Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

ANTIBACTERIAL, ANTIOXIDANT ACTIVITY AND GC-MS ANALYSIS OF *Eupatorium odoratum*

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Received:23 January 2012, Revised and Accepted: 14 April 2012

ABSTRACT

17 major and 26 minor compounds were identified in methanol and aqueous extracts of *Eupatorium odoratum* by GC –MS analysis showing significant antibacterial, antioxidant and other prophylactic activities. Antibacterial properties of aqueous and organic extracts of different parts of *E.odoratum* against nine different bacterial strains are examined. All fractions of leaf and root have significant inhibitory activity against all bacterial pathogens tested. However, flowers and stem did not show any activity. Significant protective activities against different ailments are found due to the presence of different phenols, flavonoids, alcohol derivatives and unique compounds consist of 2,4-Bis(1-phenylethy)phenol, Monoethylhexyl phthalate, Hoslundin, 2,4,6-Tris-(1-phenylethyl)-phenol; dl-α-Tocopherol, Phytol, 1,2,4-oxadiazol-5-amine, 3-(4-amino-1,2,5-oxadiazol-3-yl)-N-[2-(4-methoxyphenyl), 1-Heptacosanol, Stigmasterol, γ-Sitosterol, Tetra-O-methylscutellarein, Neophytadiene, (35)-7-O-Methoxymethylvestitol, α-Amyrin, Methylcommate D and 4-Acetyl-3-hydroxy-2,6-dimethoxytoluene. Our results revealed that, experiments conducted on different parts of this plant according to the traditional usage and several compounds identified by GC-MS analysis are principal factors for significant antibacterial, antioxidant and other prophylactic activities.

Key words: GC-MS analysis, Antibacterial activity, *Eupatorium odoratum*, Anti-oxidants

INTRODUCTION

Eupatorium odoratum is folklore medicinal plant, belongs to the family of Asteraceae, being using to treat many microbial diseases since times immemorial. Mixture of several plants with *E.odoratum* is used by tribal people for oral consumption in terms of decoction in the primary health care, and external application[1]. An ointment prepared from the leaves of *E.odoratum* has been shown to promote the healing of soft tissue wounds by enhancing the proliferation and migration of fibroblast cell, endothelial cell and keratinocytes in healing of burn. Further, studies reveal that leaf extract stimulated the expression of many proteins of the adhesion complex and fibronectin by human keratinocytes which are essential to stabilize epithelium in the healing process of wounds. Protection of cells against destruction by inflammatory mediators may be one of the ways in which the compounds of *E.odoratum*, contribute to the healing of wounds, by delaying the sequelae of trauma and to enhance the healing process[2-6]. It has been identified that the presence of antioxidants and other bioactive compounds might have helped the people to use the leaves and roots of this plant as natural medicine in the form of crude ointment, decoction or other forms of extractions by maceration, percolation and infusion techniques. This plant is still being used, by people in tribal and coastal areas working in agriculture fields and other sectors, as antiseptic for regular treatment of skin eruptions and other disorders such as diarrhea, Gonorrhea, malaria and cough[7-10]. Traditional plant based medicines are getting popular for the treatment of several ailments as it is free from side effects and less expensive when compared to the existing allopathic drugs. Tribal folks use *E.odoratum* in particular for the treatment of inflammation, to control hemorrhage after cuts, burns, dento-alveolitis, and for enhancement of fibroblasts, endothelial cell proliferation, inhibited contraction of collagen, platelet activating receptor inhibition etc[11,12].

Data collected from review of literature from web, journals, tribal's and local people of North Coastal Andhra Pradesh (NCAP) have informed that this plant contains several other compounds, so far not have been reported, are useful for treatment of many human microbial infections. Therefore, we anticipated that this plant contain many new compounds other than those observed by scientists until now, and, which might be identified and characterized from other plants but not from *E.odoratum*. Any such new compounds are screened and their structures are elucidated in this plant by latest techniques may give better understanding for folklore use of this plant by people of NCAP. With this information we can find out new drugs and also make new synthetic compounds and lead molecules with different mechanism of actions and thereby different target organisms especially against drug resistant bacteria and emerging microbes. In view of several medicinal and folklore advantages associated with *E.odoratum*; and compounds identified by several scientists until now, and correlating it with proper use of this plant by the people of tribal districts; the present work deals with screening of different antibacterial, antioxidant and other prophylactic compounds of *E.odoratum* by GC-MS analysis of aqueous and methanol extracts to be identified as authentic principal compounds in the phyto-prophylactic preparations of tribal's against various ailments.

MATERIAL AND METHODS

Plant material

Plant parts were collected from campus garden as well as outside the campus of GITAM, Visakhapatnam and different parts of North Coastal Andhra Pradesh. The plant material was washed thoroughly with tap water and then rinsed with distilled water and shade dried at room temperature. The dried plant material was finely powdered using an electric grinder and used for aqueous and organic solvent extraction.

Organic solvent extracts

A mass of shade dried, powdered plant material was soaked separately in 95% ethanol, Methanol, Chloroform and mixture of Methanol: Chloroform: Water (MCW) ratio in 12:5:3. The organic solvents were added in a ratio of 1:3 (w/v) and refluxed with the residue for six hours at their respective boiling temperatures. After filtration, the solvent was evaporated under reduced pressure in a rotary vacuum evaporator at 50°C from organic extract[13]. Stock solution for bioassay was prepared by dissolving the above extract

in the corresponding solvent to get a final concentration of 2 mg ml^{-1} (w/v).

Aqueous extract

E.odoratum plant parts were washed thoroughly with normal water followed by double distilled water. Extracts were obtained by adding water to the crushed material in a ratio 1:3 (w/v). Direct crushed leaves extract was also prepared by macerating the leaf in mortar and pestle and squeezed followed by centrifuged at 10,000 g for 10 min to get clear extract. Extracts were lyophilized and stored at -20^{0}C[13].

Microorganisms used and growth conditions

The following organisms were used in this study and they consist of both Gram positive and Gram negative bacteria: *Bacillus subtilis* (MTCC736), *Corynebacterium glutamicum* (MTCC2807), *Escherichia coli* (MTCC1572), *Klebsiella pneumonia* (MTCC7028), *Proteus vulgaris* (MTCC1771), *Salmonella typhi* (MTCC733), *Staphylococcus aureus* (MTCC87), *Streptococcus thermophilus* (MTCC1938) and *Vibrio parahaemolyticus* (MTCC451). The bacterial strains were maintained at 4^{0}C and their stock was kept in 10% glycerine saline at –20^{o}C.

Antibacterial activity

Sensitivity of different test bacterial strains to various extracts of *E.odoratum* was measured by agar well diffusion method[14,15]. Zone of inhibition was determined using Himedia zone of inhibition scale and results are expressed in millimeters (mm). For each combination of extract and the bacterial strain, the experiment was performed in triplicate. The bacteria with a clear zone of inhibition of more than 8 mm were considered to be sensitive. Respective pure solvents were used as negative controls and Cephalothin, Gentamicin have been used as positive controls.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the extracts was determined for all bacterial species using the two fold serial microdilution method with saline at a final concentration ranging from 0 to 200 mg ml^{-1} according to the National committee for clinical laboratory standards (NCCLS, 2000) [16] and Bauer *et al*[17].

GC-MS Data analysis

The Gas chromatography-Mass spectrometry (GC-MS) analysis of methanol and aqueous extracts of *E.odoratum* were performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. The column oven temperature was programmed from 50°C to 300°C for 2°C min^{-1}. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The temperature of the injector was fixed to 240°C and one of the detector to 200°C. Helium (99.9995% purity) was the carrier gas fixed with a flow rate of 1.5 ml min^{-1}. The mass range from 40-1000 m/z was scanned at a rate of 3.0 scans/s. 1.0 µL of the methanol extract of *E.odoratum* was injected with a Hamilton syringe to the GC-MS manually for total ion chromatographic analysis in split injection technique. Total running time of GC-MS is 35min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

Identification of Components

The identity of the bioactive compounds in the aqueous and methanol extracts of *E.odoratum* was carried out by Mass Spectroscopy based on the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. National Institute of Standards Technology (NIST08s), Wiley Registry of Mass Spectral Data's, New York (Wiley 8) and Fatty Acid Methyl Esters Library version 1.0 (FAME library) sources were used for matching the identified components in the extract.

DPPH radical scavenging assay

The antioxidant activity of the *E.odoratum* methanol extract was assessed by quantifying the scavenging ability to stable free radical 2, 2′-Diphenyl-1-picrylhydrazyl. The DPPH assay was performed as described by D'Mello *et al*[18]. The evaluation was carried out on Hitachi UV-Visible spectrophotometer at 516 nm using Ascorbic acid as positive control. Inhibition of free radical by DPPH in percent (%) was calculated in following way:

Percentage of inhibition (%) = (Blank - Sample / Blank) × 100

where the blank is the absorbance of the control reaction mixture excluding the test sample, and sample is the absorbance of the test sample. IC_{50} values, which represented the concentration of extract that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

Statistical analysis: **Experimental results concerning this study were mean ± S.D. of three parallel measurements. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests. *P* values <0.05 were regarded as significant and *P* values <0.01 very significant.**

RESULTS & DISCUSSION

GC-MS analysis reveals that 43compounds in methanol and aqueous extracts of *E.odoratum* with various activities are present. Most of them were not reported earlier but screened and characterized in several other plants including their biological activities. In this study, we have used nine different prominent and imperative pathogenic bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Streptococcus thermophilus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Salmonella typhi* and *Vibrio parahaemolyticus* against six different aqueous and organic fractions from different aerial parts and roots of *E.odoratum*. Most of the organisms tested are associated with human pathogeneses; *S. aureus*, *B. subtilis* and *E. coli* are common bacteria causing food poisoning and some infections ranging from minor infections to life threatening diseases of human beings. Food-borne illness to the individual is caused by eating food or drinking beverage contaminated with bacteria or by direct contact through pus. Typhoid fever is an acute illness associated with *S. typhi*, spread through contaminated water makes a significant public-health issue in developing countries. *V. parahaemolyticus* causes seafood-borne diseases due to eating of uncooked food makes wound infections and infected persons showing symptoms of watery/bloody diarrhea, vomiting, abdominal cramps, headaches and fever. These problems frequently occur to the people living in the areas of coastal districts belongs to tropical and subtropical regions. *K. pneumonia*, is a most opportunistic pathogen and second to the *E. coli* causing urinary tract infections. *C. glutamicum* is *a n*on-pathogen having more applications in fermentation processes, a model organism as species of Corynebacterium causes diphtheria.

In order to unveil the spectrum of compounds present in the *E.odoratum* extracts of different parts of *E.odoratum*, those which are showing profound antibacterial activities, were subjected to GC – MS analysis, where two extracts such as methanol and aqueous extracts of *E.odoratum* leaves found to show highest antibacterial activity (P<0.001) against all bacteria tested (Fig 1). GC-MS analysis also depicted that these extreme bioactivities are due to the presence of the different bioactive compounds which are already identified and characterized in different medicinal plants but only few of them are reported in *E.odoratum* have made us to exploit further analyses (Table 1 & Fig 6). The observed results were encouraging, as all the selected parts of *E.odoratum* aqueous and organic extracts are showing different levels of antibacterial activity. This data interpreting the extracts of *E.odoratum* contain both aqueous and organic soluble compounds useful to treat bacterial and other diseases might be the central theme behind using this plant directly as traditional tribal medicine. Antibacterial activity of aqueous leaves extract of *E.odoratum* was compared with organic solvent extracts of different parts of this plant. In fact, water extract was prepared to examine the regular use of this plant as decoction for treatment of different ailments. Different forms of *E.odoratum* extracts using for treating skin cuts or wounds, to stop bleeding,

promote quick healing, decoction of roots and leaves as an antipyretic & analgesic, leaf extract with salt is used as a gargle for sore throats and colds[13]. Phytoconstituents of *E.odoratum* leaf showed very significant antibacterial activity (P<0.001) especially water and MCW extracts against all bacterial strains tested except *P. vulgaris* and *S. typhi* (Fig 1). Results are very hopeful as their activities are higher against Gram Negative bacteria than Gram positive bacteria but their MIC are very persistent against *C. glutamicum* and *S. thermophilus* as 0.0122 and 0.003 mg ml^{-1} in methanol and aqueous extracts respectively. Very moderate MIC values were recorded in ethanol (0.78); Chloroform (0.78) and MCW (0.78) against *C. glutamicum*; *E. coli, K. pneumoniae, P. vulgaris* and *S. thermophilus* respectively (Fig 5). A similar moderate result was observed in the root extracts. The root was found to contain potent constituents against *K. pneumoniae* and *P. vulgaris* than other organisms tested (Fig 2). These results are directly correlates with the compounds identified in their corresponding extracts by GC-MS analysis (Table 1& Fig 6).

GC-MS analysis of aqueous and organic extracts of *E.odoratum* showed spectrum of compounds having strong antibacterial, antioxidant and other prophylactic activities in several plants but not this plant (Table -1). A potent antimicrobial and anti-inflammatory compound, Methyl commate D and Neophytadiene were identified as strong bactericidal compounds along with α-amyrin in Bursera simaruba (L.), a folklore plant showing anti-inflammatory activity in folklore use[19-20]. Neophytadiene, an antifungal terpenoid identified in the red alga, *Centroceras clavulatum* (C. Agardh) Montagn was also reported in several plants which were used as antipyretic, analgesic and vermifugic, including a topical application for sores and inflammation. γ –Sitosterol is a phytosterol present in many plants are being used as folklore medicine in traditional system possesses very strong antifungal, antibacterial and anti-angiogenic activity[21]. γ–Sitosterol being used as Phytomedicine in traditional medicinal system is used to treat ulcers, bronchitis, diabetes, and heart diseases[22]. Stigmasterol (24-Ethylcholesta-5, 22-dien-3β-ol) is a strong antioxidant having antibacterial activity against multi drug resistant mycobacteria[23-24]. It is hoped that these results will enable the plant extract even for the treatment of dangerous diseases causing by highly resistant bacteria. Reports available on the identified compounds in our experiments showing antibacterial activity are scarce but other compounds noticed to show antimicrobial activity are 6,8-Nonadien-2-One, 8-Methyl-5-(1-methylethyl)-, (E)-; Dihydro-neoclovene-(II), 1-Tricosanol, 5-Hydroxy-4',7-dimethoxyflavanone, Squalene, Olean-12-en-3-yl acetate. Several classes of compounds were also isolated from *E.odoratum* i.e., Coumarins, flavonoids, phenols, tannins, sterols and complex mixtures of lipophilic flavonoid, aglycones. Some of them are identified as Protocatechuic, p-Hydroxybenzoic, p-Coumaric, Ferulic and Vanillic acids with antimicrobial, anticancer, and anti-inflammatory activities[8,25-27] (Fig 6).

Aqueous and organic extracts of *E.odoratum* flowers do contain compounds against *S. aureus*, *B. subtilis*, *S. thermophilus*, *K. pneumoniae*, *P. vulgaris*, *S. typhi*, where *S. aureus*, *B. subtilis*, *S. thermophilus* and *K. pneumoniae* show moderate antibacterial activity against all bacteria tested than *P. vulgaris* and *S. typhi* (Fig 3). Extracts of aqueous, MCW and ethyl alcohol are showing appropriate activity against *P. vulgaris* and *S. typhi*. *C. glutamicum*, *E. coli* and *V. parahaemolyticus* exhibited very poor activity decipher that these plant parts do not hold any potent antibacterial compounds as present in leaves and root system of *E.odoratum* (Fig 1&2). These results are supporting the reports published by Suksamrarn *et al*[28]. Flavonoids have been reported to possess anti-bacterial activity, which could be attributed to their ability to form complex with extracellular, soluble proteins and bacterial cell walls. However, in the present study qualitatively isolated alkaloids and flavonoids showed potent antibacterial activity as revealed by gel diffusion assay. Earlier studies noticed that four flavanones i.e., Isosakuranetin (5,7-dihydroxy-4'-methoxyflavanone), Persicogenin (5,3'-Dihydroxy-7,4'-dimethoxyflavanone), 5,6,7,4'-Tetramethoxyflavanone and 4'-Hydroxy-5,6,7-trimethoxyflavanone; two chalcones, 2'-Hydroxy-4,4',5',6'-tetramethoxychalcone and 4,2'-Dihydroxy-4',5',6'-trimethoxychalcone; and two flavones, Acacetin (5,7-Dihydroxy-4'-methoxyflavone) and Luteolin (5,7,3',4'-Tetrahydroxyflavone) from flowers of *E.odoratum*. 5,7-Dihydroxy-4'-methoxyflavanone exhibited moderate antibacterial activity against *M. tuberculae* where as weak activity shown by 4'-Hydroxy-5,6,7-trimethoxyflavanone, 5,7-Dihydroxy-4'-methoxyflavone and 5,7,3',4'-Tetrahydroxyflavone (P<0.05). These two compounds also exhibited moderate anticancer activity. The exhibited poor activity due to either non-expression of compounds or below the threshold levels expression of compounds identified in leaves and roots. However, this potency is governed by their concentration in the plants. Except aqueous, ethyl alcohol and MCW extracts of stem, other extracts have not shown any antibacterial activities against bacterial tested even though they are very poor at action (Fig 4).

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-Diphenyl-2-picryl hydrazine. The ability to scavenge the free radical, DPPH, was measured at an absorbance of 517 nm. So the 1, 1-Diphenyl-2-picrylhydrazyl–radical scavenging assay (DPPH-RSA) and its percentage inhibition of aqueous and methanol extracts showed IC_{50} values of 10.5 μg ml^{-1} and 10.2 μg ml^{-1}, respectively. Ascorbic acid was taken as reference which showed 8.0 μg ml^{-1}. These results show the methanol extract to be more potent than traditionally claiming decoction. Several reports were already available claiming potent antioxidant activity of this plant especially of its leaves; hence we are not showing any data of its antioxidant activity assessed by DPPH method[9,23,29]. Phan *et al*[4]., reported that crude ethanol extract of *E.odoratum* has antioxidant activity that protects fibroblasts and keratinocytes in vitro and informed that a mixtures of lipophilic flavonoid aglycones protected cultured skin cells against oxidative damage. Our GC –MS analysis data gave more than 50% of the identified compounds are antioxidants. 2,4-Bis(1-phenylethy) phenol, Monoethylhexyl phthalate, 2,4,6-Tris-(1-phenylethyl)-phenol, Copaene, Caryophyllene, Caryophyllene oxide, beta-Eudesmol, Sakuranin, Neophytadiene, (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 3,7,11,15- Tetramethylhexadec-2-en-1-ol, Ethyl linolenate, 2,3-Dihydroxypropyl palmitate, 1-Heptacosanol, 5-Hydroxy-4',7-dimethoxyflavanone, 1-Heptacosanol, 4-Acetyl-3-hydroxy-2,6-dimethoxytoluene, 2,4,6-Tris-(1-Phenylethyl)-phenol, beta,-Tocopherol, 5,7- Dihydroxy-8-methoxychroman-4-One, dl-,alpha,-Tocopherol, N-(1,3-Benzodioxol-5-yl)- 2- (2-thienyl)-4-quinolinecarboxamide, Tetra-O-methylscutellarein, 1-Eicosanol (Table 1). These compounds are showing significant antioxidant activity (P<0.01) in different plants. Even though several reports are available on antioxidant activity of *E.odoratum* major compounds responsible for antioxidant and its protective role through their anti-inflammatory response have not been reported. Phytol is an important component of all plants used in cosmetics, shampoos, toilet soaps, household cleaners as it shows antimicrobial, anticancer, antidiuretic activity. Interestingly, phytol show high antimicrobial against the food borne pathogens (Fig 6). Phytol is important in the processing of glucose and can activate enzymes within the body that have strong positive effects on insulin level. This means that phytol in the human diet could possibly help restore the metabolic functions of those with type-2 diabetes. 4-Acetyl-3-hydroxy-2,6-dimethoxytoluene, 4-Acetyl-3-hydroxy-2,6-dimethoxytoluene, Sakuranin, Methyl linolelaidate is most dominated antioxidant compound exists in the methanol extract of *E.odoratum*. Experimental reports already noticed that 4-acetyl-3-hydroxy-2,6-Dimethoxytoluene, Methyl commate D, β-Tocopherol, Phytol, α–Amyrin, Neophytadiene, γ(gamma)–Sitosterol and Stigmasterol have significant antibacterial activity as major components in the leaves of *E.odoratum*[21]. 4-Acetyl-3-hydroxy-2,6-Dimethoxytoluene and phytol show adjuvant and immunestimulatory activity[30]. This is first report based on the results of the experiment that all these compounds existing in the methanol and aqueous extract of *E.odoratum*. Our results directly correlate with other's data and enlighten the regular use of this plant leaves for healing of wounds and other skin diseases.

Figure-1: Antibacterial activities of different fractions of *E. odoratum* leaves

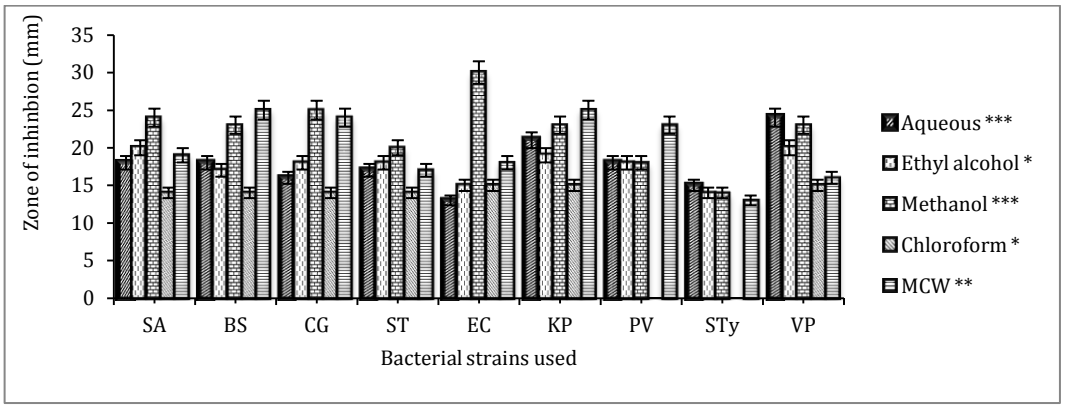


Figure-2: Antibacterial activities of different fractions of *E.odoratum* roots

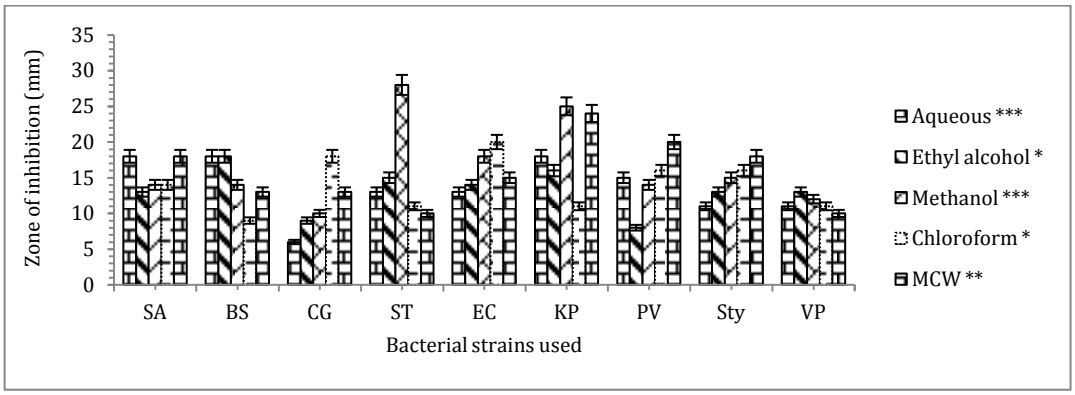


Figure-3: Antibacterial activities of different fractions of *E.odoratum* flowers

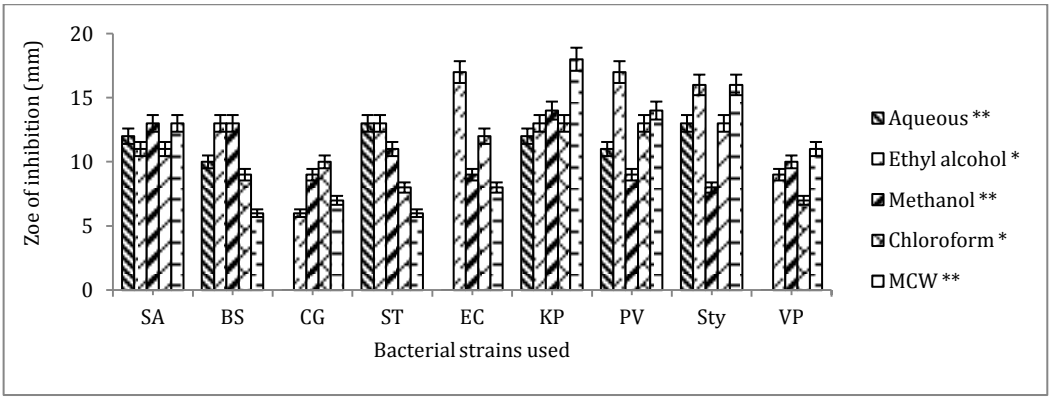
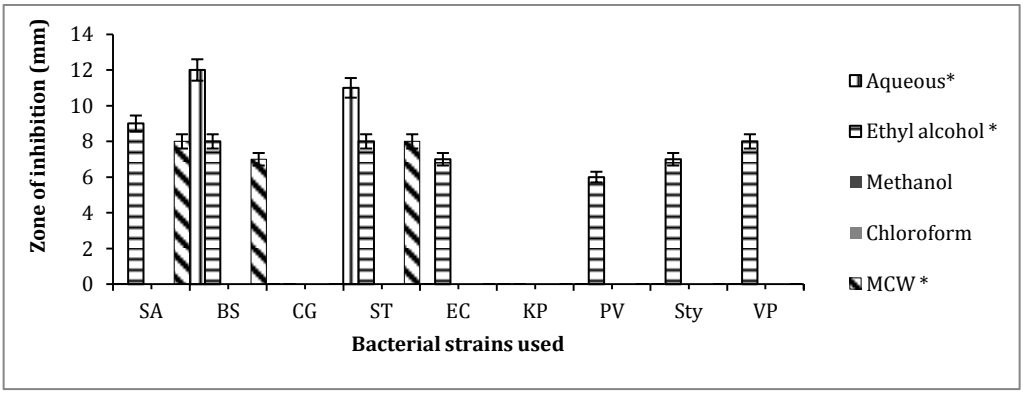
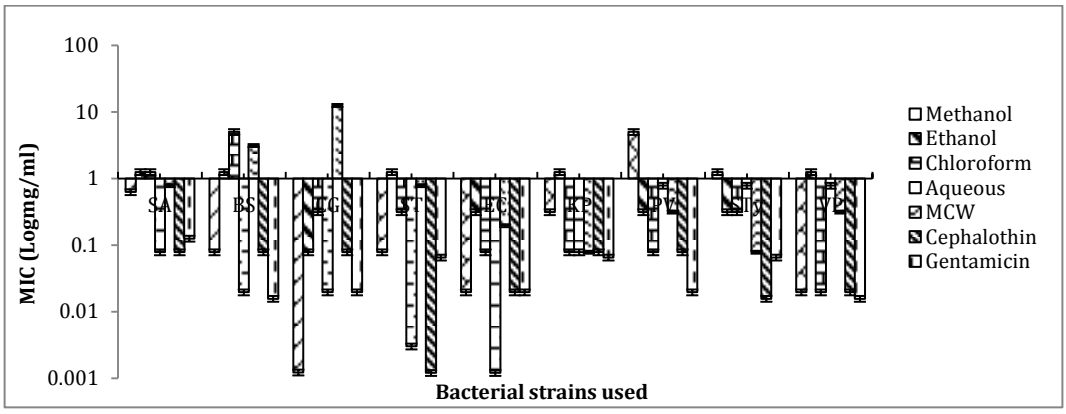


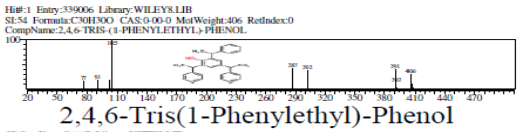
Figure-4: Antibacterial activities of different fractions of *E.odoratum* stem

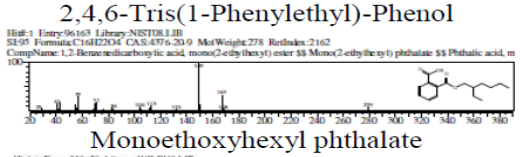


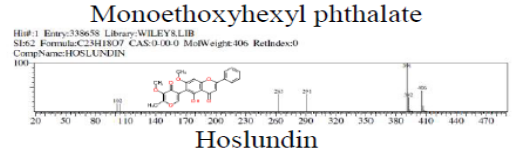
Figure–5: Minimum inhibitory concentrations of *E.odoratum* extracts

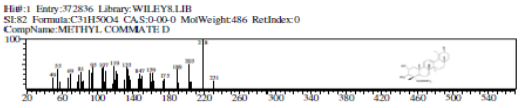


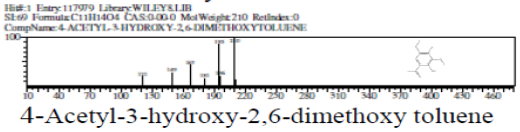
Figure–6: Mass spectrum and structure of phytocomponents identified by GC- MS in aqueous and methanolic extracts of *E.odoratum*

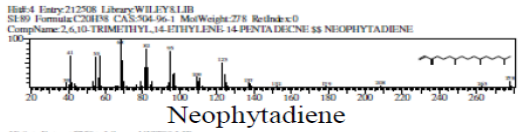

2,4,6-Tris(1-Phenylethyl)-Phenol

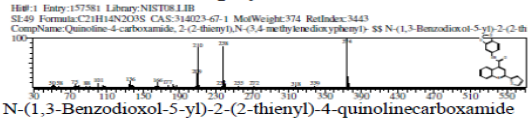

Monoethoxyhexyl phthalate

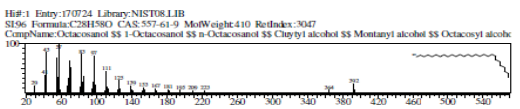

Hoslundin

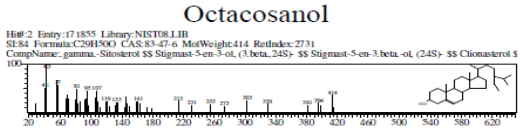

Methyl commate D

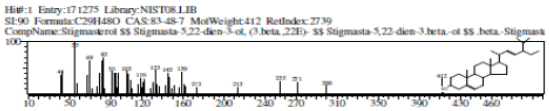

4-Acetyl-3-hydroxy-2,6-dimethoxy toluene

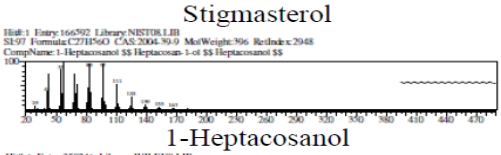

Neophytadiene

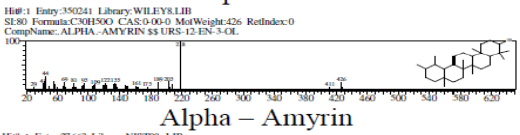

N-(1,3-Benzodioxol-5-yl)-2-(2-thienyl)-4-quinolinecarboxamide


Octacosanol


Gamma- sitosterol


Stigmasterol


1-Heptacosanol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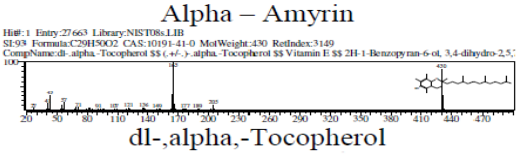
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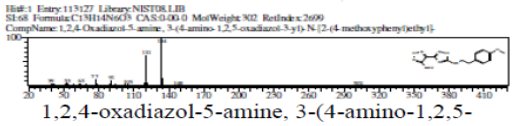
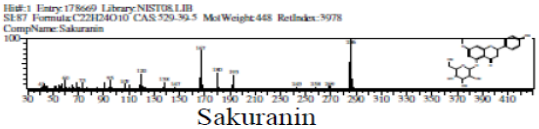
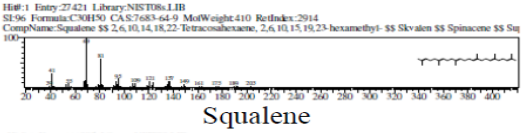
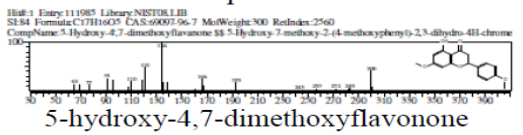
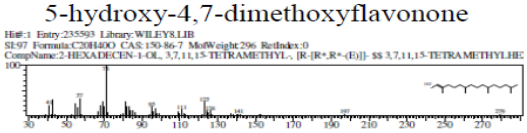

1,2,4-oxadiazol-5-amine, 3-(4-amino-1,2,5-oxadiazol-3-yl)-N-[2-(4-methoxyphenyl


Sakuranin


Squalene


5-hydroxy-4,7-dimethoxyflavonone


3,7,11,15- Tetramethylhexadec-2-en-1-ol

Inflammation is a complex biological response to the infection and tissue injury and is closely regulated by body as an inbuilt mechanism. Cyclooxygenase type-2 (COX-2), an important enzyme plays a key role in the induction of painful process by synthesizing prostaglandins and leukotriens. Several natural compounds especially flavonoids have been found to show inhibitory activity against COX-2. The following compounds identified in our analysis might have played a strong and direct anti- inflammatory role:

Methyl commate D, Phytol, β-Tocopherol, 1,2,4-Oxadiazol-5-amine, 3-(4-amino-1,2,5-oxadiazol-3-yl)-N-[2-(4-methoxyphenyl)]; 5-Hydroxy-4',7-dimethoxyflavanone, Sakuranin, Neophytadiene, Stigmasterol. α–Amyrin is a flavanoid glycoside, precursor for ursolic acid and also having many more derivatives existing abundantly in the plant kingdom and, are well known for their anti-inflammatory[31], antitumour, antimicrobial, alpha glucosidase inhibitory and anticancer, antiarthritic activity[32]. This α–Amyrin has three times more active than aspirin in their anti-inflammatory activity (Anti-nociceptive) where it is identified as strong COX-2 inhibitor and also inhibits collagen activated platelet aggregation[33]. Polymethoxy flavonoid (PMF) could be an important plant constituent as a natural anti-inflammatory compound with antipruritic, hepatoprotective and gastroprotective properties[34]. In an experiment α–amyrin showed consistent reduction of vascular endothelial growth factor (VEGF) expression, an effect that could account for a decreased angiogenesis-induced inflammatory response on colon tissues[35]. Tetra-O-methylscutellarein present in all citrus fruits is also a PMF found to exhibit a wide spectrum of biological activities and also showed antimutagenic activity[36]. These compounds prevent cancer and inflammation having excellent inhibitory activities against CoX-2 enzyme. Stigmasterol is another phytochemical identified as strong PAF receptor binding inhibitor, anti-inflammatory, antioxidant compound[23]. Data presented in this article provides more authentic and clear insight of phytoconstituents of *E.odoratum* to be used as plant based medicine (Table 1).

Table 1: Phytoconstituents identified in the aqueous and methanol extracts of *E.odoratum* by GC-MS

S.No	RT	Name of the compound	Molecular formula	Mol. Wt.	Peak area%	Activity*
Aqueous extract						
1.	16.825	o-(.alpha.-methylbenzyl) Phenol	$C_{14}H_{14}O$	198	2.07	Good antioxidant
2.	19.497	2-(2,4-di-tert-pentylphenoxyl)-butyric acid	$C_{20}H_{32}O_3$	320	3.41	Agrochemical intermediate
3.	21.419	n-Octadecyl ethanoate	$C_{20}H_{40}O_2$	312	2.44	Ant-repellent
4.	23.259	2,4,6-tris-(1-phenylethyl)-phenol**	$C_{30}H_{30}O$	406	20.77	Antibacterial and antioxidant
5.	24.143	Monoethylhexyl phthalate	$C_{16}H_{22}O_4$	278	15.71	Potent antimicrobial, antioxidant, anticancer
6.	27.713	Hoslundin	$C_{23}H_{18}O_7$	406	18.92	Significant action on Gonorrhooea, cystitis, hookworm, cough, fevers, colds, wounds bilharzias and also shows anti-malarial
7.	27.827	2,4,6-tris-(1-phenylethyl)-phenol	$C_{30}H_{30}O$	406	20.77	Antibacterial and antioxidants
Methanol extract						
8.	11.578	4-hydroxy-2-methylproline	$C_6H_{11}NO_3$	145	0.38	Anti–inflammatory
9.	12.490	Copaene	$C_{15}H_{24}$	204	0.35	Antimicrobial and antioxidant
10.	13.103	Caryophyllene	$C_{15}H_{24}$	204	0.54	Antimicrobial and antioxidant, Anti-tumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
11.	15.197	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.71	Antimicrobial, Anti-inflammatory, antioxidant, uses in manufacturing of Fragrances and Flavors of all types
12.	15.519	6,8-Nonadien-2-One, 8-Methyl-5-(1-Methylethyl)-, (E)-	$C_{13}H_{22}O$	194	0.69	Antimicrobial
13.	16.060	beta.-Eudesmol	$C_{15}H_{26}O$	222	0.55	Antimicrobial and antioxidant
14.	16.399	Mome Inositol	$C_7H_{14}O_6$	194	1.96	No activity reported
15.	17.829	Neophytadiene	$C_{20}H_{38}$	278	5.40	antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant
16.	18.085	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.13	Antituberculosis , insecticidal, anti-inflammatory, antioxidant, antimicrobial
17.	18.276	3,7,11,15- Tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296	1.71	Anti-inflammatory, antioxidant, antimicrobial
18.	20.548	Phytol	$C_{20}H_{40}O$	296	3.29	Antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immunostimulatory and anti-diabetic
19.	21.007	Methyl linolelaidate	$C_{19}H34O_2$	294	0.27	Antioxidant, catalase activator
20.	21.070	Ethyl linolenate	$C_{20}H_{34}O_2$	306	0.39	Antioxidant
21.	22.903	Dihydro-Neoclovene-(II)	$C_{15}H_{26}$	206	0.39	Antimicrobial
22.	24.052	2,3-Dihydroxypropyl palmitate	$C_{19}H_{38}O_4$	330	1.26	Antioxidant and anti-inflammatory
23.	25.349	1-Tricosanol	$C_{23}H_{48}O$	340	1.66	Antibacterial and antifungal
24.	25.581	5-Hydroxy-4',7-dimethoxyflavanone	$C_{17}H_{16}O_5$	300	1.87	Anti-inflammatory, Immuno Co-stimulatory enhancer, anticancer, antimicrobial, antioxidant
25.	26.150	Squalene	$C_{30}H_{50}$	410	2.35	Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and pathology, and Adjuvant activities.
26.	26.221	Sakuranin	$C_{22}H_{24}O_{10}$	448	1.28	Antiinflammatory, antiallergic, anticancer, Cox-2inhibitor
27.	26.291	1,2,4-oxadiazol-5-amine, 3-(4-amino-1,2,5-oxadiazol-3-yl)-N-[2-(4-methoxyphenyl]-	$C_{13}H_{14}N_6O_3$	302	3.31	Anti-inflammatory
28.	26.784	1-Heptacosanol	$C_{27}H_{56}O$	396	3.34	Nematicidal , anticancer, antioxidant and antimicrobial
29.	27.135	(3S)-7-O-Methoxymethylvestitol	$C_{17}H_{18}O_4$	286	5.85	Antioxidant

30.	27.460	4-Acetyl-3-Hydroxy-2,6-Dimethoxytoluene	$C_{11}H_{14}O_4$	210	8.34	Antioxidant activity, Food additive
31.	27.715	2,4,6-Tris-(1-Phenylethyl)-Phenol**	$C_{30}H_{30}O$	406	0.92	Antibacterial and antioxidants
32.	27.841	Beta,-Tocopherol	$C_{28}H_{48}O_2$	416	2.03	Antioxidant, anti-inflammatory antimicrobial, oestrogenic and insecticidal
33.	28.011	5,7- Dihydroxy-8-methoxychroman-4-One	$C_{10}H_{10}O_5$	210	1.97	Antioxidant, antibacterial, anti-inflammatory, antifungal, anticancer
34.	28.126	Octacosanol	$C_{28}H_{58}O$	410	2.52	Anticancer, cholesterol lowering effect, Anticoagulant, Increase stamina and improve strength and reaction time for athletes.
35.	28.358	dl-,alpha,-Tocopherol	$C_{29}H_{50}O_2$	430	3.06	Anti-inflammatory, antioxidant, antimicrobial , radical scavenging, antispasmodic
36.	28.685	N-(1,3-Benzodioxol-5-yl)-2-(2-thienyl)-4-pquinolinecarboxamide	$C_{21}H_{14}N_2O_3S$	374	2.09	Antimicrobial, antioxidant, anti-inflammatory, antifungal
37.	28.874	Tetra-O-methylscutellarein	$C_{19}H_{18}O_6$	342	5.22	Antioxidant, anti-diabetic, anti-inflammatory, antibacterial, anti-mycobacterial, Anticancer
38.	29.422	Stigmasterol	$C_{29}H_{48}O$	412	3.88	Anti-inflammatory, inhibit tumor promotion, anti-HIV reverse transcriptase, anti-inflammatory
39.	29.683	1-Eicosanol	$C_{20}H_{42}O$	298	1.49	Antimalarial, antifungal, Antioxidant
40.	29.971	gamma.-Sitosterol	$C_{29}H_{50}O$	414	4.38	Anti-diabetic, Anti-angeogenic, Anticancer, antimicrobial, anti-inflammatory, antidiarrhoeal and antiviral
41.	30.461	Alpha-Amyrin	$C_{30}H_{50}O$	426	7.57	Anti-diabetic, anti-inflammatory, Anti-arthritic Activity, anticancer, Three times more potent than aspirin
42.	30.969	Methyl commate D	$C_{31}H_{50}O_4$	486	8.55	Antimicrobial, anti-inflammatory
43.	31.304	Olean-12-en-3-yl acetate	$C_{32}H_{52}O_2$	468	1.18	Antimicrobial, anti-diabetic, anti-amylase inhibitor
44.	32.225	1,2-Epoxyoctadecane	$C_{18}H_{36}O$	268	2.96	No Activity reported

*** Source: Dr. Duke's Phytochemical and Ethnobotanical Databases; ** Present in both aqueous and methanol extract**

Several new compounds are also identified such as O-(alpha-methylbenzyl) phenol, 2-(2,4-di-tert-pentylphenoxyl)-butyric acid, Hoslundian, 1,2-Epoxyoctadecane, 4-Hydroxy-2-methylproline, n-Octadecyl ethanoate, (3S)-7-O-Methoxymethylvestitol. These molecules may add extra support to the antioxidant and anti-inflammatory activity of this plant. Octacosanol has been used to increase stamina and improve strength and reaction time in top athletes and also showed anticancer, anti-diabetic, cholesterol lowering effect. *E.odoratum* is uses as folklore medicine for treatment of various ailments where poultice of leaves applied on cuts or wounds to stop bleeding, promote quick healing. These have been thoroughly described in this study, improving our understanding of the folklore use of this plant for the treatment of different skin based problems by tribal's must be considered to effectively use them in various experimental systems. Our reports claiming that this activity is due to the presence of a potent anticoagulant, Octacosanol reported in other plants[37]. Therefore, we conclude that *E.odoratum* is a highly valuable medicinal plant having different compounds with antioxidant, Anti-inflammatory, wound-healing and other activities proving the folklore use of this plant by the tribal's. Compounds identified in the aqueous and methanol extracts are highly precious showing extra pharmacologically activities along with anticancer, Immuostimulatory, anti-diuretic, antipyretic and analgesic activities. This in-depth investigation on compounds present in the aqueous and methanol extracts that make this study novel and useful. In addition, this study provides evidence that the compounds we identified are well characterized in various other rare plants. Getting of rare plants for treatment of different diseases is a difficult task. This might be the reason for preparation of several mixtures of folklore medicine with *E. odoratum* as cost effective and safe medicine like Eupolin for the treatment of different ailments. The results are in accord with tribal belief for which they use as traditional medicine for different bioactivities and treatment of ailments.

Acknowledgments

Dr. B.V.Raman gratefully acknowledges UGC, New Delhi for the financial support in the form of UGC Research Award.

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