MGO) variety was found to contain the maximum amount of lutein (Bhattacharyya et al. 2010). It also had the highest DPPH radical scavenging activity and ABTS radical scavenging activity, with an EC₅₀ value of 0.344 mg/ml. It was also the most effective against lipid peroxidation and hydroxyl radical scavenging activities. The MGO extract also had the maximum reducing power. Hepatic cell damage in iron-mediated Fenton reaction caused by free radicals was reduced by the Marigold extracts. The results suggested that Marigold flowers of Indian variety can be effectively utilized to produce lutein ester, which could be used as a food supplement or as an accessible source of natural antioxidants. Polar extracts, fractions and phases of the flowers demonstrated better antioxidant activity (Faizi et al. 2011a). Of the isolated constituents, methyl protocatechuate showed IC₅₀ value of 2.8 μg/ml, whereas patuletin (IC₅₀=4.3 μ g/ml) was comparable to quercetin and rutin but significantly better than patulitrin ($IC_{50}=10.17 \mu g/ml$). Toxicity test for the methanol extract and compound 2 did not elicit any behavioural changes or cause mortality in mice.

Antimicrobial Activity

Alpha-terthienyl (αT), a thiophene compound isolated from Tagetes patula (Asteraceae), exhibited antifungal activity towards five strains of dermatophytes (Trichophyton mentagrophytes, T. rubrum, T. violaceum, Epidermophyton floccosum, Microsporum cookei) (Romagnoli et al. 1994). α-T plus UVA irradiation for 90 minutes acted as a fungistatic at concentrations between 6 and 24 µM. Between 1 and 10 days after irradiation, the fungal growth was reduced or arrested with marked responses for T. mentagrophytes, T. rubrum and M. cookei. After UVA irradiation the photoactive compound caused damage to membranes of the nucleus, mitochondria and endoplasmic reticulum. Plasmolytic and autolytic changes resulted in plasma membrane breakage and in cell wall aberrations. 5-(4-hydroxy-1butynyl)-2,2'-bithienyl (BBTOH), the thiophene from the roots, was found to be biocidal to Fusarium oxysporum (Arroo et al. 1995).

The essential oil of the aerial parts of *Tagetes* patula showed strong antibacterial activity against important human pathogenic with MIC values for Staphylococcus aureus (30 µg/ml), Enterococcus faecalis (30 µg/ml), Escherichia coli (60 μg/ml), Klebsiella pneumoniae (90 μg/ ml) and Pseudomonas aeruginosa (130 µg/ml) (Rondon et al. 2006). The hot aqueous extracts of Tagetes patula exerted higher antibacterial activity as compare to cold aqueous extract and methanol extract at 40 mg/ml concentration (Jain et al. 2012). It was highly inhibitory towards *Proteus* vulgaris, moderately towards Aeromonas sobria, Aeromonas hydrophila, Staphylococcus aureus (MTCC7405) and *Bacillus subtilis* but least so against Staphylococcus aureus (clinical isolate). Minimal inhibitory concentrations (MICs) were between concentrations of 20-160 mg/ml with both aqueous or methanol extracts. The methanol flower was found to possess antimicrobial activity, and its principal flavonoid patuletin was isolated as the active antibacterial principle with minimum inhibitory concentration (MIC) value of 12.5 µg/disc against *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Micrococcus luteus* (Faizi et al. 2008). Its glucoside, patulitrin (4), was found to be weakly active, except against *Staphylococcus saprophyticus*, *Streptococcus faecalis* and *Streptococcus pyogenes*. The patuletin cinnamate derivative displayed antibacterial activity comparable with the parent flavonoid with a MIC value of 50 µg/disc against *Corynebacterium* spp., whereas patuletin benzoate derivative was found to be devoid of any antibacterial activity.

Two intensely mauve UV fluorescent compounds 5-(3-buten-1-ynyl)-2,2′-bithienyl and α-terthienyl isolated from *Tagetes patula* roots were found to be phototoxic to *Candida albicans* (Chan et al. 1975). The UV-mediated antibiotic activity of polyacetylenes and their thiophene derivatives from *Tagetes* species with their phototoxic effects on *Candida albicans* and certain pathogenic microorganisms and their apparent lack of phototoxicity towards the skin (except for alpha-terthienyl) suggested a potential topical therapeutic role for them in yeast, fungal and bacterial infections and light-responsive dermatoses (Towers et al. 1979).

Antiinflammatory Activity

The methanol extract of *Tagetes patula* florets inhibited acute and chronic inflammation in mice and rats when administered orally (Kasahara et al. 2002). The extract significantly suppressed hindpaw oedema induced by gamma-carrageenan in mice. Further, the extract not only inhibited the hind-paw oedema induced by various acute phlogogens, such as histamine, serotonin, bradykinin and prostaglandin E1, but also suppressed the increase of vascular permeability by acetic acid, indicating that it primarily acted at the exudative stage of inflammation. In the chronic inflammation model, the extract did not inhibit the proliferation of granulation tissue when tested by the cotton pellet method; however, it was effective on the development of adjuvant arthritis in rats.

Podiatric Therapeutic Activity

In a double-blind placebo-controlled trial carried out for 8 weeks, involving patients with bilateral hallux abducto valgus and its associated condition, bunion, administration of *T. patula* preparations, plus protective pad, was effective in reducing the width of the lesion and level of pain of hallux abducto valgus (Khan 1996).

Analgesic Activity

Patuletin, isolated from the flower, demonstrated mild analysic activity in the acetic acid-induced writhing test and hot-plate test in mice (Faizi et al. 2011a).

Hypotensive Activity

Citric and malic acids isolated from the methanol root extract caused 71 and 43 % fall in mean arterial blood pressure (MABP) of rats at the doses of 15 and 30 mg/kg, respectively (Saleem et al. 2004). LD₅₀ and LD₁₀₀ of citric acid had been determined as 545 and 1,000 mg/kg, respectively.

Hypertensive Activity

Pyridine hydrochloride isolated from the methanol root extract produced 34 % rise in the mean arterial blood pressure of rats at the dose of 30 mg/kg (Saleem et al. 2004).

Anticataract Activity

Patulin extracted from *Tagetes patula* inhibited aldose reductase of Wistar rat lens by 100 % at 10^{-1} M, 780.0 % at 10^{-5} M and 22.7 % at 10^{-6} M (Li et al. 1991).

Acaricidal Activity

A 70 % ethanol extract from aerial parts of *T. patula* exhibited acaricidal activity against

Rhipicephalus sanguineus, the thick involved in the transmission of pathogens such as *Babesia canis*, *Ehrlichia canis*, *Coxiella burnetii*, *Rickettsia rickettsii* and *Rickettsia conorii* (Politi et al. 2012). At 50.0 mg/ml, the extract decreased oviposition rate by 21.5 % and eliminated 99.78 % of the larvae. Also it was determined that the best results were obtained with 5 minutes of immersion.

Larvicidal Activity

The thiophenes produced in callus cultures of *T. patula* showed larvicidal effect against mosquito larvae (Rajasekaran et al. 2003). *Tagetes patula* essential oil exhibited larvicidal activity against the fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Dharmagadda et al. 2005). *A. aegypti* (LC₅₀ 13.57, LC₉₀ 37.91) was most susceptible followed by *An. stephensi* (LC₅₀ 12.08, LC₉₀ 57.62) and *C. quinquefasciatus* (LC₅₀ 22.33, LC₉₀ 71.89).

Toxicity Studies

The essential oils of *Tagetes* flowers were tested for their toxicological potential on mice; the results showed that the oils had a low toxicity with a TD_{50} 99.6 mg/kg for *T. erecta* and 112 mg/kg for *T. patula* (Martínez et al. 2009).

Allergic Dermatitis Problem

Topically applied alpha-terthienyl evoked biphasic phototoxic dermatitis and the appearance of 'sunburn' cells in human epidermis (Towers et al. 1979). None of 11 polyacetylenes had the same effect although they mimicked alpha-terthienyl in their phototoxic effects on *Candida albicans* and certain pathogenic microorganisms. Bilsland and Strong (1990) reported a case of allergic contact dermatitis from the essential oil of French Marigold (*Tagetes patula*) in an aromatherapist.

Traditional Medicinal Uses

The whole herb is aromatic, digestive, diuretic and sedative (Bown 1995). It is used internally in the treatment of indigestion, colic, severe constipation and to treat sore eyes.

In the Philippines, a flower decoction is taken internally as a carminative and used as a refreshing drink (Guerrero 1921). In Argentina, the plant extract is taken internally as a calmative and diuretic; a plant decoction is taken internally as a stimulant and stomachic (stomach strengthener) (Neher 1968). In Columbia and in Venezuela, the plant infusion is used as a bath or rub for rheumatism (Neher 1968).

Other Uses

T. patula is a popular ornamental plant especially in temperate and subtemperate areas around the world. In Argentina, the plant is used as a forage crop for sheep and goat but not cattle (Neher 1968). T. patula produces a significant amount of essential oil, which has characteristic antibacterial, antifungal and insecticide effects (Szarka et al. 2006). The 'Genda Attar' perfume has a beautiful and refreshing fragrance and is made from Marigold essential oil and sandalwood oil. Marigold flower garlands are used for adornment during Indian marriage ceremonies and on other special ceremonies.

Studies showed that *T. patula* essential oil (aerial parts) at concentration of 10 µl effectively control the stored grain pest, Sitophilus zeamais adults (Restello et al. 2009). The capitula essential oil exerted a good antifungal activity against two phytopathogenic fungi, Botrytis cinerea and Penicillium digitatum, providing complete growth inhibition at 10 and 1.25 ul/ml, respectively (Romagnoli et al. 2005). The two main compounds, piperitone and piperitenone, caused large alterations in hyphal morphology and a multisite mechanism of action. Methanol Tagetes patula plant extract exhibited antifungal activity against three phytopathogenic fungi: Botrytis cinerea, Fusarium moniliforme and Pythium ultimum (Mares et al. 2002, 2004). The extract proved to

have a dose-dependent activity on all the fungi with a marked difference between treatments in the light than in the dark. The presence of a luminous source enhanced the antifungal activity, with small differences between UVA and solar spectrum light. *Tagetes patula* extract induced alterations on *Pythium ultimum* cell membranes.

The plant contains polyacetylenic thiophenes in their roots that confer strong biocidal activity, useful for suppressing nematode populations in the soil and as sources of safe and natural pesticides (Marotti et al. 2010). T. erecta and T. patula were employed as a cover crop in Indian tea plantations to suppress nematodes (Neher 1968). Greenhouse studies by Ploeg (1999) demonstrated that preplanting of Marigold cultivars of Tagetes patula, T. erecta, T. signata and a Tagetes hybrid all reduced galling and numbers of second-stage juveniles of Meloidogyne incognita, M. javanica, M. arenaria and M. hapla in subsequent tomato plantings. Field studies found that T. patula diminished Pratylenchus penetrans populations in strawberries (Evenhuis et al. 2004). The effect of T. patula on P. penetrans population densities lasted longer than the effect of chemical soil fumigation. Strawberries were grown for 3 consecutive years after T. patula without damage by the root lesion nematode.

The polar extract and fractions of yellow flowers of Tagetes patula showing nematicidal activity against cyst nematode *Heterodera zeae* led to the isolation of phenolic compounds (flavonoids and phenolic acids). In the nonpolar extract, a few fatty acids, their methyl esters and thiophenes (including α -terthienyl) were detected. In studies of compounds obtained commercially, α-terthienyl and gallic and linoleic acids showed 100 % mortality at concentrations of 0.125 % after 24 hours. N-hexane extract of the roots showed nematicidal activity against *Pratylenchus* penetrans which was due predominantly to α -terthienyl (Kyo et al. 1990). Studies found that population levels of root-lesion nematode (Pratylenchus penetrans) were consistently lower under Marigolds (Tagetes tenuifolia cv. Nemakill and cv. Nemanon, T. patula ssp. nana unidentified cv. and *T. erecta* cv. Crackerjack) compared to the other cover crops tested (Kimpinski et al. 2000). Correspondingly, average potato tuber yields were significantly higher (8–14 %) when potato followed Marigolds.

Comments

Cytological and morphological evidence indicated that $Tagetes\ patula\ L$. to be an allotetraploid species (2n=48) which probably originated by hybridization between the diploids $T.\ erecta$ L. and $T.\ tenuifolia$ Cav., or species closely related to them (Towner 1961).

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