International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 2- ICESMT| 2019 | 69-76
International Conference on "Empowering Society with Microbial Technology"
Held at Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati - 413 102,
Dist. Pune, Maharashtra, India, 7-9 February 2019
| Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Phytochemical Analysis of Flower Extracts of *Tagetes Erecta* L. and their Antibacterial Efficacy Against *Streptococcus* Mutans

S N Deshpande and D G Kadam*
Department of Microbiology, D.B.F.Dayanand college of Arts and Science, Solapur - 413002, Maharashtra, India

Received: 30 Jan 2019 / Accepted: 20 Feb 2019 / Published online: 01 Apr 2019
***Corresponding Author Email:** kadamdgin@rediffmail.com

Abstract
Dental caries is the common chronic oral disease prevalent particularly in children. *Streptococcus mutans* is the most common causative agent of the dental caries. In the present study the antibacterial activity of methanol and chloroform extracts of *Tagetes erecta* L. flowers was evaluated against *Streptococcus mutans* by agar diffusion method. The Preliminary Phytochemical screening of methanol and chloroform extracts of *T. erecta* L. flowers was done which revealed that alkaloids, carbohydrates, proteins, anthraquinones, tannin, cardiac glycosides, fatty acids and oils were present in methanol and chloroform extracts *of T. erecta L.* flowers. However, saponins and amino acids were absent in both the extracts. Chloroform extract showed absence of flavinoids. GCMS analysis revealed seven compounds in methanol extract. Thymine [72.5%] was the major compound followed by N hexadecanoic acid, phenol, 2-6 dimethoxy- and benzoic acid, 4hydroxy-3, 5 dimethoxy- and 1-eicosonol. The most abundant compound in chloroform extract of *Tagetes erecta* L. flower was N- hexadeconoic acid [31.79%] followed by tetradecanoic acid and 2,2';5,2"-terthiophene. Other compounds present were 9, 12-octadecadienoic acid (z,z)- , octadecaane,2methyl- and Heptacosane. Antibacterial activity of the extracts was determined by agar diffusion method. The antibacterial studies of methanol extract showed significant activity [23.3±0.6] compared with chloroform extract. The antibacterial activity of methanol and chloroform extracts of *T. erecta L.* flowers against S. *mutans* indicated that the flower extracts of this plant could be used for management of Dental caries.

Keywords
Dental caries, *Streptococcus mutans, Tagetes erecta* L., antibacterial activity, GCMS analysis

*****

INTRODUCTION:
Dental caries is one of the oral diseases mainly caused by *Streptococcus mutans.* Many attempts have been made to investigate preventive measures for dental caries. Antimicrobial agents, fluorine compounds have been used as preventive agents. However,

development of antibiotic resistance and cytotoxicity of fluoride compounds limits their use. Plant derived medicines have been used as prophylactic measures and antibacterial activity of plant derived compounds is well documented. Natural products have been used in folk medicine for treatment of oral diseases. In Japan, it is the custom to drink green tea after every meal. It inhibits the growth of *Streptococcus mutans* due to presence of polyphenols [1]. There is evidence for use of plants and their products in preventing caries disease. Bark of *Acacia leucophloea, Albizia lebbeck, Syzygium cumini*, leaves of *Legenaria sicerania, Mentha arvenis*, *Hamamelis verginiana*, root of *Anacyclus pyrethrum, Erythrina variegate,* fruit of *Rheedia brasiliensis, Embelia ribes, Caesalpinia maritus*, flower of *Physalis angulata*, whole plant of *Albizia leebbeck, Breynia nivosus, Cocos nusifera, Euclea natalensis, Ginkgo biloba, Milkania glomerata*, *Mangolia grandiflora, Piper cubeca, Rhus corriaria* and *Thuja plicata* have been used in curing dental caries [2]. Dental caries yet remains a widespread public disease that highlight an urgent need to find new effective strategies. If some remedies are not initiated, there could be negative impact upon the future oral health of global community [3]. In the light of these observations, the present study was designed to evaluate the antibacterial activities of *Tagetes erecta* L. flower extract against *Streptococcus mutans.*

Tagetes erecta L. belongs to family *Asteraceae.* The origin of *Tagetes* genus is in North and South America. Now-a-days it is also cultivated in Asian countries like India, Bhutan, Nepal and China [4]. It is also a small shrub which grows 1-2 m height [4]. Different parts of *T. erecta* L. are used for treatment of various diseases in folk medicine. The leaves are effective against piles, kidney troubles, muscle pain, ulcers and wounds. Leaves of this plant are used externally to treat boils and carbuncles [5]. Flowers are used in fever, epileptic fits, astringent, carminative, stomachache, scabies and liver complaints and to treat the different eye diseases. Flower juice is used for treatment of rheumatism, bronchitis, colds and bleeding piles [6].The essential oil of *T. erecta L.* possesses properties like antibiotic, antimicrobial, antiparasitic, antiseptic and spasmodic [4]. Taking into consideration these therapeutic properties present study was designed to evaluate the antibacterial activities of *T. erecta L.* flower extract against *Streptococcus mutans*.

MATERIALS AND METHODS:

Plant material: *T. erecta* L. flowers were purchased from the local market. The flowers were identified in the department of Botany, D.B.F.Dayanand College of Science, Solapur [Maharashtra].

Test Microorganism: Pure culture of *Streptococcus mutans* was obtained from MTCC, Chandigarh, India

Plant extracts preparation and phytochemical analysis study:

Preparation of plant extract: Fresh flowers were washed under the running tap water and dried under shade at room temperature. Dried flowers were powdered in electronic grinder. The powdered flowers were then packed in Soxhlet apparatus and then the extraction was done by using methanol & chloroform as solvents [7]. The solvent from extract was allowed to evaporate. Dried extracts were kept in freeze until further use.

Preliminary Phytochemical Analysis of Extract:

Preliminary phytochemical analysis was done to find out the active chemical principle of the particular plant [7].

Physical characteristics of plant Extract:

Physical characteristics of the plant extracts like colour, odour and consistency were studied.

Percentage yield of Plant Extract:

Percentage yield of the plant extracts in methanol and chloroform was determined in terms of total quantity of powder in grams taken for preparation of extract.

Detection tests of plant extracts:

Detection of Alkaloids:

50 mg of Solvent free extract was mixed with few ml of dilute HCL and filtered. The filtrate was used for various tests as follows.

1. Mayer's test -To a small aliquot of filtrate in a test tube, a drop of Mayer's reagent was added. Development of white or creamy precipitate indicated the positive test.

2. Wagner's test - To a small aliquot of filtrate in a test tube, a few drops of Wagner's reagent were added. Development of reddish-brown precipitate indicated the positive test.

3. Hager's test - To a small aliquot of filtrate in a test tube one ml of Hager's reagent was added. Development of yellow precipitate indicated the positive test.

Detection of Carbohydrates:

Benedict's test - To a 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated for 2 min in a boiling water bath. A characteristic colored filtrate indicated the presence of sugar.

Detection of Amino acids and proteins:

100 mg extract was dissolved in 10 ml distilled water and filtered through Whatman no.1 filter paper. The filtrate was used to test presence of proteins and amino acids.

Biuret test - One drop of 2% copper sulphate solution was added to 2 ml of filtrate. To this, 1ml of ethanol was added followed by addition of excess of potassium hydroxide pellets. Development of pink color in the ethanol layer indicated presence of proteins.

Ninhydrin Test-Two drops of ninhydrin solution were added to 2 ml of aqueous filtrate. Development of purple color indicated presence of amino acids.

Detection of Saponins:

Foam test - 50 mg of extract was dissolved in 20 ml of distilled water. The suspension was shaken in a graduated cylinder for 15 min. Development of two cm layer of foam indicated the presence of Saponins.

Detection of Tannins:

Ferric chloride test – 50 mg of extract was dissolved in 5 ml of distilled water and then a few drops of 5% Ferric chloride were added. Development of dark green color indicated the presence of tannins.

Detection of flavonoids:

Magnesium and hydrochloric acid reduction test - 50 mg of the extract was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise. Development of pink to crimson colour indicated presence of flavonoids.

Detection of anthraquinones:

50 mg of extract was dissolved in distilled water. 1ml dilute ammonia solution was added to 2 ml of extract and shaken vigorously. Development of pink color in ammonia layer indicated presence of anthraquinones.

Detection of Cardiac glycosides:

Killer kiliani test - 50 mg of the extract was dissolved in distilled water and filtered. Then 1ml of glacial acetic acid and a drop of Ferric chloride and a drop of concentrated sulfuric acid were added to 2 ml of filtrate. Development of green blue color to upper layer and reddish-brown color at the junction of two layers indicated the presence of cardiac glycosides.

Detection of fixed oils and fat:

Spot test- A small aliquot of extract was pressed between two filter papers. Development of oil stain on the paper indicated the presence of fixed oils.

GCMS analysis of extracts:

GCMS analysis of methanol and chloroform extracts of *Tagetes erecta* L. flowers was carried out in Indian Institute of Technology; Mumbai. GCMS was performed by using Hewlett Packard, GCD 1800. An electron ionization detector was used in the instrument with an operating mass range 10 – 425. Helium was used as a carrier gas. 1 µl of extract was used to inject in injection port of GC column. The temperature program was 100-10-250-2M-30-270-3M which indicates that initial temp was 100°C that rose to 250 °C at a ramp of 10 °C/min – isothermal for 2 min, then to 270 °C at a ramp of 30 °C /min and remained isothermal at 270 °C for 3 min.

Identification of compounds:

The mass spectrum of unknown components was compared with spectrum of the known components stored in the NIST and Wiley library. Interpretation of mass spectrum of GCMS was done using data base of NIST library having more than 75,000 compounds. The molecular weight, structure and names of components were then ascertained. The relative percentage was determined by comparing its average peak area to the total area.

Antibacterial activity of Plant Extract:

Antibacterial activity of the extract was determined by agar diffusion method [8]. For this, fresh [overnight] isolated colony of *S. mutans* was suspended in sterile saline to get turbidity of 0.5 McFarland standards. 0.1 ml amount of this suspension was spread aseptically on sterile Muller Hinton agar medium [Hi media]. Then wells [6 mm diameter] were bored by sterile cork borer. 0.2 ml amount of each extract [100 mg /ml in 10% DMSO] was added to the wells. It was allowed to diffuse by keeping the plates in freeze for 20 min. 10 % DMSO in one of the wells served as negative control. Antibiotic chloramphenicol [300mcg, Hi Media] disc was used as standard positive control. After diffusion of extracts the plates were incubated at 37 °C for 24 hrs. Diameter of zone of inhibition was then measured in mm. For each extract three replicates were maintained.

Statistical analysis:

For the antibacterial activity, experiment was carried out in triplicates. Zone of inhibition was expressed as mean ± standard deviation.

RESULTS AND DISCUSSION:

Phytochemical analysis study:

Physical characteristics of extracts *T. erecta* L. flowers:

The physical characteristics of methanol & chloroform extracts of *T. erecta* L. flowers are depicted in table 1. Methanol and chloroform extracts of *T. erecta* L. were dark brown in color while the consistency was solid sticky. Organic odour was exhibited by extracts of *T. erecta* L.

Table 1: Physical characteristics of extracts *T. erecta* L. flowers

Sr No	Solvent used	Physical characteristics		
		Colour	Consistency	Odour
1	Methanol	Dark brown	Solid sticky	Organic
2	Chloroform	Dark brown	Solid sticky	Organic

Table2: Percentage yield of extracts *of T. erecta* L. flowers

Sr No	Solvent used	Physical Characteristics	Amount[g]
1	Methanol	Wt. of dry powder[g]	15
		Wt. of extract[g]	2.25
		% yield	15
2	Chloroform	Wt. of dry powder[g]	15
		Wt. of extract [g]	1.15
		% yield	7.6

Table 3: Preliminary Phytochemical analysis of *T. erecta* L. flowers extract

Sr.No.	Phytochemical	Methanol extract	Chloroform extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Saponin	-	-
4	Protein	+	+
5	Amino acids	-	-
6	Antraquinones	+	+
7	Tannin	+	+
8	Flavonoids	+	-
9	Fatty acids and oils	+	+
10	Cardiac glycosides	+	+

Table 4: Antibacterial activity of *T. erecta* L. flower extracts and chloroform against *Streptococcus mutans*

Solvent used	Diameter of Zone of inhibition[mm.] [±SD]
DMSO	-
Methanol	23.3±0.6
Chloroform	20±0.0
Chloramphenicol	30±0.0

Table 5: Gas chromatographic and Spectral data for methanol extract *T. erecta* L. flower

Peak No.	Retention time	Name of compound	Molecular weight	Molecular formula	% peak area
1	4.0	Thymine	126	C5H6N2O2	72.5%
2	4.9	4H-Pyran-4-one,2,3-dihydroxy-6-methyl-	144	C6H8O4	10%
3	7.9	phenol ,2-6 dimethoxy-	154	C8H10O3	24.05%
4	14.6	Benzoic acid, 4 hydroxy-3 ,5 dimethoxy-	198	C9H1005	23.28%
5	17.2	N hexadeconoic acid	256	C16H32O2	50%
6	20.2	9,12-octadecadienoic acid (z,z)-	280	C18H32O2	19.30%
7	28.0	1-eicosonol	298	C20H42O	6.42%

Table 6: Structures of Compounds found & Identified in *T. erecta* L. flower Methanol Extract

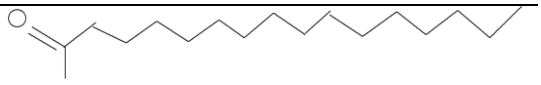
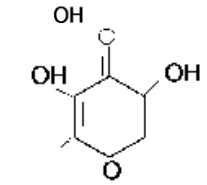
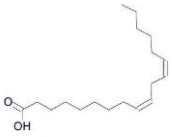
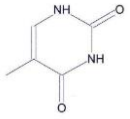
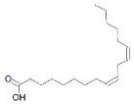
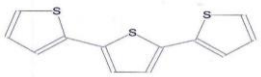
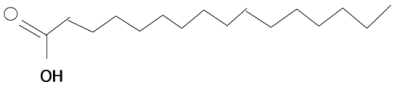
Sr.no.	Name of compound	Structure of compound
1	n-Hexadecanoic acid	
2	4,4-pyra-4-one 2,3 dihydro-3,5-dihydroxy 6 methyl	
3	9,12-octadecadienoic acid (z,z)-	
4	Thymine	

Table 7: Gas chromatographic and Spectral data for chloroform extract of *T. erecta* L. flower

Peak no.	Retention time	Name of compound	Molecular weight	Molecular formula	% Peak area
1	14.3	tetradecanoic acid	228	C14H28O2	13.98%
2	17.3	N- hexadeconoic acid	256	C16H32O2	31.79%
3	20.1	9,12-octadecadienoic acid (z,z)-	280	C18H32O2	9.95%
4	20.8	2,2';5,2"-terthiophene	248	C12H8S3	10.57%
5	22.7	Heptacosane	380	C27H56	6.60%
6	28.4	Octadecaane,2methyl-	268	C19H40	9.15%

Table 8: Structures of Compounds found & Identified in *T. erecta L.* flower chloroform Extract

Sr.no.	Name of compound	Structure of compound
1	9,12-octadecadienoic acid (z,z)-	
2	2,2';5,2"-terthiophene	
3	nHexadecanoic acid	
4	Heptacosane	
5	Tetradecanoic acid	
6	Octadecaane,2methyl-	

Percentage yield of extract of *T. erecta* L. flowers:

Table 2 shows the percentage yield of *T. erecta* L. flower extract in methanol and chloroform as solvent. More yields were obtained in methanol extract compared to chloroform extract.

Preliminary Phytochemical Analysis *of T. erecta* L. flowers extracts:

The Preliminary Phytochemical screening revealed the presence of alkaloids, carbohydrates, proteins, anthraquinones, tannin, cardiac glycosides, fatty acids and oils in methanol and chloroform extracts *of T. erecta* L. flowers. However, saponins and amino acids were absent in both the extracts. Chloroform extract also showed absence of flavonoids [Table 3].

GCMS analysis:

In methanol extract of flower of *T. erecta* L. seven compounds were identified by GCMS analysis. Thymine [72.5%] was the major compound followed by N hexadecanoic acid, phenol, 2, 6-dimethoxy- and benzoic acid, 4-hydroxy-3, 5 dimethoxy- and 1-eicosonol [Table5]. Structures of compounds identified and detected in methanol extracts of *T. erecta* L. are shown in Table 6. Figure 1 show the GCMS chromatogram of methanol extract of *T. erecta* L. GCMS analysis in the present study was carried out to explore the potential of bioactive compounds found in *T. erecta* L. Antibacterial activity observed in the present study might due the presence of n-hexadecanoic acid. The antibacterial activity of this compound has been reported previously also [9]. Phenol, 2, 6- dimethoxy- was reported as an antioxidant and had demonstrated marked antimicrobial activity inhibiting the growth of gram-positive bacteria at a higher degree [10]. Earlier investigations on GCMS analysis of essential oil of flower of *T. erecta* L. showed the presence of 2- methyl - 6 - (4-methyl cyclohexadienyl) hept-4-en-2-ol, a-sesquiphellandrene, b-sesquiphellandrene, myristoleic acid and trieicosane [11]. The present study showed the qualitative difference in the composition of flower of *T. erecta* L. from earlier reports [11]. This variation might be due to difference in extraction method, habitat conditions, geographical locations, altitudes and season of sample collection [12].

The most abundant compound found in chloroform extract of *T. erecta* L. flower was N- hexadeconoic acid [31.79%] followed by tetradecanoic acid and 2,2';5,2"-terthiophene as shown in Table 6. Other compounds present were 9, 12-octadecadienoic acid (z,z)- , octadecaane, 2methyl- and Heptacosane. Structures of compounds found and identified in chloroform extracts of *T. erecta* L. are shown in Table 8. Figure 2 shows the GCMS chromatogram of chloroform extract of *T. erecta* L. flower.

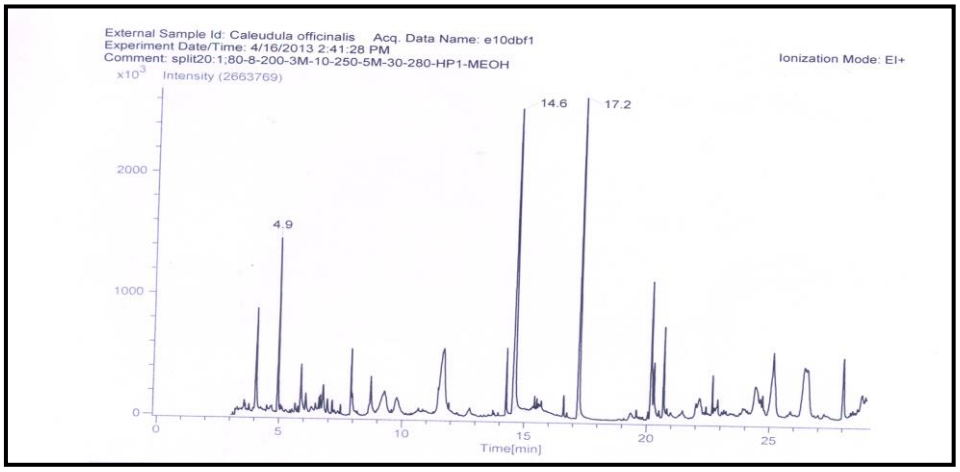


Figure 1: GCMS chromatogram of methanol extract of *T. erecta* L. flower

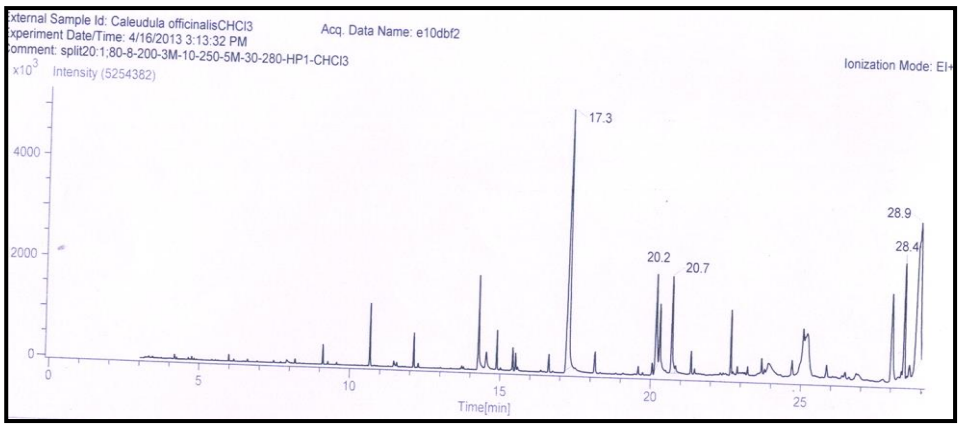


Figure 2: GCMS chromatogram of chloroform extract of *T. erecta* L. flower.

Antibacterial activity of *T. erecta L.* flower extracts against *S. mutans*:

Table 4 depicts the antibacterial activity of *T. erecta* L. flower extracts in methanol and chloroform against *S. mutans*. Methanol extract showed higher antibacterial activity compared to chloroform extract against *S. mutans*. Chloramphenicol (300mcg) was used as positive control. It showed 30 mm diameter of zone of inhibition against *S. mutans.*

Among the identified phytochemicals in the chloroform extract of *T. erecta* L. flower, tetradecanoic acid and n-hexadecanoic acid have antioxidant and antibacterial properties [13]. Fatty acids with its main constituents as palmitic, stearic and oleic acid showed antibacterial activity against pathogenic strains of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* [14, 15]. The fatty acids such as lauric, palmitic, linolenic, linoleic, oleic, stearic and myristic acids are reported have potent antibacterial and antifungal values [16, 17]. In the present study, tetradecanoic [myristic], n-hexadecanoic and 9, 12- octadecanoic acids [stearic acid] were found in chloroform extract of *T. erecta* L. flowers. The antibacterial activity of this extract might due to presence of these fatty acids in it. These findings are consistent with several previous studies [11, 13, 14, 16, and 17].

CONCLUSION:

In the present study, Methanol extract of *T. erecta* L. flower showed maximum antibacterial activity against *S. mutans* compared to chloroform extract. Both methanol and chloroform extracts of *T. erecta* L. flowers showed presence of alkaloids, carbohydrates, proteins, anthraquinones, tannin, cardiac glycosides, fatty acids and oils. GCMS analysis revealed seven compounds in methanol extract. Thymine [72.5%] was the major compound followed by N hexadecanoic acid, phenol, 2-6 dimethoxy- and benzoic acid, 4hydroxy-3, 5 dimethoxy- and 1-eicosonol. The most abundant

compound found in chloroform extract of *T. erecta* L. flower was N- hexadeconoic acid [31.79%] followed by tetradecanoic acid and 2,2';5,2"-terthiophene. Such phytochemical constituents have been reported to possess antioxidant, antibacterial and antifungal value. Thus, the phytochemical analysis, GCMS studies and antibacterial activity studies of plant extracts indicate that the *T. erecta* L. flower constituents possess potential therapeutic value and may be used to design a drug for the treatment and control of dental caries caused by *Streptococcus mutans.*

REFERENCES:

1. Set S, Kim M, Taniguchi M, Yamamo T**.** Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. Agric Biochem, 53:2307-11, (1989).
2. Parimala DB, Ramsubramaniaraja R**.** Dental caries and medicinal plants-An overview. Journal of Pharmacy Research, 2(11):1669-1675, (2009).
3. Parashar K, Zaidka S, Somani R, Jayanti S**.** Anti-cariogenic effects of polyphenol plant products-A review. International Journal of Research in Ayurveda and Pharmacy, 2(3): 736-742, (2011).
4. Dasgupta N, Shivendu R, Proud S, Jain R, Malhotra S, Saleh AM**.** Antibacterial activity of leaf extract of Maxican Marigold (*Tagetes erecta*) against different gram positive and gram-negative bacterial strains. Journal of Pharmacy Research, 5(8): 4201-4203, (2012)
5. Ibrahim M, Kiranmai M. Antibacterial potential of different extracts of *Tagetes erecta* L. International Journal of Pharmacy, 291):90-96, (2012).
6. Kirtikar KR, Basu BD. Indian Medicinal Plants, 4(2 Ed), Bishan Singh Mahendra Pal Singh, Deharadun, (1935).
7. Raaman N**.** Phytochemical techniques. New Age Publications, New Delhi, (2006).
8. Cruickshank R, Duguid JP, Marmion BP, Swain RHA. Medical Microbiology vol. II (12th Ed.) Churchill Livingstone, Edinburgh, London and New York, (1975).
9. Ragasa CY, Tepora M, Rideout JA. Antimicrobial chromenes from *Eupatarium topingianum* ACGC Chem Res Com, 7:54-61, (1998).
10. Turcotte P, Saheb SA**.** Antimicrobial activity of phenolic antioxidants. Can J Microbiol, 24(11):1306-1320, (1978).
11. Ray DP, Srivastava S, Singh RP. Gas chromatographic-mass spectrometric [GCMS]analysis of marigold (*Tagetes erecta*) flower oil. International Journal of Agriculture, Environment and Biotechnology, 5(3):215-218, (2012).
12. Seth R, Manindra MP, Syed ZH, Gupta S, Bajapai I, Singh D, Dobhal R**.** Chemical composition and antibacterial properties of the essential oil and extracts of *Lantana camara* (L) from Uttarakhand (India). Asian Pacific Journal of Tropical Biomedicine, S1407- S1411 (2012).
13. Copping LG. Crop protection agents from nature: Natural products and analogues. The Royal Society of Chemistry, Cambridge, U.K. (1996).
14. Bhattacharjee I, Ghosh A, Chandra G. Antimicrobial activity of the essential oil of *Cestrum diurnum* L. Af J Biotechnol., 4(4):371-374, (2005).
15. Amer WM, Abouwarda AM, Garf EL, IA Dawoud GTM, Abdelmohsen G. Phytochemical composition of *Solnum elaeagnifolium* cav. and its antibacterial activity. Inter J Bio Pharm and Allied Sci, 2(6):1282-1306, (2003).
16. McGaw IJ, Jager AK, Van SJ. Isolation of antibacterial fatty acids from Schtia brachypetala Fitoter, 73:431-433,(2007).
17. Seidel V, Taylor PW. In vitro activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. Int J Antimicrob Agen. 23:613-619, (2004).