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Antimicrobial activity of supercritical fluid extracted *Acorus calamus* Oil against different microbes

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Abstract
Sweet flag rhizome *(Acorus calamus* L.) which belongs to family *Araceae* and is a perennial, semi-aquatic plant found in both temperate and sub-temperate zones. Super critical fluid extracted sweet flag rhizome oil has good antimicrobial activity. The rhizome oil showed maximum antimicrobial response against Gram positive bacterium *Staphylococcus aureus* with a diameter of zone of inhibition about 9.2 mm. Whereas the zone of inhibition diameter for *Escherichia coli* and *Pseudomonas aeruginosa* was observed to be 7.5 mm and 6.9 mm respectively. Maximum activity of sweet flag rhizome oil was also reported against fungal pathogens. Sweet flag rhizome recorded zone of inhibition of 12.5 mm against *Mucur species.* Whereas zone of inhibition of 11.5 and 10.3 mm against *Aspergillus species* and *Pencillium.* The minimum inhibitory concentration of SC-CO, extracted sweet flag rhizome oil found maximum zone of inhibition for *Psedomonas aeruginosa* (9.3 mm) and for *Staphylococcus aureus* (8.1 mm) and these were more sensitive at concentration of 100 µl/ml for sweet flag rhizome oil, whereas *Escherichia* coli was not at all sensitive at the concentrations used.

Keywords: *Acorus calamus*, antimicrobial activity, supercritical fluid extraction

Introduction
The interest to study the *Acorus calamus* plants is because of its medicinally and pharmacologically important active ingredients. Plant produces a plethora of natural products, such as alkaloids, flavonoid, glycoside, steroid, saponin and resin which has often been correlated with medicinal and pharmacological properties of the plants. The development of resistance to existing antibiotics and increasing public concern over environmental pollution and toxicity generated a continuing need for new antibiotic agents.
Mother earth has gifted the mankind with lots of plants which has the ability for curing the health disorders of human beings. This feature was identified in the pre-historic times [3], resulting in the world wide use of herbal therapies and health care preparations that are prescribed in the ancient books for discovers of natural products with medicinal values [5]. Eighty percent of the world population meet their primary health care through traditional medicine. As is estimated by World Health Organization (WHO). Medicinal plants possess secondary metabolites which are the main sources of medicinal drugs having curative nature. Seven thousand five hundred species are being used as medicinal plants in India [3]. One of them is sweet flag, whose botanical name is *Acorus calamus.*
Acorus calamus is a native of Eastern countries and is indigenous to the marshes of the mountains of India. It is cultivated throughout India in the marshy tracts of Kashmir, Shirmaur (Himachal Pradesh), Manipur, Nagahills and Koratagere taluka of Karnataka state in peninsular India [24]. The rhizomes of *Acorus calamus* possess spasmolytic [12], ectoparasiticide and insect repellant [11], antisecretagogue, antiulcer and cytoprotective [23], antidiarrheal [12], hypolipidemic [20], anthelmintic and antibacterial [10], neuroprotective [22], antioxidant [31], larvicidal [29], bio pesticide [25], antiproliferative and immunosuppressive [17] and antifungal [14], properties.
It is a good sedative so that the extract is used for epilepsy, insanity and as a tranquillizer along with *valeriana jatamansi* and *nardostacys grandiflora*. It is an ingredient of an ayurvedic preparation "Brahmi Bati" (Budhivardhav) which is used cases of epilepsy, coma, hysteria and mental retardation; the same uses are prescribed for an *Acorus* containing Unani drug "Majun Baladur". The component β-asarone obtained from sweet flag rhizome has antimicrobial, insecticidal and pesticidal properties [3].
In the present work, the super critical fluid extracted *Acorus calamus* rhizome oil was analysed

for its antimicrobial activity against different microbes.

Materials and Methods

Plant material

The sweet flag rhizome (*Acorus calamus* L.) was procured from the local market of Raichur, Karnataka (India). The dried rhizome was grinded by using water cooled pulverizer and sieved. The chemicals, reagents (analytical and HPLC grade) and pure standard of β-asarone were purchased from M/s. Himedia Chemical Co., Bengaluru.

Supercritical Fluid/ Supercritical carbon dioxide (SC-CO_2) Extraction

Sweet flag rhizome oil is extracted by supercritical fluid extraction method at 200 bar pressure and 45 °C temperature at constant dynamic extraction time of 120 min. Extraction yield (16), extraction efficiency [19] and β-asarone content is calculated using HPLC (2 & 26).

Antimicrobial activity

Evaluation of antimicrobial properties of sweet flag rhizome extract

Antimicrobial sensitivity test

Disc agar diffusion technique described by Bauer *et al*. (1996) and demonstrated by Cakir *et al*. (2004) was employed for antimicrobial bioassay.

Sweet flag rhizome with their wide variety of chemical constituents offer a promising source of antimicrobial agents, with general as well as specific antimicrobial activity. There are several reports on the presence of anti-microbial compounds in sweet flag rhizome. Rhizome extracts have been screened for their antimicrobial properties especially due to the presence of phenolic compounds like β-asarone [21].

Test organisms, bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus* and fungi such as *Mucor species* and *Aspergillus species* were used for this assay (MTCC, Chandigarh, India).

The inoculam were prepared from the stock cultures, which were maintained on nutrient agar slant at 4 °C and sub cultured to nutrient broth using a sterilized wire loop and maintained for further study [8, 18].

Paper discs (6 mm) were made from Whatman No.1 filter paper. Stock solution (400 mg/ml) of the rhizome extract was prepared by dissolving 0.8 g of each extract in 2 ml dimethyl sulphoxide (DMSO) [6].

For testing antimicrobial activity against bacteria and fungi, 15 ml of growth medium and 5 ml of inoculum were mixed and poured in separate sterilized petriplates. Each mixture sample was thoroughly shaken to ensure uniform distribution of inoculum. Sterile paper discs were immersed in 0.1 ml of the solution for test in test samples. All the test petriplates were kept at 5 ºC for 40-50 min so as to allow the diffusion of the substances and then incubated at 35-37 ºC for 18 h. The inhibition zones formed by the sweet flag rhizome extract were measured [30]. Experiment was carried out in three replicates.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration for bacterial and fungal isolates was carried out using test tube dilution technique as described by Akinyemi *et al*. (2005). Stock solution of 80 µl in 10ml sterilized distilled water was serially diluted to get different concentrations of 25 µl /ml, 50 µl /ml, 75 µl /ml and 100 µl /ml [6].

Results and Discussion

Anti-microbial profile of SC-CO_2 extracted sweet flag rhizome oil against selected foodborne pathogens

SC-CO_2 extracted sweet flag rhizome oil had varying degree of antibacterial and antifungal activities against the test organisms. Sweet flag rhizome oil recorded highest diameter zone of inhibition of *Staphylococcus aureus* 9.2 mm (Table 1). Zone of inhibition formed by the sweet flag rhizome oil against *Escherichia coli* and *Pseudomonas aeruginosa* were 7.5 mm and 6.9 mm (Fig. 1) respectively. Antimicrobial activity of sweet flag rhizome oil was not effective against *Escherichia coli* and *Pseudomonas aeruginosa*. Similar findings were reported by Manikandan *et al*. (2010).

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 less antibacterial activity on properties of *Acorus calamus* rhizome. Devi *et al.* (2009) showed that the ethyl acetate extract of the rhizome had no effect against *Staphylococcus aureus* while in the present study the ethanolic and methanolic extracts were identified that had anti-bacterial effect against these bacteria. It is suggested that this difference originated from the different location where the plants had grown and it resulted in the difference in the profile of secondary metabolites which had antibacterial properties [13].

Sweet flag rhizome recorded a zone of inhibition of *Mucur species* 12.5 mm, Where as sweet flag rhizome oil recorded zone of inhibition of 11.5 and 10.3 mm against *Aspergillus species* and *Pencillium*, respectively. Similar findings were reported by Srividya *et al.* (2014).

Determination of MIC of SC-CO_2 sweet flag rhizome oil for food borne pathogens

Sweet flag rhizome oil had the broadest spectrum activity on the test bacteria species. The results showed that the maximum zone of inhibition recorded by *Psedomonas aeruginosa* (9.3 mm) and *Staphylococcus aureus* (8.1 mm) and these isolates were more sensitive at concentration of 100 µl/ml for sweet flag rhizome oil, whereas *Escherichia coli* was not at all sensitive at the concentrations used.

The fungal isolates were more sensitive compared to bacterial isolates. The data found that *Aspergillus species* (11.3 mm) and *Mucor species* (11.2 mm) were more sensitive at concentration of 100 µl/ml for sweet flag rhizome oil.

The different concentrations of SC-CO_2 extracted sweet flag rhizome oil were effective against some food borne pathogens. SC-CO_2 extracted sweet flag rhizome oil was more active against bacterial isolates viz, *P. aeruginosa* (9.3 mm) and *S. aureus* (8.1 mm). These organisms were more sensitive to the concentration 100 mg/ml of sweet flag rhizome oil.

The antibacterial properties of the sweet flag rhizome shown in the present study corroborate the earlier claims by Sujina *et al*. (2012) and Haghighi *et al.* (2014), who reported on the antibacterial properties of sweet flag rhizome.

A recent study by Phongpaichit *et al.* (2005) has revealed strong antimicrobial properties of crude methanol extracts of sweet flag rhizome. In their study, they found that methanol extract of the rhizome exhibited high activity against filamentous fungi. From the different studies it is clear that the differences in the effectiveness (MIC values) could be due to the solvents used for extraction of active ingredients from the concerned plants parts or climatic and geographical differences. The sensitivity of the microorganisms to the

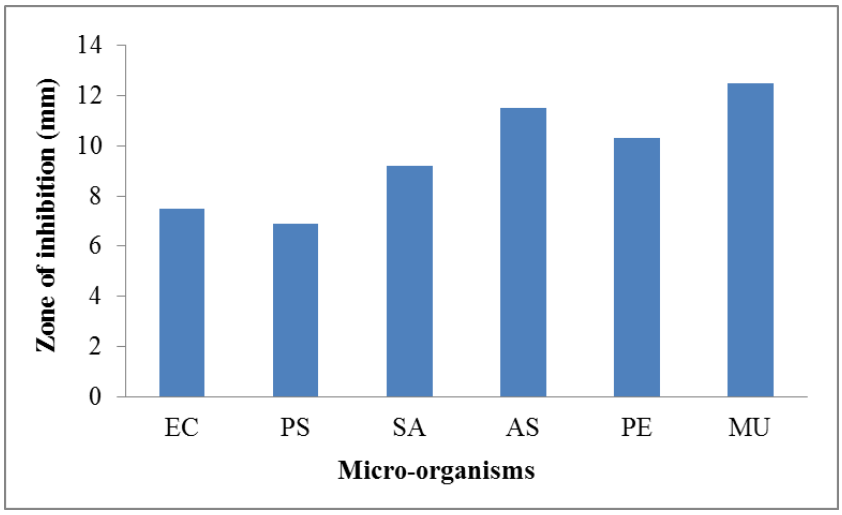
rhizome and leaf extract could be due to morphological as well as difference in the cell wall constitution of the microorganisms tested by Devi *et al.* (2009).

Table 1: Antimicrobial activity of SC-CO_2 extracted sweet flag rhizome oil

Sweet flag rhizome oil	Zone of inhibition (mm)					
	EC	**PS**	**SA**	**AS**	**PE**	**MU**
Sweet flag rhizome oil	7.5	6.9	9.2	11.5	10.3	12.5
EC= *Escherichia coli*			PS= *Pseudomonas aeruginosa*			
SA= *Staphylococcus aureus*			AS= *Aspergillus species*			
PE= *Pencillium*			MU= *Mucor species*			

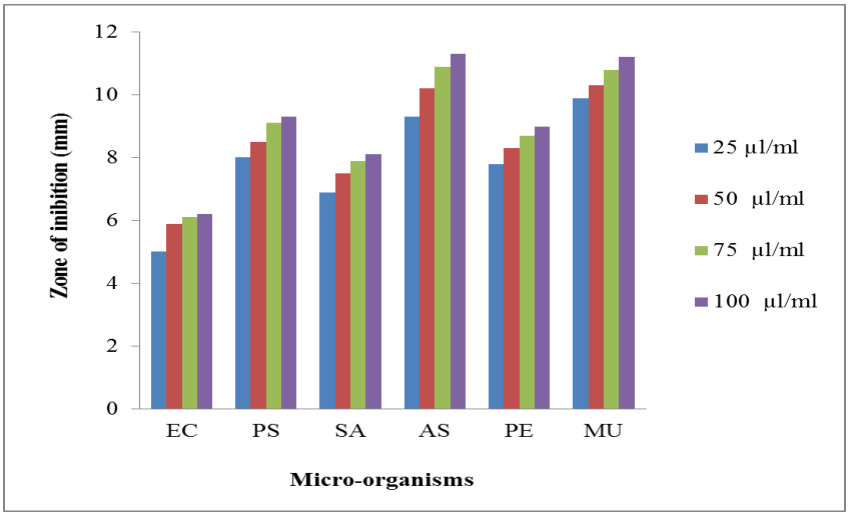
Table 2: Determination of minimum inhibitory concentration (MIC) of SC-CO_2 extracted sweet flag rhizome oil against bacterial and fungal pathogens

Sweet flag rhizome oil	Conc. (µl/ml)	Zone of inibition (mm)					
		EC	**PS**	**SA**	**AS**	**PE**	**MU**
Sweet flag rhizome oil	25	5.0	8.0	6.9	9.3	7.8	9.9
	50	5.9	8.5	7.5	10.2	8.3	10.3
	75	6.1	9.1	7.9	10.9	8.7	10.8
	100	6.2	9.3	8.1	11.3	9.0	11.2
EC= *Escherichia coli*		PS= *Pseudomonas aeruginosa*					
SA= *Staphylococcus aureus*		AS= *Aspergillus species*					
PE= *Pencillium*		MU= *Mucor species*					



EC= *Escherichia coli* PS= *Pseudomonas aeruginosa*
SA= *Staphylococcus aureus* AS= *Aspergillus species*
PE= *Pencillium* MU= *Mucor species*

Fig 1: Antimicrobial activity of SC-CO_2 extracted sweet flag rhizome oil



EC= *Escherichia coli* PS= *Pseudomonas aeruginosa*
SA= *Staphylococcus aureus* AS= *Aspergillus species*
PE= *Pencillium* MU= *Mucor species*

Fig 2: MIC of different micro-organisms at different concentrations of the extracted sweet flag rhizome oil

Conclusion

The extraction yield of sweet flag rhizome oil is 3.15 % and efficiency is 90.08% and the concentration of β-asarone was 26.80 per cent in sweet flag rhizome oil. The results showed that the zone of inhibition was recorded at maximum of *Staphylococcus aureus* (9.2 mm). The oil was found to have strong antifungal activity against *Mucur species* (12.5 mm) and less activity against *Aspergillus species* (11.5 mm) and *Pencillium* (10.3 mm), respectively. The highest MIC value of antibacterial activity was found in *Psedomonas aeruginosa*. MIC value of antifungal activity was found high in *Aspergillus species* (11.3 mm) and *Mucor species* (11.2 mm) and the lowest value was found in *pencillium* (9 mm), at concentration of 100 µl /ml. The SC-CO_2 extracted sweet flag rhizome oil was found to be strong activity against *Staphylococcus aureus*, *Mucor* and *Aspergillus.*

References

1. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for antimethicillin resistant *S. aureus* activity. BMC Complimentary Alternative Medicine. 2005; 5(6):175-268.
2. Avadhani MMN, Selvaraj IC, Rajasekharan PE, Rajasekaran C, Tharachand C, Rao VK *et al*. Asarone content and cytogenetic studies of medicinally important Indian *Acorus calamus* L., Accessions. Int. J Pharm. Sci. Rev. Res. 2013; 22(2):73-78.
3. Balakumbahan P, Rajamani K, Kumanan K. An overview of Acorus calamus. J Med. Plants Res. 2010; 4(25):2740-2745.
4. Bauer AW, Kibry WMM, Sherris, Turk M. Antibiotic suceptibality testing by a standardized single disc method. Am. J Clin. Pathol. 1996; 45(1):493-496.
5. Bhuvaneswari R, Balasundaram C. Anti-bacterial activity of *Acorus calamus* and some of its derivative. J Med. Plants Res. 2007; 3(7):538-547.
6. Bukar A, Uba A, Oyeyi TI. Antimicrobial profile of *Moringa oleifera* extracts against some food borne microorganisms. Bayero J Pure Applied Sci. 2010; 3(1):43-48.
7. Cakir A, Kordali S, Zengin H, Izumi SH, Hirata T. Composition and antifungal activity of essential oils isolated from *Hypericumhus sopifolium* and H. heterrophyllum. J Flavour Frag. 2004; 19(1):62-68.
8. Cheesbrough M. Medical laboratory manual for tropical countries ELBS edition. *Tropical health publications*, UK, 2002.
9. Devi A, Ganjewala D. Antomicrobial activity of *Acorus calamus* (L.) rhizome and leaf extract. *Acta Biologicalzegediensis.* 2009; 53(1):45-49.
10. Gaw MC, Jager AK, Staden JV. Isolation of β- asarone an antibacterial and anthelmintic compound from *Acours calamus* in South Africa. S Afr. J. Bot., 2002; 68(1):31-35.
11. Ghosh S, Sharma AK, Kumar S, Tiwari SS, Rastogi S, Srivastava S *et al*. *In vitro* and *in vivo* efficacy of *Acorus calamus* extract against Rhipicephalus (Boophilus) microplus. *Parasitol. Res*., 2011; 108(2):361-370.
12. Gilani AU, Shah AJ, Ahmad M, Shaheen F. Antispasmodic effect of *Acorus calamus* Linn. is mediated through calcium channelblockade. *Phytother. Res.* 2006; 20(12):1080-4.
13. Haghighi SR, Asadi MH, Madvar AR, Baghizadeh A. Antibacterial effect of *Acorus calamus* extractions against gram positive and negative bacteria. Ethno-Pharm. Prod. 2014; 1(1):1-7.
14. Jaripa-Begum, Sohrab H, Yusuf M, Chowdary JU, Husaain MM, Begum HA *et al*. Invitro antifungal activity of asaron isolated from the rhizome extract of Acorus calamus L. Pak. J Biol. Sci. 2004; 7(8):1376-1379.
15. Kumar V, Singh R, Joshi V. Antimicrobial activity of Rhizome extract of *Acorus calamus* against different Micro-Organisms, Octa J. Biosci. 2014; 2(1):59-63.
16. Marongiu, Bruno, Piras, Alessandra, Porcedda, Silvia *et al*. "Chemical Composition of the Essential Oil and Supercritical CO2 Extract of *Commiphora myrrha*(Nees) Engl. and of *Acorus calamus L*.". J Agri. Food Chem. 2005; 53(20):7939-7943.
17. Mehrotra S, Mishra KP, Maurya R, Srimal RC, Yadav VS, Pandey R *et al*. Anticellular and immune suppressive properties of ethonolic extract of *Acorus calamus* rhizome. Intenational Immunopharmacology. 2003; 3(1):53-61.
18. Murugan S, Anand R, Devi UP, Vidhya N, Rajesh KA. Efficacy of *Eupobiya milli* and *E. Pulcherrima* on aflatoxin producing fungi (*Aspergillus flavus and A. parasiticus*). African J. Bio. Technol. 2007; 6(6):718-719.
19. Olawale SA. Solid-liquid extraction of oils of African elemi's (*Canarium schweinfurthii*'s) fruit. J Agric. Engg. Int: CIGR., 2012; 14(2): 155-172.
20. Parab RS, Mengi SA. Hypolipidemic activity of *Acorus calamus* L. in rats. Fitoterapia. 2002; 73(6):451-455.
21. Pongpaichit S, Pujenjob N, Rukachaisirikul V, Ongsakul M. Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn. Songklanakarin. J Sci. Technol. 2004; 27(2):517-523.
22. Pradeep K, Shukla Vinay K, khanna Ali, MM, Maurya RR, Handa SS *et al*. Protective effect of *Acorus calamus* against Acrylamide Induced Neurotixicity. Phytother. Res. 2002; 16(3):256-260.
23. Rafatullah S, Tariq M, Mossa JS, Al-Said MS, Ageel AM. Anti-secretagogue, anti-ulcer and cytoprotective properties of *Acorus calamus* in rats. Fitoterapia. 1994; 65(1):19-23.
24. Raja AE, Vijayalakshmi M, Devalarao G. *Acorus calamus* Linn: chemistry and biology. Res. J. Pharm. and Tech. 2009; 2(2):256-261.
25. Rani AS, Satyakala M, Devi VS, Murty US. Evaluation of antibacterial activity from rhizome extract of *Acorus calamus* Linn. J Sci. Ind. Res. 2003; 62:623-625.
26. Shailajan S, Menon S, Swar G, Singh D, Nair S. Estimation and quantitation of β-asarone from *Acorus calamus* rhizome and its formulations using validated RP-HPLC method. Pharm. Methods. 2015; 6(2):94-99.
27. Srividya G, Adilaxmamma K. Ameliorative effect of *Acorus Calamus* in drug induced hepatotoxicity in rats. Int. J Sci. Tech. 2014; 3(5):7-9.
28. Sujina I, Prabhu V, Hemalal H, Ravi S. Essential oil composition, isolation of β-asarone and its antibacterial and MRSA activity from the rhizome of *Acorus calamus*. J Pharm. Res. 2012; 5(6):3437-3440.
29. Suryadevara P, Khanan P. Screening of plant extracts for larvicidal activity against *Culexquinquefasciatus*. J Nat. remedies. 2002; 2(6):186-188.

30. Talreja T. Screening of crude extract of flavonoids of *Moringa oleifera* against bacterial and fungal pathogen. J Phytology. 2010; 2(11):31-35.
31. Ulyana MA, Daniel EA, Jun M, Michael HN, Edward J. Antioxidant capacities of ten edible North American plants. Phytother. Res. 2002; 16: 63-65.