Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF ROOTS OF *HERACLEUM RIGENS* WALL EX DC.

LINGARAJU D P[*1] AND SUDARSHANA M S[2]

*[*1]Department of Botany, AVK college for women, Hassan, Karnataka, India.*
[2]Department of studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India.

ABSTRACT

The purpose of present work is to study antimicrobial activity and medicinally active principles present in different solvent extracts obtained from roots of *Heracleum rigens.* The active principles were isolated by Soxhlet extractor using petroleum ether, chloroform, ethyl acetate and methanol and identified by preliminary phytochemical test. The results of analyses of each extract confirm the active substances were alkaloids, sterols, triterpenes, glycosides and flavonoids. The antimicrobial tests of isolated substances were performed against bacteria- *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomons aeruginosa,* and a fungus *Candida albicans.* The results revealed that ethyl acetate extract has significant antimicrobial activities. *Heracleum rigens* revealed the highest antibacterial activity at a minimum inhibitory concentration (6.25µg/ml) against *P.aeruginosa* and highest antifungal activity at a MIC (1.56µg/ml) against *C.albicans*. The results provide justification for the use of the *H.rigens* in folk medicine to treat various infectious diseases.

KEYWORDS: ***Heracleum rigens*, Phytochemical screening, Secondary metabolites, Antibacterial activity, Antifungal activity.**

LINGARAJU D P
Department of Botany, AVK college for women, Hassan, Karnataka, India.

***Corresponding author**

INTRODUCTION

Plants are principal source of raw material for plant-based medicine since ancient times. Globally about 85% of the traditional medicines used for primary health care are derived from plants[1]. This is because, medicinal plants are easily available natural products and cost effective with negligible or no side effects[2]. Even today 80% of the world population in developing countries relies on traditional medicine for their health care[3].The phytochemical research based on ethno-pharmacological information is generally considered as an effective approach in the discovery of new anti-infective agents from higher plants[4]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, glycosides and tannins[5] . These substances are useful to control the growth of microorganisms and plants are the possible source of antimicrobial agents[6]. It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects[7,8,9]. Now microorganisms have become resistant to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines[10]. So, it has become necessary to find out new antimicrobial agents. The aim of the present study is to investigate the antimicrobial properties of *Heracleum rigens* (Fam: Apiaceae), an endangered aromatic medicinal plant. In this study we are reporting the results of preliminary phytochemical screening and antimicrobial activity of different solvent extracts of roots of *H.rigens* in order to orient future investigations towards the finding of new potent and anti-infectious compounds.

Majority of the plants belonging to the family Apiaceae are aromatic with hollow stems commonly known as umbellifers, used as traditional ethnomedical remedies and more than100 cultivated species are registered for several uses[12]. *Heracleum rigens* is a perennial herb belonging to Apiaceae distributed in the high altitudes of Western Ghats of India. In Ayurveda, *H. rigens* has been traditionally used for urinary disorders, cough, hyperacidity, wounds, abdominal disorders, and cardiac diseases and vomiting. In Siddha, it is used for treating constipation, stomachache, diarrhoea, headache, phlegm, gastric disorders and indigestion[11]. The seeds of *H. rigens* are reported to contain a group of phyto-constituents called as coumarins like 5-(3-methyl but-2-enyloxy) 7 methoxy coumarin, isopimpinellin and 8-hydroxy, 5-methoxy furanocoumarin[13]. Coumarins comprise a very large class of phenolic derivatives and consist of fused benzene and α-pyrone rings. *H.rigens* is reported to have anticancer and anti-inflammatory activity[14]. Despite its extensive use in traditional treatment, detailed studies focusing on antibacterial and antifungal properties have not been carried out. In view of this, in the present study an attempt is made to evaluate the antimicrobial activities of different solvent extracts of root of *H. rigens.*

MATERIALS AND METHODS

(i) Collection of plant materials

Fresh roots of *H. rigens*, collected from the hilly slopes of Thalthare shettyhalli village (Somwarpet taluk, Kodagu district, Karnataka state) were used for the preparation of aqueous and different organic solvent extracts. A voucher specimen of the plant has been deposited in the herbarium of Department of studies in botany, University of Mysore, Mysore.

(ii) Preparation of Solvent extracts

The roots of *H. rigens* were washed thoroughly 2-3 times with running water and once with sterile water, chopped into small pieces, shade dried, coarse powdered in a mechanical grinder, sieved and used for extraction. The dried material (50gm) was extracted with petroleum ether, chloroform, ethyl acetate, and methanol in the increasing order of their polarity by using Soxhlet apparatus[15].The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated using a rotary flash evaporator and preserved at 5^{o}C in air tight bottle until further use. All the extracts were subjected to antibacterial activity assay and preliminary phytochemical analysis.

(iii)Test microorganisms

The test microorganisms used were *Escherrichia coli* MTCC 7410*, Pseudomonas aeruginosa* MTCC 7408*, Bacillus subtilis* MTCC 121 *, Staphylococcus aureus* MTCC 7443 and a fungus - *Candida albicans* MTCC 183*.* All these microorganisms were obtained from Herbal Drug Technology Laboratory, Department of studies in Microbiology, University of Mysore, Mysore, Karnataka.

(iv) Evaluation of antimicrobial activity by agar well diffusion method

The antimicrobial activities of different solvent extracts of roots of *H. rigens* were evaluated by agar well diffusion method[16]. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5 ×10^{8} cru/ml. 20 ml of agar media was poured into each Petri plate and plates were swabbed with 100 µl inoculate of the test microorganisms. Using sterile cork borer of 6 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 50µl volume with

concentration of 50 mg/ml of different solvent extract constituted in the respective solvents used for extraction. All the plates were incubated at 37˚C for 24 h. Antimicrobial activity of different solvents extracts were evaluated by measuring the zone of growth inhibition against the test microorganisms. The well with pure solvents used for extraction was used as a negative control whereas Gentamicin (standard antibacterial drug) and Nystatin (standard antifungal drug) were used as positive control at concentration of 10 µl/well from 1mg/ml of stock solution to confirm that all the microorganisms tested were inhibited by the antibiotic. The experiments were performed in triplicates and results analyzed for statistical significance.

(v) Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) were evaluated by the microbroth dilution test in an 96-well microtitre plates using standard inocula consisted of 2×10^6 CFU/ml for bacteria and 2×10^5 cfu/ml respectively. Two-fold serial dilutions of the test compounds, dissolved in solvents used for extraction were prepared to final concentrations of 200 - 0.18 µg/ml in Mueller-Hinton Broth and Sabourauds dextrose broth for bacteria and fungi respectively. To each well 10 µL of microbial inocula were added. In the tests, triphenyltetrazolium chloride (TTC) and (MTT) methyl thioazyltetrazolim bromide (Aldrich Chemical Company Inc., USA) at concentration of 0.05ml was added to the culture medium as a growth indicator. The microbial growth was determined by the absorbance at 600 nm using a universal microplate reader after incubation at 37 ˚C for 24 h for the bacteria, and at 26 ˚C for 48h for the fungi. The MIC is defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth.

(vi) Phytochemical screening

The preliminary phytochemical tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, flavonoids, triterpenes, sterols, tannins and reducing sugar with method of Trease[17], Harborne[18] and Edeoga et al[19].

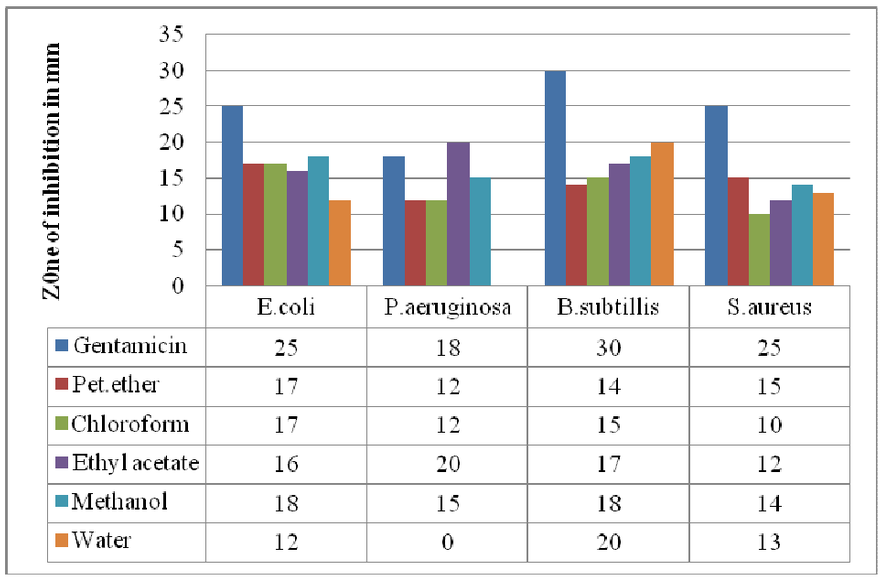
RESULTS AND DISCUSSION

1. Results of antibacterial activity

Results of Antibacterial activity of different solvent extracts of roots of H.rigens by agar well diffusion method (zone of inhibition in mm)

Compound	*E.coli*	*P.aeruginosa*	*B.subtilis*	*S.aureus*
Gentamycin	25	18	30	25
Pet .ether extract	17	12	14	15
Chloroform extract	17	12	15	10
Ethyl acetate extract	16	20	17	12
Methanol extract	18	15	18	14
Aqueous extract	12	12	20	13

The antibacterial activity of the all solvent extracts is presented in Table 1. All the extracts showed broad spectrum activity against the bacterial strains used. The ethyl acetate and methanol extract showed significant antibacterial activity as compared to chloroform and petroleum ether extracts. The aqueous and ethyl acetate extracts showed highest inhibitory activity (20 mm) against *P. aeruginosa* and *B. subtilis* respectively. The lowest inhibitory activity (10 mm) was recorded for *S. aureus*. The maximum inhibition zone was observed for *S.aureus* followed by *P.aeruginosa* and *B.subtilis.* Gentamicin (standard antibacterial drug) have shown inhibitory activity ranged from 18 mm to 30mm at a concentration of 10µl/well from 1mg/ml of stock solution. Comparative efficacy with gentamicin is highly encouraging. The zone of inhibition of Gentamicin against *P.aeruginosa* is 18mm and that of ethyl acetate is 20mm (Fig 1.). Antibacterial characteristics of plants from *Heracleum* genus have relatively slightly been researched. Brkovic et al., reported that ethanol, ethylacetate extracts of plant species showed significant antibacterial effect on pathogenic bacteria[20].



	E.coli	P.aeruginosa	B.subtillis	S.aureus
Gentamicin	25	18	30	25
Pet.ether	17	12	14	15
Chloroform	17	12	15	10
Ethyl acetate	16	20	17	12
Methanol	18	15	18	14
Water	12	0	20	13

Figure 1
Antibacterial activity of different solvent extracts of roots of H. rigens (Zone of inhibition in mm)

The result of MIC assay is shown in Table 2. Ethyl acetate and methanol extracts exhibited the highest antibacterial efficacy against *P.aeruginosa* at 6.25 µg/ml concentration when compared to Gentamicin(12.5 µg /ml)- a standard antibacterial drug. Ethyl acetate extract showed the highest antifungal efficacy against *C.albicans* at 1.56 µg /ml concentration when compare to Nystatin (3.12 µg /ml). In this study, *P.aeruginosa* and *C,albicans* were found to be sensitive to ethyl acetate extract of root of *H.rigens.* This may be due to the presence of flavonoids and triterpenes.

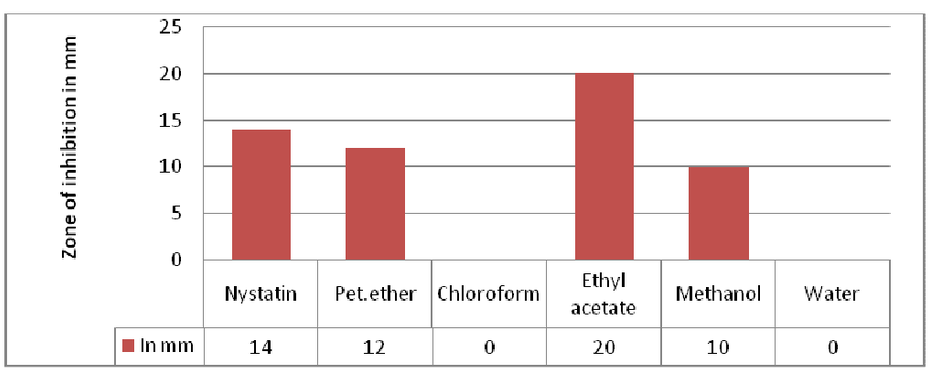
Table 2
MIC (µg/ml) of solvent extracts of root of H.rigens against bacteria.

Compound	*E.coli*	*P.aeruginosa*	*B.subtilis*	*S.aureus*	*C.albicans*
Gentamycin for bacteria	3.12	12.5	0.39	1.56	NT
Nystatin for fungi	NT	NT	NT	NT	3.12
Pet .ether extract	100	50	100	100	6.55
Chloroform extract	100	50	100	200	-
Ethyl acetate extract	100	6.25	100	200	1.56
Methanol extract	150	6.25	50	100	25
Aqueous extract	200	-	50	200	-

NT – Not tested, '- ' Not sensitive

2. Results of antifungal activity

Except chloroform and aqueous extracts, all other solvent extracts showed antifungal against *C.albicans* (Fig 2). The methanol extract showed lowest inhibitory activity (12 mm) and the ethyl acetate extract showed highest inhibitory activity (20 mm). Nystatin (standard antifungal drug) have shown 14mm inhibitory activity at a concentration of 10µl/well. This is mainly due to the presence of phenolic compounds and terpenoides in the root extracts of *H.rigens*.



	Nystatin	Pet.ether	Chloroform	Ethyl acetate	Methanol	Water
In mm	14	12	0	20	10	0

Figure 2
Anticandidial activity of solvent extracts of roots of Heracleum rigens

3. Results of phytochemical screening

Table 3
Qualitative preliminary phytochemical analysis of root extracts of H. rigens.

Phytocontituents	Tests	PE	CL	EA	MN	AQ
Alkaloids	Dragendroff's test	+	+	+	+	-
	Wagner's test	+	+	+	+	-
	Meyer's test	+	+	+	+	-
Glycosides	Keller Killani test	+	+	+	+	+
	Molisch's test	+	+	+	+	+
Flavonoids	Shinoda test	-	-	+	+	+
	Ferric chloride test	-	-	+	+	+
	Lead acetate test	-	-	+	+	+
Triterpenes	Libermann-Burchard test	-	+	+	+	-
	Salkowski's test	-	+	+	+	-
Sterols	Libermann-Burchard test	-	+	+	-	-
	Salkowski's test	-	+	+	-	-
Tannins	Gelatin test	-	-	-	-	-
	Ferric chloride test	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-
Carbohydrates	Benedict's test	-	-	+	+	+
	Fehling's test	-	-	+	+	+

' + ' Present ' - ' Absent
PE- Petroleum ether, CL- Chloroform, EA-Ethyl acetate, MN- Methanol, AQ- Aqueous

It is clear from the experimental data presented in Table-3 that the substances like alkaloids, triterpenes, sterols, glycosides, and flavonoids are medicinally active components of roots of *H. rigens*. The petroleum ether extract confirms the presence of alkaloids and glycosides. The chloroform extract confirms the presence of Alkaloids, sterols, triterpenes and glycosides. The ethyl acetate extracts confirms the presence of alkaloids, glycosides, flavonoids, triterpenes, sterols and sugars. The methanol extract confirms the presence of alkaloids, glycosides and flavonoids. But the extracts do not contain the tannins and saponins. Alkaloids are widely used in medicinal purposes which have positive and negative effects even to human beings. Most of the plants have alkaloids in different organs with different chemical configurations[21] . Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against diseases and endurance against stress[22]. Many researchers reported that the presence of alkaloids in the plants cure asthma[23]. High degree precipitation of alkaloids found in all extracts of *H. rigens* root. The presence of alkaloid may be the reason why the infusion of roots and seeds of *H.rigens* are given orally in tribal areas to cure asthma.

Flavonoids show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity[24] . They exhibit activity against a wide range of gram positive bacteria as well as fungi[25]. Flavonoids are found in ethyl acetate, methanol and aqueous extracts. Glycosides were present in all solvent extracts. Phenolic compounds are reported to act as anti-tumor agents and to exhibit antiviral and antimicrobial activities[26] and antioxidant properties[27]. Ethyl acetate and methanol extracts of root of *H.rigens* showed highest phenolic content. The presence of the phenolic compounds in these studied samples proved that they had antimicrobial and antifungal effect. Similar reports were reported in methanol leaf extracts of *Oxalis corniculata*[28]. Mahato and Sen reported that terpenoids had wound healing properties[29]. *Staphylococcus aureus, Escherrichia coli, Pseudomonas aeroginosa, Streptococcus pneumonia, streptococcus pyogens* are some important organisms causing wound infection[30].Terpenoids are active against bacteria[31] and fungi[32] . They show excellent activity against *B. subtilis, S. aureus* and lesser activity against Gram negative bacteria as well as *C. albicans*[33]. Chloroform, ethyl acetate and methanol extracts showed the presence of triterpenes. The present study records the scientific validation of this plant for use as an anti-infective agent. The presence of triterpenes may be the reason why the infusion of roots of *H. rigens* is given orally in folk-medicine to cure diarrhoea ; and root paste is applied on wounds to heal up early .

CONCLUSION

It is expected that screening and scientific evaluation of different solvent extracts of roots of *H.rigens* for its antimicrobial activity may provide new antimicrobial substances ; hence the present investigation clearly reveals the antibacterial and antifungal nature of this plant and suggests that *H.rigens* could be exploited in the management of diseases caused by these bacteria in human systems. It is an evident that the findings of the present study can provide a basic concept for synthesizing a new drug. It will also help to isolate new antibiotic substances that control the infectious disease causing microbial pathogens. The presence of antimicrobial activity in *H.rigens root* extracts give support to their traditional use for treating conditions associated with

microorganisms in humans and consequently seems to fight against multi-resistant microbes.

ACKNOWLEDGEMENT

We extremely thankful to the Principal, AVK College for Women, Hassan, HOD of department of Botany and Department of Microbiology, University of Mysore, Mysore and also to our colleagues for their constant support and valuable suggestions during this study.

Conflict of Interest

Conflict of interest declared none.

REFERENCES

1. Farnsworth J D, Screening plants for new medicines, Inwilson E O, (Ed), Biodiversity, National academy press, Washington D C: 83-97(1988).
2. Kamboj V P, Herbal medicine, Curr sci., 78(1) :35-39 (2000).
3. Anonymous, ethonobotany in India – A status report : Ministry of Environment and forests, Government of India, (1994)
4. Duraipandiyan V, Ayyanar M and Ignacinthu S, Antimicrobial activity of some ethnomedicinal plants used by paliyar tribe from Tamilnadu ,India, BMC complimentary and alternative medicine, 635 (2006).
5. Edeoga H O, Okwu DE and Mbaebre BO, Phytochemical constituent of some Nigerian Medicinal Plant, Afr .J. biotechnol., 4(7) : 685-688 (2005).
6. Sibi G, Parul Chatly, Sayak Adhikari and Ravikumar K R, Phytoconstituents and their influence on antimicrobial properties of *Morinda citrifolia* L, Res J of Med Pl., 1-8 (2012).
7. Vaghasiya Y, Dave R and Chanda S, Phytochemical analysis of some medicinal plants from Western region of India, Res J of Med Pl., 1- 6 (2011).
8. Mahesh B and Sathish S, Antimicrobial activity of some important medicinal plants against plant and human pathogens, Word J Agric Sci., 4:839-843 (2008).
9. Thenmozhi M and Rajeshwari Sivaraj, Phytochemical analysis and antimicrobial activity of Polyalthia longifolia, Int J Pharm bio sci., 1(3):1-7(2010).
10. Amit Pandey and Parul Singh, Antibacterial activity of *Syzygium aromaticum(*clove*)* with metal ion effect against food borne pathogens , Asian J plant sci res.,1(2):69-80 (2011).
11. Ana CT, Maria JG, Maria TC, Carlos C and Maria Clorge C, Essential oil from Distichoselinum tenuifolium: chemical composition , cytotoxicity, antifungal and anti-inflammatory properties, J ethnopharm., 130: 593-598 (2010).
12. Yoganarasimhan SN, Medicinal plants of India: Karnataka,vo l I, Interline publishing PVT Ltd (1996).
13. Saraswathy A, Sasikala E and Purushothaman K K, Chemical investigation on *Heracleum rigens* wall, Ind drugs., 27: 316-319 (1990).
14. Jagannath N, Hanumanthaiah, Ramakrishnaiah and Venkatarangaiah Krishna., Antiinflammatory and anticancer activity of Heracleum rigens, J phytopharm., 3(1): 61-67 (2012).
15. Kokate CK, Practical Pharmacognosy, Vallabh Prakashan, New Delhi, 145-147(1999).
16. Anonymous, Pharmacopoeia of India, 3rd Edition,(Ministry of health and Family welfare)' Government of India, New Delhi (1994).
17. Trease G E and Evans WC Pharmacognosy, 13th Edition, Balliere Tindall, Landon, 176-180 (1989).
18. Harborne J B, Phytochemical Methods ; A Guide to Modern Techniques of Plants Analysis,Chapman & Hall, London, England, 3rd Edition (1998).
19. Edeoga H O and Eriata D O, Alkaloid, tannin and saponin contents of some Nigeria medicinal plants. *J Med Aromatic Pl Sci.,* 23: 344-49 (2001).
20. Brkovic L. Dusko , Ljiljana Comic and Slavic Sukdolac , antibacterial activity of some plants from family Apiaceae in relation to selected phytopathogenic bacteria, Kra J Sci.,28: 65-72 (2006).
21. Harborne J B, Introduction to Ecological Biochemistry, 3rd Edition, Academic Press, London: 10- 15 (1984).
22. Gupta S S, Prospects and perspectives of natural plant products in medicine, Ind J Pharmacol., 26: 1-12 (1994).
23. Mary J, Lobelia herb treats respiratory problems and more. Http://ezine articles.com. screening of Suran. Pharmacolonline., 1: 189-94(2009).
24. Aiyelaagbe O O and Osamudiamen P M, Phytochemical screening for compounds in *Mangifera indica* leaves from Ibadan, Oyo state., Pl. Sci. Res., 2: 11-13 (2009).
25. Afolayan A J and Meyer J J, The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens.* Ethno pharmcol., 57: 177-78 (1997).
26. Robbins R, Medical and nutritional aspects of citrus bioflavonoids. In: Nagy S. and Attaway J, Eds). Citrus nutrition and

quality, American chemistry society, Washington, DC: 43-59 (1980).
27. Robak J and Gryglewski RJ, Flavonoids are scavengers of superoxide anions. Biochem Pharmacol., 37: 837-41 (1988).
28. Raghavendra M P, Sathish S and Raveesha K A., Phytochemical analysis and antibacterial activity of *Oxalis corniculata* - A known medicinal plant, My Sci., 1: 72-78 (2006)
29. Mahato SB and Sen S., Advances in triterpenoids research, 1990-1994, Phytochem*.,* 44: 1185-1236 (1997).
30. Puttanayak S P and Sunitha P, Wound healing, antimicrobial and antioxidant potential of Dendrophthoe falcate, J Ethno pharmcol., 120 :241-247(2008).
31. Amaral J A, Ekins A, Richards SR and Knowles R, Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture, Appl Environ Microbiol.,64: 520-25 (1998).
32. Ayafor J F, Tchuendem MHK and Nyasse B, Novel bioactive diterpenoids from *Aframomum aulacocarpos.* Int J Nat Prod., 57: 917-23(1994).
33. Hufford C D, Jia Y, Croom E M, Muhammed I, Okunade AE and Rogers, Antimicrobial compounds from *Petalostemum purclim*, J Nat Prod.,56: 1878-89 (1993).