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RESEARCH ARTICLE

Antioxidant and cytotoxic potential of a new thienyl derivative from *Tagetes erecta* roots

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Abstract

Context: The search for newer compounds against pathogenic species continues unabated due to drug resistance. Traditionally, *Tagetes erecta* Linn. (Compositae) has been used for the treatment of various parasitic and microbial diseases.

Objective: To evaluate the antioxidant activity of the ethanol extract of *Tagetes erecta* roots and its cytotoxicity against prostate and HeLa cancer cell lines followed by activity-guided isolation.

Materials and Methods: The antioxidant screening was carried out using diphenylpicrylhydrazyl (DPPH) radical scavenging assay with serial concentrations ranging from 2 to 100 µg/mL, and cytotoxicity was evaluated against prostate (PC-3) and HeLa cell lines using microculture tetrazolium test (MTT) assay with concentrations ranging from 500 to 1.89 µg/mL. Isolation of the ethanol extract was carried out using column chromatography whereby 21 isolates were obtained (T₁-T₂₁), and the most active isolate was subjected for characterization using ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectroscopic techniques.

Results: The ethanol extract scavenged DPPH free radicals thereby exhibiting antioxidant activity with an IC₅₀ of 35.9 µg/mL. In addition, the extract conferred noticeable cytotoxicity against the HeLa (LD₅₀ of 164.28 µg/mL) and PC-3 cell lines (LD₅₀ of 407.3 µg/mL). Among all the isolates, T₃ showed antioxidant activity with IC₅₀ of 11.56 µg/mL and cytotoxicity with LD₅₀ of 12.5 µg/mL against HeLa and 30.25 µg/mL against PC-3 cell lines and was characterized as 2-ethynyl-5-(thiophen-2-yl) thiophene.

Discussion: The new thienyl compound (T₃) exhibited profound antioxidant activity and cytotoxicity at relatively lower concentrations than the extract.

Conclusion: The observations provide support for the ethnobotanical use of the plant.

Keywords: *Tagetes erecta*, marigold, Compositae, thiophenyl, cell lines, radical scavenging

Introduction

Cultivating ornamental plants, once considered to be gardener's activity, has now become a commercial venture throughout the world. The cultivation of such plants even as a hobby may yield a lucrative business if done with a little care. The plants for decoration, termed as ornamentals, constitute a major component of the floriculture

industry, and although India is very rich in ornamental flora, yet there arise a need for further exploration of the untapped wild potential ornamental plants. Marigold is a potential ornamental plant grown commercially in different parts of the world. Two widely cultivated marigolds from the Compositae family, the African (*Tagetes erecta* Linn.) and the French (*Tagetes patula* Linn.) are native

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to Mexico and South America (Kaplan, 1960). In India, these were introduced by the Portuguese. 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 (Raghava, 1998). The name *Tagetes* was given after Tages, a demigod, known for his beauty. There are about 33 species of the genus *Tagetes* (Rydberg, 1915). Five species have been introduced into the Indian gardens viz. *Tagetes erecta* Linn. (Aztec or African marigold), *Tagetes minuta* Linn. (*Tagetes glandulifera* Schrank), *Tagetes patula* Linn. (French marigold), *Tagetes lucida* Cav. (sweet-scented marigold), and *Tagetes tenuifolia* Cav. (striped marigold).

Thiophenyl moieties have been an integral part of the genus *Tagetes*, and *T. erecta* has been previously reported for several thiophenes such as 5-(but-1-ol-3-ynyl)-2,2-bithienyl (Tripathi et al., 1992), and the "hairy root" cultures of *T. erecta* have also shown the presence of thienyl moieties such as α -terthienyl (Flores, 1992). The roots of *T. erecta* have been previously reported for their insecticidal and nematocidal activities (Rasoanaivo et al., 1992; Vasudevan et al., 1997). Previous studies from our laboratory evaluated the antiparasitic and antimicrobial potential of the crude successive extracts viz. petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts of the roots of *T. erecta* (Gupta & Vasudeva, 2010). In the present study, we undertook an evaluation of the root extract for antioxidant activity using diphenylpicrylhydrazyl (DPPH) radical scavenging assay and its cytotoxic potential against prostate and HeLa cancer cell lines. Henceforth, the ethanol extract was subjected for activity-guided isolation.

Materials and methods

Plant material and extraction

The roots (4 kg) of *T. erecta* (Compositae) were collected in April, 2005 from Hisar district, Haryana, India. The plant was authenticated by Dr. H. B. Singh, taxonomist at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, and a voucher specimen (no. GJU/HS-509) has been retained at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana. The roots were washed with water, air-dried at room temperature (30–40°C), and then ground to pass through a sieve of 1 mm. The air-dried and coarsely powdered roots (750 g) were exhaustively extracted with 5 L ethanol (95%) in a Soxhlet apparatus for three times repeatedly, and the combined extracts were evaporated to dryness in a rotary evaporator.

Column chromatography

Isolation was carried out using column chromatography as per the reported procedure (Vasudeva et al.,

2007). Briefly, the dark brown extract (60 g) was shaken with 20% ethanol and subsequently refluxed with ethyl acetate and methanol. The ethyl acetate fraction was further concentrated in a rotary evaporator to yield a 20 g fraction which was mixed with 30 g silica gel (100–200 mesh); the resulting mixture was chromatographed over prepacked column consisting of 400 g silica gel (100–200 mesh) in 1.3 L of petroleum ether. The column was subjected to successive elutions starting with 100% petroleum ether followed by varying ratios of petroleum ether and ethyl acetate (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95), and finally 100% ethyl acetate and these were labeled T₁ to T₂₁. The fractions of column eluents collected were subjected to thin layer chromatography (TLC) to check uniformity and homogeneity. Chromatographically identical fractions of column eluents were combined, concentrated, and labeled as T₁ to T₂₁. The spots on TLC were either visualized under ultraviolet (UV) light or with spray reagent, ceric ammonium sulfate in sulphuric acid, followed by heating at 120°C for 15 min.

All the isolates were screened for antioxidant activity and cytotoxicity against cancer cell lines. Among all, the most active isolate was selected for characterization using various techniques, viz., UV, infrared (IR), nuclear magnetic resonance (NMR), and gas chromatography coupled with mass spectroscopy (GC-MS). The melting point was determined in open capillary tube and the UV spectrum was obtained on Shimadzu spectrometer. IR spectrum (KBr cm⁻¹) was recorded on a Perkin Elmer spectrophotometer, using KBr pellets. The ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer (chemical shift in δ ppm). The electrospray ionization (ESI) positive mode mass spectra was obtained in Agilent 5975C GC-MS using Agilent 19091S-433 (30 m \times 250 μ m \times 0.25 μ m) column.

DPPH radical scavenging assay

In order to measure antioxidant activity, the DPPH free radical scavenging assay was carried out according to the procedure described by Blois (1958). The ethanol extract and isolates at various concentrations (2–100 μ g/mL) were added to a 0.1 mM solution of DPPH in methanol, and the reaction mixture was shaken vigorously. The amount of DPPH remaining was calculated by the decrease in absorbance at 520 nm after 30 min, and the radical scavenging activity was obtained from the following equation:

$$\text{Radical scavenging activity (\%)} = \left[\frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})}{\text{OD}_{\text{control}}} \right] \times 100$$

The antioxidant activity of the ethanol extract and isolates was expressed as IC₅₀. The IC₅₀ value is defined as the concentration (μ g/mL) of the extract required for inhibiting the formation of DPPH radical by 50%. Sodium ascorbate was used as a positive control.

MTT assay

Prostate (PC-t3) and HeLa cancer cell lines were purchased from the National Centre for Cell Science (NCCS), Pune, India. The (microculture tetrazolium test) MTT assay was performed by a modification of the method described by Mosmann (1983). Briefly for the assay, both the cancer cells were plated in 96-well falcon plates at a density of 10^4 cells/well in 100 μ L DMEM (Dubelco modified eagle medium). Before treatment, the cells were allowed to adhere to the plate for 24 h. Afterward, the medium was replaced by the same volume of test medium containing the specific substance. Each concentration ranging from 500 to 1.89 μ g/mL for the ethanol extract and isolates, including the control, which contained vehicle alone (1% dimethyl sulfoxide [DMSO]), was applied to 3 wells. The cells were then incubated for 24 h at 37°C. The tetrazolium salt is cleaved to purple formazan crystals by metabolically active cells. MTT labeling reagent (10 μ L) was added to each well after 24 h of treatment, respectively, and the plates were incubated for 4 h at 37°C. Solubilization solution (100 μ L) was then added and incubated overnight at 37°C. Spectrophotometric absorbance of each well was measured at 570 nm using a Benchmark Microplate Reader (BioRad Laboratories, Hercules, California, United States). The percentage cell viability was calculated for each tested concentration, and the result expressed as LD₅₀. The assay was performed in triplicates and repeated in three independent experiments, and paclitaxel (Admac, India) was used as a positive control.

Results

Characterization of active compound

Among all the isolates, T₃ conferred profound antioxidant activity and noticeable cytotoxicity against both the cancer cell lines and was therefore selected for characterization in order to get a profound idea of the putative active principle responsible for the observed effect. T₃ was subjected for characterization using various spectroscopical techniques, viz., UV, IR, NMR, and mass and was identified as compound **1** (Figure 1). The compound was further purified by crystallization from methanol. The compound was isolated as a light brown crystalline adduct, and the following parameters were obtained: melting point. 213°C; UV (CHCl₃) λ_{\max} 241, 305 nm. IR (KBr) γ_{\max} 2363, 2189 cm⁻¹ spectrum indicated the presence of acetylenic group. ¹H NMR (CDCl₃, 300 MHz) spectrum of the compound **1** demonstrated the presence of five well-resolved signals in the aromatic region which were assigned to thiophene moieties: 7.15 (1H, dd, J =5.08, 1.08 Hz, H-5'); 7.12 (1H, dd, J =2.7, 1.08 Hz, H-3'); 7.02 (1H, d, J =3.8 Hz, H-4'); 6.98 (1H, d, J =3.6 Hz, H-3'); 6.96 (m, 1H, J =3.4 Hz, H-4'). The GC-MS scanning was carried out using EI positive mode with m/z 191 showing the molecular ion peak. On the basis of these evidences, the probable structure of the isolated compound

(Figure 1) has been elucidated as 2-ethynyl-5-(thiophen-2-yl) thiophene.

Antioxidant activity

The ethanol extract of the roots of *T. erecta* showed antioxidant activity (Figure 2) at relatively higher concentration in comparison with the standard (sodium ascorbate). The IC₅₀ for the ethanol extract was found to be 35.9 μ g/mL whereas the IC₅₀ for sodium ascorbate was found to be 5.32 μ g/mL. Among all the isolates, the new thienyl constituent (T₃) exhibited DPPH free radical scavenging activity with IC₅₀ value of 11.56 μ g/mL (Table 1).

MTT assay

The ethanol extract and isolates were solubilized in 1% DMSO and tested with varying concentrations ranging from 500 to 1.89 μ g/mL, for evaluation of cytotoxicity against the prostate and HeLa cancer cell lines. The ethanol extract showed noticeable cytotoxicity against both the cells lines with LD₅₀ of 164.28 μ g/mL against HeLa cells (Figure 3) and 407.3 μ g/mL against PC-3 cancer cell lines. Among all the isolates, compound **1** (T₃) conferred cytotoxicity against the prostate and HeLa cancer cell lines with relatively lower LD₅₀ concentrations of 12.5 μ g/mL against HeLa and 30.25 μ g/mL against PC-3 cell lines (Table 1).

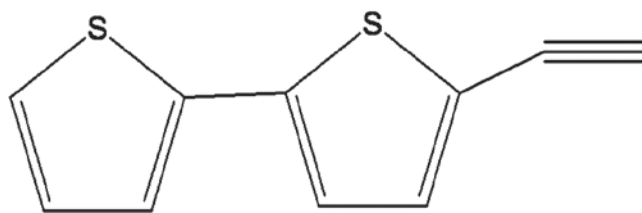


Figure 1. Structure of compound **1**.

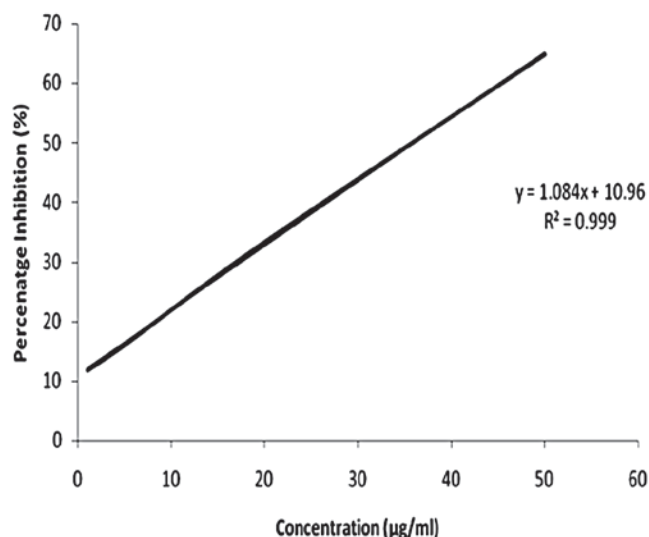


Figure 2. Antioxidant activity of the ethanolic extract of *Tagetes erecta*.

Table 1. IC_{50} against DPPH free radical depicting antioxidant activity and LD_{50} against HeLa and prostate (PC-3) cancer cell lines obtained for the ethanol extract and isolates of the roots of *Tagetes erecta* Linn.

Extract/isolate	IC_{50} ($\mu\text{g/mL}$)	LD_{50} ($\mu\text{g/mL}$) against HeLa	LD_{50} ($\mu\text{g/mL}$) against PC-3
Ethanol	35.9 ± 0.35	164.28 ± 0.69	407.3 ± 0.56
T ₁	29.93 ± 0.11	50.15 ± 0.32	86.92 ± 0.72
T ₂	25.23 ± 0.16	39.36 ± 0.09	79.13 ± 0.06
T ₃	11.56 ± 0.18	12.5 ± 0.84	30.25 ± 0.35
T ₄	19.03 ± 0.71	26.81 ± 0.31	45.13 ± 0.56
T ₅	21.08 ± 0.36	34.64 ± 0.07	57.05 ± 0.25
T ₆	17.01 ± 0.97	31.25 ± 0.74	53.37 ± 0.09
T ₇	19.9 ± 0.55	43.27 ± 0.64	42.19 ± 0.12
T ₈	25.91 ± 0.23	37.22 ± 0.21	48.08 ± 0.26
T ₉	28.06 ± 0.09	36.25 ± 0.19	47.05 ± 0.21
T ₁₀	29.15 ± 0.82	27.81 ± 0.05	44.74 ± 0.37
T ₁₁	24.57 ± 0.91	29.13 ± 0.09	56.74 ± 0.48
T ₁₂	27.81 ± 0.07	37.28 ± 0.27	49.23 ± 0.03
T ₁₃	22.23 ± 0.14	28.17 ± 0.26	45.03 ± 0.35
T ₁₄	29.98 ± 0.85	31.03 ± 0.17	56.29 ± 0.39
T ₁₅	21.05 ± 0.83	38.87 ± 0.21	58.23 ± 0.06
T ₁₆	32.76 ± 0.67	43.02 ± 0.31	49.65 ± 0.85
T ₁₇	36.86 ± 0.34	44.21 ± 0.92	59.97 ± 0.87
T ₁₈	49.17 ± 0.88	65.22 ± 0.59	56.13 ± 0.15
T ₁₉	34.92 ± 0.27	59.28 ± 0.62	73.22 ± 0.53
T ₂₀	57.05 ± 0.98	45.13 ± 0.15	79.05 ± 0.26
T ₂₁	63.03 ± 0.86	39.14 ± 0.29	84.22 ± 0.19
Sodium ascorbate	5.32 ± 0.09	–	–
Paclitaxel	–	5.02 ± 0.75	8.56 ± 0.93

Values are mean \pm standard error mean. "–" denotes not tested.

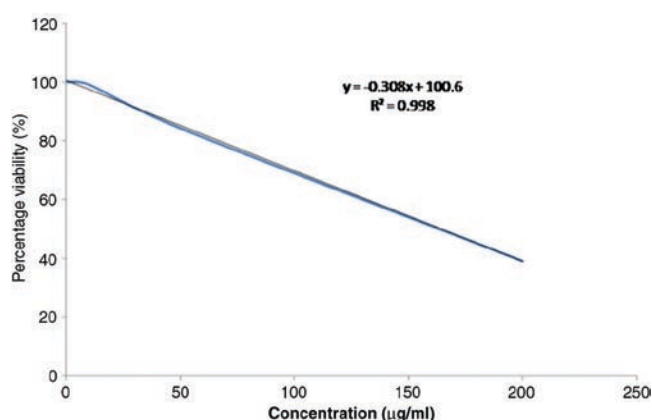


Figure 3. Cytotoxicity exhibited by the ethanolic extract of *Tagetes erecta* against HeLa cancer cell lines.

Discussion

The last two decades have witnessed a clear global inclination for drugs and health foods from natural resources. With the realization of health hazards and toxicity associated with indiscriminate use of synthetic

drugs and remedies, introduction of postmarketing surveillance and active adverse drug reaction monitoring, more than ever a need has been felt for an integrated system of medicine which would minister to the human body as a whole, safety being a major concern. The solution may perhaps be hidden into the traditional system of medicines such as "Ayurveda" or the "Science of life" (Kalia, 2005).

With time, there has been an ever increasing anticipation by researchers worldwide with their growing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body (Molyneux, 2004). The univalent reduction of molecular oxygen results in reactive oxygen species (ROS). In addition to its fatal effects on human body such as tissue degeneration, ROS may also join in generation as well as in malign transformation of cancer cells. If newer compounds from natural sources can enhance the level of antioxidation within the body and clear the ROS in cancer cells, they may inhibit the cell's growth and may thereby play a vital role to treat cancer in combination with the anticancer drugs (Lee et al., 2003; Leng et al., 2005). In our research, the ethanol extract of *T. erecta* was found to scavenge the DPPH free radical indicating that this property may contribute equally or enhance its cytotoxic potential. The observed antioxidant activity of the extract of *T. erecta* may be due to the presence of tannins and flavonoids found in preliminary phytochemical investigation (Gupta et al., 2009).

The ethanol extract of the flowers of *T. erecta* was recently evaluated for antioxidant activity using the DPPH radical scavenging assay, and it was found that the IC_{50} for the ethanol extract ($3.4 \mu\text{g/mL}$) was half than that of the IC_{50} reported for the standard ascorbic acid ($7 \mu\text{g/mL}$) indicating the tremendous antioxidant potential of the flowers which was presumably attributed to the presence of carotenoids in the flowers, such as lutein and zeaxanthin (Chivde et al., 2011). In contrast, the essential oil of the flowers of *T. erecta* also exhibited antioxidant activity but relatively lesser in comparison with the flower extract, whereby it was found to scavenge only 64.3% of the DPPH free radicals (Gutierrez et al., 2006). The antioxidant potential of the aerial parts of *T. erecta* find application in a wide range of therapeutics, for example, the hydroalcoholic extract of the leaves of *T. erecta* was recently reported for its wound-healing activity which may be duly attributed to the free radical scavenging action of the phytoconstituents such as flavonoids present in it (Kiranmai et al., 2011). The present study explored the untouched domain of the plant, that is, the roots of *T. erecta* and evaluated its antioxidant and cytotoxic potential for the first time. The results indicated the antioxidant activity of the ethanol extract of the roots of *T. erecta* whereby it showed noticeable free radical scavenging effect but at a IC_{50} that was seven fold higher than the standard drug (sodium ascorbate)

thereby arising a need for the exploration of putative active principles present in the extract. The antioxidant activity of the new thienyl derivative was observed at relatively lesser concentration in comparison with the ethanol extract, and this observation strongly marks to the antioxidant potential of the thiophenyl moieties present in the roots of *T. erecta* that remained unexplored till date.

The MTT assay, which evaluates the mitochondrial enzyme succinate dehydrogenase activity, is a preliminary assay technique that have been used since time for the evaluation of cytotoxicity of several herbs against a wide range of tumor cell lines, for example, the butanol extract of *Garcinia mangostana* Linn. (Clusiaceae) at $LD_{50} \geq 85 \mu\text{g}$ showed noticeable cytotoxicity against HEP2 cells (Maleeka Begum & Kavitha, 2011), the ethanol root extracts of *Polyathia laui* Merr., and *Polyathia rumphii* Merr. (Annonaceae) inhibited proliferation of four human cancer cell lines, viz., lung cancer, hepatocellular carcinoma, gastric cancer, and myelogenous leukemia cell lines with 50% inhibitory concentrations ranging between 6 and 100 $\mu\text{g/mL}$, demonstrating potential antitumor activities tested using MTT assay (Yuan et al., 2011). The ethanol and aqueous extracts of the aerial parts of *Tagetes lucida* Cav. exhibited cytotoxicity against human breast cancer T47D and cervix cancer HeLa cell lines with ethanol extract showing profound activity with LD_{50} of 1.82 $\mu\text{g/mL}$ and $\geq 50 \mu\text{g/mL}$ against T47D and HeLa cancer cell lines whereas the aqueous extract was effective with LD_{50} of 13.2 and 18.94 $\mu\text{g/mL}$ against HeLa and T47D cell lines (Vega-Avila et al., 2009). In the present study, the ethanol extract of the roots of *T. erecta* exhibited noticeable cytotoxicity against both the tested cells lines with LD_{50} of 164.28 $\mu\text{g/mL}$ against HeLa and 407.3 $\mu\text{g/mL}$ against PC-3 cancer cell lines. Among all the isolates, the new thienyl compound, T_3 , showed profound cytotoxicity against the prostate and HeLa cancer cell lines at concentrations that were 14-fold lower than the ethanol extract but 3-fold higher than the standard drug, paclitaxel. All the other isolates were found to exhibit LD_{50} in the concentration range of 25–70 $\mu\text{g/mL}$ for HeLa and 40–90 $\mu\text{g/mL}$ for PC-3 cancer cell lines, which were relatively higher than T_3 . These observations further signify the potential of T_3 for its development as a compound of therapeutic intervention for the treatment of various types of cancers.

Conclusions

The ethanol extract conferred noticeable antioxidant activity and cytotoxicity against the prostate and HeLa cancer cell lines. Comparatively, the newly isolated thienyl constituent (compound **1**) exhibited antioxidant efficacy and cytotoxicity at much lower concentrations than the extract, supporting toward its development as a potential therapeutic agent for the treatment of cancer with further insight toward the possible mechanisms

of actions. The *in vivo* efficacy studies and further optimization of the active constituent (compound **1**) are in progress. This is the first report on the antioxidant and cytotoxic potential of the roots of *T. erecta*, and the results obtained strongly provide support for the ethnobotanical use of the plant.

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Declaration of interest

There are no conflicts of interest.

References

- Blois MS. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199–1200.
- Chivde BV, Biradar KV, Shiramana RS, Maoj KV. (2011). *In vitro* antioxidant activity studies on the flowers of *Tagetes erecta* L. (Compositae). *Int J Pharm Biol Sci*, 2, 223–229.
- Flores HE. (1992). Plant roots as chemical factories. *Chem Ind*, 10, 374–377.
- Gupta P, Vasudeva N. (2010). *In vitro* antiplasmodial and antimicrobial potential of *Tagetes erecta* roots. *Pharm Biol*, 48, 1218–1223.
- Gupta P, Vasudeva N, Sharma SK. (2009). Pharmacognostical study and preliminary phytochemical screening of the roots of *Tagetes erecta* Linn. *Hamdard Med*, 52, 153–160.
- Gutierrez RMP, Luna HH, Garrido SH. (2006). Antioxidant activity of *Tagetes erecta* essential oil. *J Chil Chem Soc*, 51, 883–886.
- Kalia AN. (2005). *Textbook of Industrial Pharmacognosy*, first edition, New Delhi, India: CBS publishers and distributor.
- Kaplan L. (1960). Historical and ethnobiological aspects of domestication in *Tagetes*. *Econ Bot*, 14, 200–202.
- Kiranmai M, Kazim SM, Ibrahim M. (2011). Combined wound healing activity of *Gymnema sylvestre* & *Tagetes erecta* Linn. *Int J Pharmaceut Appl*, 2, 135–140.
- Lee SE, Shin HT, Hwang HJ, Kim JH. (2003). Antioxidant activity of extracts from *Alpinia katsumadai* seed. *Phytother Res*, 17, 1041–1047.
- Leng B, Liu XD, Chen QX. (2005). Inhibitory effects of anticancer peptide from *Mercenaria* on the BGC-823 cells and several enzymes. *FEBS Lett*, 579, 1187–1190.
- Maleeka Begum SE, Kavitha. (2011). Antimicrobial, antioxidant and antitumor activity of the *Garcinea mangostana*. *Int J Current Res*, 2, 27–31.
- Molyneux P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol*, 26, 211–219.
- Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65, 55–63.
- Raghava SPS. (1998). Pusa Narangi Gaiinda and Pusa Basanti Gaiinda: New marigolds. *Indian Horticulture*, 43, 31.
- Rasoanaivo P, Petitjean A, Ratsimamanga-Urverg S, Rakoto-Ratsimamanga A. (1992). Medicinal plants used to treat malaria in Madagascar. *J Ethnopharmacol*, 37, 117–127.
- Rydberg, PA. (1915). *Tagetes*. *North Am Flora*, 34, 148–159.

- Tripathi AK, Khan SA, Vaishnava MM, Gupta KR. (1992). Compounds of taxonomic significance in *Tagetes*. *Fitoterapia*, 63, 85.
- Vasudeva N, Gupta P, Sharma SK. (2007). A new bithienyl constituent from *Tagetes erecta* Linn roots. *Indian J Heterocycl Chem*, 16, 303-304.
- Vasudevan P, Kashyap S, Sharma S. (1997). *Tagetes*: A multipurpose plant. *Biosci Technol*, 62, 29-35.
- Vega-Avila E, Espejo-Serna A, Alarcón-Aguilar F, Velasco-Lezama R. (2009). Cytotoxic activity of four Mexican medicinal plants. *Proc West Pharmacol Soc*, 52, 78-82.
- Yuan Y, Huang G, Wang T, Chen G. (2011). *In vitro* screening of five Hainan plants of Polythia (Annonaceae) against human cancer cell lines with MTT assay. *J Med Plant Res*, 5, 837-841.