Iranian Medicinal Plants Society

Ethno-Pharmaceutical products

Journal homepage:Http://js.kgut.ac.ir

Ministry of Science Research and Technology
Graduate University of Advanced Technology

Antibacterial effect of *Acorus calamus* extractions against gram positive and negative bacteria

Samaneh Rahamoz-Haghighi[1], Malek Hossein Asadi[2*], Ali Riahi-Madvar[2], Amin Baghizadeh[2]

1- Department of Plant breeding, Faculty of Sciences and Modern Technologies, Graduate University of Advanced Technology, Kerman, Iran
2- Department of Biotechnology,Institute of Science and High Technology and Environmental Sciences,Graduate University of Advanced Technology, Kerman, Iran

Article information

Article history:
Received: 5 May 2013
Accepted:12 Sep. 2013
Available online: 15 Mar. 2014
EPP 2014; 1 (1):1-7

Keywords:
Acorus calamus
Anti-bacterial effect
Staphylococcus aureus
Escherichia coli

*Corresponding author:
Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran.
E-mail:
Hossein_a1359@yahoo.com

Abstract

Acorus calamus that grows in province of Baluchistan and Kerman in Iran plays a major role in the revival of traditional medicine. Despite the useful role of this plant in many ailments, its anti-bacterial properties are not well understood. In this study, we investigated anti-bacterial activities of rhizomes of *A. calamus* against various strains of bacteria such as *Staphylococcus aureus* and *Escherichia coli*. The anti-microbial activity of extracts of *A.calamus* was assessed by disc diffusion method. Rhizomes extracted by ethanol, methanol solvents and essential oil were obtained by clevenger apparatus. Antibacterial effects of the extracts tested at different concentrations (100, 200, 250, 300 and 400 mg/ml). The Minimal Inhibitory Concentration (MIC) of extracts ranged from 25-100 mg/ml against the susceptible bacteria. All the Minimal Bactericidal Concentrations (MBCs) were the same as the MICs. Our results revealed that ethanolic and methanolic extracts have an inhibitory effect on all gram positive and negative strains and it is comparable with kanamycin, an anti-bacterial reference drug. Amongst extracts of *A.calamus* , ethanolic extracts showed maximum inhibitory activity (16mm) against *Staphylococcus epidermidis.* So we can conclude that this plant has anti-bacterial properties on all gram positive and negative bacteria. The result can be related to the nature of the compounds found in this plant. The effective GC-MS method was performed for the determination of essential oil compounds of *A.calamus*. The Gas Chromatography and Mass Spectrometry (GC-MS) analysis revealed that the presence of phenyl propanoids, monoterpenes, sesquiterpenes and β-asarone in essential oil of the plant caused its antibacterial properties. Our results showed that the ethanolic and methanolic extracts of *A. calamus* could be useful for the development of effective treatment for the control of infectious diseases.

Introduction

The clinical efficacy of many existing antibiotics is being endangered by the appearance of multi drug resistant pathogens (Bandow et al., 2003). It is important and urgent to discover new antimicrobial compounds with varied chemical structures and novel mechanisms of action for new and reappear infectious diseases (Benkeblia., 2004). Therefore, researchers are increasingly turning their attention to herbal medicine, looking for new leads to improve better drugs against microbial infections (Murugaian et al., 2009 & Manikandan et al., 2010). The increasing failure of chemotherapeutics and antibiotic resistance, showed by pathogenic microbial infectious agents has been led to the screening of some medicinal plants for their potential antimicrobial activity (Manikandan et al., 2010 & Srikumar et al., 2007). Plants have been a significant source of medicine for thousands of years. Even today, the World Health Organization (WHO) estimates that up to 80 percent of people still use traditional remedies such as herbs for their medicines and more than 50% of chemotherapeutic agents immediately in using have been derived from herbaceous products (Gurib-Fakim., 2006) and plants are a potential source of many modern medicines. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active

ingredients obtained from plant substances (Tripathi L & Tripathi JN., 2003).

Acorus calamus is classified as Kingdom: Plantae, Division: Magnoliophyta, Class: Liliopsida, Order: Acorales, Family: Acoraceae, Genus: Acorus, Species: calamus and Other species: *Acorus gramineus*, *A. aromaticus* , *A. calamus* var. *americanus* (Balakumbahan et al., 2010 ; Alaf et al., 2010 & Singh et al., 2011). *Acorus calamus Linn*, known as Sweet Flag, belongs to the Araceae family (Adoraceae) and also called as *Acorus odoratus*. *A.calamus* is a herbaceous and perennial with a long indefinite branched cylindrical rhizome which is about 3.4 inches in diameter, smooth, pinkish or pale green. Its leaf scars are brown, white and spongy. It possesses slender roots, few leaves and distichously alternate (Raja et al., 2009).Several previous and recent studies have described many important biological activities, particularly antimicrobial properties of *A. calamus* rhizome (Grosvenor et al., 1995; Mungkornasawakul., 2000; MacGaw et al., 2002; Rani et al., 2003 & Phongpaichit et al., 2005). *A.calamus* is traditionally used as medicinal plants for treatment of bacterial infection in India (Panchal et al., 1989). This plant possesses important medicinal properties (Vohora et al., 1990; Rahman & Shereen., 2002) and many commercial drugs have been prepared from that (Ka et al., 2005). In this study, we have investigated the potential antibacterial effect of methanolic and ethanolic extractions of *A. calamus* against gram positive and negative bacteria. Furthermore, GC-MS was performed for identification of bioactive compounds of essential oil of *A.calamus*.

Materials and Methods

- **Preparation of alcoholic extracts**

Approximately 20 grams of dried rhizome of *A. calamus* was ground to a coarse powder and placed in a soxhlet extractor containing 500 ml of each of solvents as ethanol or methanol for 24 hrs. Then obtained extracts were concentrated in a rotatory evaporator under reduced pressure at 45°C for 75 minutes. Extracts were stored in refrigerator at 4 °C for further use. The extracts of *A.calamus* were dissolved in dimethyl sulphoxide (DMSO) for preparation of different concentrations (100, 200, 250, 300, 400 mg/ml). Aqueous extract was obtained by maceration for 24 hrs, and then the extractions were boiled for 3 hrs. Extracts were stored in refrigerator (4°C) for further use.

- **Microorganisms**

The standard pathogenic bacterial culture (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*) were obtained from the Graduate University of advanced Technology and are used in the present study. Culture collection of bacteria were grown in Mueller- Hinton broth (MHB) (QUELAB) at 37 °C for 18hrs and then in Mueller-Hinton Agar (MHA) (Lin et al., 1999). Bacteria suspensions were prepared on the basis of 0.5 McFarland and inoculated on the MHA agar.

- **Preparation of Disc for antibacterial activities**

The ethanolic and methanolic extracts were prepared in solvents, the sterile blotting paper disc (Whatman #2paper, 6 mm in diameter) was soaked in the diluted extract, and the disks left to dry and remove the excess of solvents. These disks were used for antibacterial activity assessment.

- **Disc Diffusion Method**

The Disc diffusion method was employed to determine the antibacterial activity of both ethanolic and methanolic extracts of *A.calamus*. Turbidity of inoculums was matched with the McFarland turbidity standard. Inoculums were spread over the MHA using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc was placed over the lawn and pressed slightly along with positive and negative controls. The sterile 6mm diameter paper discs were impregnated with 3µl of extracting dissolved in dimethylsulfoxide (DMSO) and Kanamycin 30 µg/ml were used as positive control while disc soaked in DMSO as negative control. The plates were incubated for 24 hours at 37°C. The antibacterial activity and diameter of inhibition zones were then evaluated. An experiment was carried out in three times and the average diameter of area of inhibition was recorded.

- **MIC and MBC determination**

For determination of anti-bacterial effect of *A. calamus* the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ethanolic and methanolic extracts were performed. The MIC was determined by tube dilution assay which is standard bacterial suspension and ranging concentrations of extracts (25, 50 and 100 mg/ml) were added to the tubes, that each tube was containing 1 ml of MHB, 1ml suspension bacteria and 1ml of extract. These tubes were incubated at 37 °C for 24 hrs. The first tube in the above series with no sign of apparent growth was considered as the MIC. The MBC was specified by culturing one standard loop of the tubes with no apparent growth

on MHA and subsequent incubation at 37 °C for 24 hrs. The lowest concentration that inhibited colony configuration on agar was considered as the MBC.

- **Preparation of Essential oil**

The shade dried rhizome (100 g) of *A. calamus* was ground to a coarse powder for hydrodistillation in a clevenger apparatus for 3hour to derive the volatile constituents in the form of essential oils. Each volatile oil was dried over anhydrous sodium sulphate and then kept separately in sealed clean glasses vials at 4°C until needed.

- **Gas chromatography-Mass spectrometry analysis**

Gas chromatography–mass spectrometry analysis of the *A.calamus* essential oil performed by 0.1 µl of the pure oil sample,as subjected to GC-MS analysis. The GC-MS was composed of an Agilent Technology GC 7890A , MS 597SC , capillary column (30m × 250µm × 0.25µm, ID = 0.32mm). The carrier gas was helium (1.0 ml min^{-1}), the injector temperature was 250°C, and the oven temperature was programmed from 50 to 260°C at a rate of 5°C min^{-1} and finally held isothermally for 52 minutes.

- **Statistical analysis**

The data were analyzed using SAS and SPSS softwares. Duncan test was used to determine siginificant differences among treatments.

Results

- **Determination of antibacterial activity by using Disc Diffusion**

Antimicrobial activity of extracts showed that the ethanolic extract has maximum inhibition at concentrations of 400 mg/ml against *Staphylococcus epidermidis* (16mm) and methanoli extracts at 100mg/ml has minimum inhibition against Escherichia Coli (7mm).

Two extracts have higher effect against bacteria in higher concentrations in comparison to lower concentration. The ethanolic extract of *A. calamus* was active against both gram positive and negative bacterial strains, and its antibacterial effects was higher than metanolic extracts against all strains(fig.1). In the other hand, the extracts have the most effects in 400mg/ml concentration against bacteria specially *Staphylococcus epidermidis*(fig.1). Aqueous extract of *A. calamus* was inactive against all strains (data not showed). *Staphylococcus epidermidis* was the more sensitive gram positive strain than *Staphylococcus aureus* when in treating with the extracts.

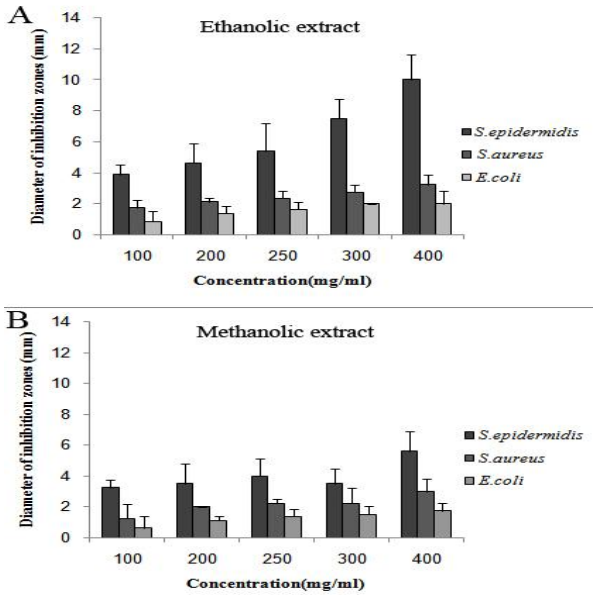

Fig. 1) Disk diffusion assay performed by the effect of the extracts against clinical isolate of bacteria.

A) Ethanolic extract has a significantly higher effect against *Staphylococcus epidermidis* in comparison to other bacteria, *Staphylococcus aureus* and *Escherichia Coli.*

B) Methanolic extract also has a similar effect against the bacteria as similar as ethanolic extract.

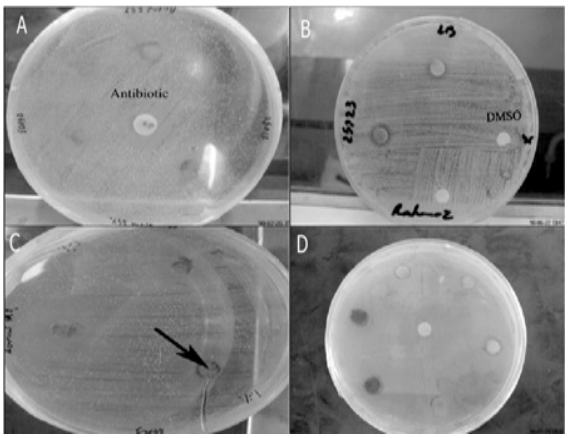

Fig.2) Zone of inhibition of ethanolic extract (250 mg /ml) of *A.calamus.* Diameter of zone of inhibition of kanamycin (A) as a positive control, *Staphylococcus aureus* (B), *Staphylococcus epidermidis* (C), *Escherichia coli* (D). Dimethylsulfoxide in the middle plate is showed in as negative control (D).

Table 1. MBC in the presence of *A.calamus* extracts (25-100 mg/ml)

Extracts	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Escherichia Coli*
Ethanolic extract	25	50	100
Methanolic extract	25	50	100

- **Minimum Inhibitory Concentration (MIC)**

To determine the MICs of ethanolic and methanolic extracts, concentrations of 25, 50, and 100 mg/ml were prepared. Our results showed that extractions in concentration of 25 mg/ml caused inhibition of growth of *S.epidermidis* after 24 hours of treatment. Further more, the extractions caused inhibition of growth of *S.aureus* in concentration of 50 mg/ml, and ethanolic extract is more effective than metanolic extract in 100 mg/ml dose against *E.coli.*

- **Minimal Bactericidal Concentration (MBC)**

Minimal Bactericidal Concentration (MBC) of ethanolic and methanolic extracts are showed in table 1. All the Minimal Bactericidal Concentrations (MBCs) were as same as the MICs.

- **GC-MS**

The compositions of the essential oil of *A.calamus* have been analyzed by GC-MS. The components identified by according to Wiley7n.1 library. We have reviewed the Chemical constituents and biological activity of *A.calamus.* Different parts of the plant showed the presence of a large number of phenyl propanoids, monoterpenes and sesquiterpenes as isomers of asarone (Table 2). Our present study directed that *A.calamus* essential oil having anti-bacterial compounds. According to the library database, the spectra of the compounds match with the Anethole,Cymene, Asarone, Camphor, Carvacol, Aristolene spectra and other anti-bacterial compounds includedthymol, γ-cadinene, β-cedrene, terpinolene linalool, trans-methylisoeugenol, zingiberene, limonene, eugenol, s-carvone, cinnamaldehyde, α-cedrene and terpinenol-4-ol. The peak of one of important components of essential oil showed a small amount of asarone-type derivatives (0.23-3.37%) (Table 2).

Table 2. Identified antimicrobial compounds in Essential oil of *Acorus calamus* by GC-MS analysis.

Peak number	Compound	*Area*
1	*Camphene	*0.58%*
2	*o-cymene, o-cymol and p-Cymene	*4.75%*
3	*Limonene, dl-limonene	*0.40%*
4	*Gamma-terpinene	*3.80%*
5	* alpha-terpinolene, L-linalool, β-Linalool	*0.98%*
6	*Camphor	*3.36%*
7	*3cyclohexen-1-ol,4-methyl-1-(1-meth…) or Terpinenol-4-ol	*0.19%*
8	*Estragole	*2.35%*
9	*Carvacrol	*3.08%*
10	*2-cyclohexan-1-one,2-methyl-5-(1-methye..) and ((S)-Carvone)	*0.26%*
11	*Cinnamaldehyde	*0.21%*
12	*Anethole, trans-anethole	*12.22%*
13	*Thymol	*1.87%*
14	*Eugenol	*0.30%*
15	*1h-3a 7-methanoazulene or α-Cedrene	*0.20%*
16	*Beta-elemene	*0.42%*
17	*Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-or γ-Cadinene and β-Cedrene	*1.35%*
18	(-)-aristolene, *Aristolene or Aristolen	*2.10%*
19	*1(5),3-aromadenedradiene and 5-n-butyltetralin and 4,5-dehydro-isolongifolene	*0.23%*
20	*Calarene	*3.10%*
21	(-)-5-epiprezizaene and (-)-piprezizaene	*0.42%*
22	(-)-5-epiprezizaene and (-)-piprezizaene	*1.90%*
23	*Isohomogenol, *trans-methyl isoeugenol and cis-methyl isoeugenol	*0.73%*
24	(-)-beta-acoradiene and gamma-curcumene	*0.21%*
25	*Aristolene and Zingiberene	*0.42%*
26	benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- or Ar-Curcumene	*0.41%*
27	*Valencene l and *α-Gurgujene	*1.67%*
28	*Alpha-selinene	*3.78%*
29	*cyclohexanone,3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(.. Or shyobunone	*7.57%*
30	*delta-cadinene and beta-cadinene	*1.03%*
31	2-hexyl-1-decan-3-yne and 2 ,5-cyclohexadiene-1 4-dione and 2 6-bis(1 1-dimethylethyl)-4a…	*5.78%*
32	*Calacorene and 5,8-dimethylisoquinoline	*1.21%*
33	*cyclohexanemethanol,4-ethenyl-alpha,alpha,4-trimethyl-3-…and Elemol	*0.20%*
34	*Calacorene and alpha-calacorene	*0.87%*

35	*1h-cycloprop[e]azulen-7-ol,decahydro--… or Spathulenol, (+)spathulenol and (-)-spathulenol	*0.91%*
36	*isoaromadendrene epoxide and vulgarol B and Junipene or longifolene	*0.52%*
37	*trans-isoelemicin and *cis-asarone or β-asarone and *Isoelemicin	*0.23%*
38	*dehydroxy-isocalamendiol	*14.73%*
39	*cis-asarone or Asarone- trans	*3.37%*
40	2,5-diethyl-3,6-dipropylpyrazine and 5-methylene-10-oxo-10,…and 4a,5,8,8a.beta-tetrahydro-2-methoxy-4a...,	*2.69%*
41	1-t-buty-2,4-dimethyl-1-cyclohexene and 3,4-dihydro-6-fluorocoumarin and spiro[4.5]dec-8-en-7-ol,4,8-dim…	*1.08%*
42	Valencene	*0.36%*
43	Isocyclocitral	*0.64%*
44	*spiro[4.5]decan-7-one,1,8-dimethyl-…, 1-t-buty-2,4-dimethyl-1-cyclohexene and 3,4-dihydro-6-fluorocoumarin	*0.42%*
45	(-)-isoledene	*1.14%*
46	*1,4-cis-1,7-cis-acorenone , *1,4-cis-1,7-trans-acorenone	*5.68%*
47	*Isocalamendiol	*0.28%*

*These compounds have been obtained by Wiley7n.1Library. With using of their CAS numbers,the popular names were determined and based on previous review, natural of the compounds and some of their properties were also determined. Antibacterial properties of the compounds were given and each of the compound may be have other properties. The specified compounds which determined by * have been repeated in similar articles.

Discussion

Because of the excessive using of antibiotic, it seems that the clinical efficacy of many existing antibiotics is being endangered by the appearance of multidrug resistant pathogens (Bandow et al., 2003). The researchers had tried to discover new antimicrobial compounds with varied chemical structures and novel mechanisms against the infectious diseases from new sources (Benkeblia., 2004). Hence, Researchers' attentions are increasingly focused on herbal medicine, are looking for better and improved drugs against microbial infections(Murugaian et al., 2009 & Manikandan et al., 2010). Plants have been considered as an important source of secondary metabolites which are useful in medicine. The World Health Organization (WHO) estimates that the most of people in the world still use traditional remedies such as herbs for their medicines and more than 50% of chemotherapeutic agents immediately in use were derived from Herbaceous products (Gurib-Fakim., 2006). Despite the assessment of antimicrobial activities of *A. calamus* by different researchers, its antimicrobial activity is not well understood.In the present study, we examined the effects of extracts of *A.calamus* against three strains of bacteria to evaluate their antimicrobial activity by using MIC and MBC. At first, we observed the ethanolic extract has a better antibacterial effect in comparison to methanolic extract. It was concluded that ethanol is a better solvent for secondary metabolites which have a better antibacterial effects. Our results showed that the methanolic extract as same as ethanolic extract exhibited maximum zone of inhibition at 400mg/ml concentration against bacteria, and *Staphylococcus epidermidis* was the most sensitive strain to the extracts in comparison to other strains.

Our result showed that the ethanolic extract has a most antimicrobial effect on *S. epidermidis* and the minimum effect on *E.coli*, among all of the bacteria were tested. Our obtained data are in agreement with the previous reports that showed the plant extracts were more active against gram positive bacteria than gram negatives (Rabe & Staden., 1997). We have also examined the antibacterial activity of the methanolic extracts on the species, so our data revealed that the *S. epidermidis* was the most sensitive and *E.coli* has the most resistance to methanolic extract. According to the study of Phongpaichit et al., (2005) crude methanolic extract of *A. calamus* showed inhibition activity against *S. aureus* and *E. coli* and have observed more less antibacterial activity on properties of the *A. calamus* rhizome. Devi et al., (2009) showed that the ethyl acetate extract of the rhizome has no effect against *Staphylococcus aureus* while in the present study the ethanolic and methanolic extracts were identified that has anti-bacterial effect against these bacteria. It is suggested that this difference originated from the difference location where the plants growth and it results in the difference in secondary metabolites which have antibacterial properties. Finally, our data revealed that the extracts of rhizome of *A.calamus* have anti-bacterial activity, so we identified the components of essential oil of *A.calamus* by using GC-MS. Many antibacterial compounds were observed in essential oil such as thymol, γ-cadinene, β-cedrene, terpinolene linalool, trans-methylisoeugenol, zingiberene, limonene, eugenol, s-carvone, cinnamaldehyde, α-cedrene and terpinenol-4-ol. It must be mentioned one of the important compounds which observed in essential oil was the asarone , the known antibacterial compounds.

Altogether, our results showed that the alcoholic extracts of rhizome of *A.calamus* possess components that can be used as a new source of antibacterial drugs for the therapy of infections caused by pathogenic bacteria.

Acknowledgements

This study was supported by Graduate University of advanced Technology, grant no. 1/984.

References

1- Altaf A, Shashidhara S, Rajasekharan PE, Hareesh KV, Honnesh NH. Invitro regeneration of AcorusCalamus – an important medicinal plant . J Curr Pharm Res. 2010; 2(1): 36-39.

2- Balakumbahan R, Rajamani K, Kumanan K. Acorus Calamus: An overview. J Med Plants Res. 2010; 4(25): 2740-2745.

3- Bandow JE, Leichert HB, Leichert LIO, Labischinski H, Hecker M. Proteomic approach to understanding antibiotic action. Antimicrob Agents Chemother. 2003; 47(3): 948-955.

4- Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). Lebensm.-Wiss. u.-Technol. 2004;37:263-268.

5- Devi A, Ganjewala D. Antimicrobial activity of Acoruscalamus (L.) rhizome and leaf extract. Acta Biologica Szegediensis. 2009;53(1):45-49.

6- Grosvenor PW, Suprino A, Gray DO. Medicinal plants from Riau Province, Sumatra,Indonesia. Part 2; antibacterial and antifungal activity. J Ethnopharmacol. 1995; 45(2):97-111.

7- Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Asp Med. 2006; 27(1): 1-93.

8- Ka MH, Choi EH, Chun HS, Lee KG. Antioxidative activity of volatile extracts isolated from Angelica tenuissimae roots, peppermint leaves, pine needles, and sweet flag leaves. J Agric Food Chem. 2005; 18(53):4124-9.

9- Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK, Staden JV. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol. 1999; 68: 267-274.

10- Manikandan S, Sheela DR, Srikumar R, Thangaraj R, Ayyappan R, Jegadeesh R, Hariprasath L. In-vitro Antibacterial activity of aqueous and ethanolic extracts of Acorus calamus. Int J Appl Biol Pharm Tech. 2010; 1 (3):1072-1075.

11- McGaw LJ, Jager AK, Van SJ. Isolation of B-asarone, an anti-bacterial and anthelmintic compound, from Acoruscalamus in South Africa. S Afr J Bot. 2002; 68(1):31-35.

12- Mungkornasawakul P. Fungicide from Acorus calamus Linn, Eugenia caryophyllus Bullock ET Harrison and MammeasiamensisKosterm. And their Residues after Application. M. Sc. Thesis. Chiang Mai: Chiang Mai University, 2000.

13- Murugaian P, Srikumar R, Thangaraj R. Phytochemical estimation and antibacterial activity of Heliotropium indicum(L) against the common bacteria isolated from human beings. J Biomed. 2009; 29(1): 48-51.

14- Panchal GM, Venkatakrishna BH, Vajpayee S. Pharmacology of Acorus calamus L. Indian J Exp Biol. 1989; 27(6): 561-567.

15- Phongpaichit S, Pujenjob N, Rukachaisirikul V, Ongsakul M. Anti-microbial activities of the crude methanol extract of Acorus calamus Linn. M J Sci Technol. 2005; 27(2):517-523.

16- Rabe T, Staden JV. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol. 1997; 56: 81-87.

17- Rahman M, Shereen G. Antibacterial activity of hydrodistilled essential oil of Psammogeton canescens N.O. umbelif-erae. Pak J Biotechnol. 2002; 1: 155-160.

18- Raja AE, Vijayalakshmi M, Devalarao G. Acorus calamus linn: Chemistry and Biology Research. Pharm J Tech. 2009; 2 (2): 256-261.

19- Rani AS, Satyakala M, Devi VS, Murty US. Evaluation of antibacterial activity from rhizome extracts of Acorus calamus Linn. J Sci Indust Res 2003; 5:621-623.

20- Singh R, Sharma PK, Malviya R. Pharmacological Properties and Ayurvedic Value of Indian Buch Plant (Acorus calamus): A Short Review, J Adv Biol Res. 2011;5 (3): 145-154.

21- Srikumar R, JeyaParthasarathy NJ, Shankar EM, Manikandan S, Vijayakumar R, Thangaraj R, Vijayananth K, Sheeladevi R, Usha A, Rao A. Evaluation of growth inhibitory activities of Triphala against common Bacterial Isolates from HIV infected patients. J Phytother Res. 2007; 21(5): 476-480.

22- Tripathi L, Tripathi JN. Role of biotechnology in medicinal plants. Trop J pharm Res. 2003; 2:243-53.

23- Vohora SB, Shah SA, Dandiya PC. Central nervous system studies on an ethanol extract of Acoruscalamus rhizomes, J Ethnopharmacol. 1990; 28(1):53-62.