International Journal of Phytomedicine 2 (2010) 436-439

http://www.arjournals.org/ijop.html

Research article

ISSN: 0975-0185

Chemical composition and antimicrobial activity of the essential oil of *Desmostachya bipinnata* linn.

K. Ashok Kumar [1*], Sharvanee [1], Jitendra Patel [2], Ram Kumar Choudhary[1]

***Corresponding author:**

K. Ashok Kumar
[1] Department of Pharmacognosy, Bharat Institute of Technology-School of Pharmacy, Mangalpally, Ibrahimpatnam, RR Dist-501510, AP, India.
email-akumar181974(at)gmail.com
[2]Department of Pharmacognosy, Navabharat Institute of Pharmaceutical and Medical Sciences, Mangalpally, Ibrahimpatnam, R.R Dist - 501510, AP, India.

Abstract

The present study describes the phytochemical profile and antibacterial activity of *Desmostachya bipinnata*. The sample of essential oil was obtained from the aerial parts of the plant by hydrodistillation and analyzed by GC–MS. From the 16 compounds representing 99.97% of the oils: camphene (16.79%), isobornyl acetate (9.92%), tricyclene (4.30%), (+,-) trans-2,6-gamma-Irone (2.21%), Caryophyllene diepoxide (12.29%) , β-eudesmol (11.16%) Eseroline (25.15%) and Calarene (3.48%) appear as the main components. The oil also contained smaller percentages of Diphenyliodinium bromide, 1.limenone, 2-cyclohexene-1-one and 8-nitro-12-tridecanolide. Furthermore, an antibacterial study of the oil was evaluated using agar diffusion and broth dilution methods. The antibacterial studies showed that the oil had significant inhibitory effect against all four bacteria strains included in the study. Results, suggest potential antibacterial activity of the essential oil of *Desmostachya bipinnata*, which may find its application in future research for the therapy, food and pharmaceutical industry.

Key Words: *Desmostachya bipinnata*, Essential oil, Antimicrobial activity, GC-MS.

Introduction

Desmostachya bipinnata Linn, belonging to family Graminae, is a perennial grass widely distributed in India. The root stock and leaves are used in Indian traditional system of Medicine. Many medicinal properties and use of Kush as medicine have been reported in reference literatures. According to Ayurveda, Kush is acrid, cooling, oleaginous, aphrodisiac, diuretic and useful in treatment of blood diseases, biliousness, asthma (Dama), thirst, stranguary, jaundice, vaginal discharge, vesical calculi, diseases of skin, bladder and uterus etc. Powdered root stock is used as diuretic agent, galactagogue and as an astringent [1]. The present study involves extraction of essential oils from the powdered leaves by hydro-distillation, analysis of the components by GC-MS technique and antimicrobial studies.

Material and Methods

Plant material

The fresh arial parts were collected from osmania university campus, Hyderabad, Andhra Pradesh, India in November 2009. A voucher specimen of the authenticated plant was deposited in the herbarium of botany department of Kakatiya University, Warangal, India. The plant material

doi:10.5138/ijpm.2010.0975.0185.02062

was collected, washed with double distilled water to remove earthy impurities and shade dried for seven days. The dried material was powdered with help of a grinder and stored in a air tight container until further use.

Isolation of essential oil

A weighed amount of powdered plant material (250 g) was subjected to hydro-distillation for 6 hours in a Clevenger apparatus. The oil obtained was dried with anhydrous Na_2SO_4 to remove moisture. The fraction obtained was stored in a refrigerator at - 4^{o}C until use.

Gas chromatography-mass spectrometry (GC-MS) analysis

Sample Preparation

The volatile oil was dissolved in n-hexane (1mg/mL) and sonicated for 10 minutes. The contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India) to remove particulate matter. The sample of 0.1µl was used for the analysis.

Chromatographic conditions

The sample was analyzed using Shimadzu GC-MS-QP2010 Plus apparatus equipped with quadrapole detector and split injection system. The GC was fitted with a ZP-624 capillary column (30mm x 1.4 mm, film thickness 0.25µm). The temperature programmed was as follows: injector temperature 220^{0}c , initial oven temperature at 50^{0}c for 2 minutes, then rise to 250^{0} at the rate of 10^{0}c per minute for 25 minutes, transfer line temperature 220^{0}c. Helium was used as carrier gas at 35.6 Kpa pressure with flow 2.5 ml/min and electronic pressure control on. The EM voltage was 952.9 V with lower and upper mass limits set at 30& 350 m/z. Samples were solved in n-hexane and injected automatically. MS spectra of separated compounds were compared with one from Wiley 7 Nist 05 mass spectral database. The identity of the spectra above 95% was needed for the identification of compounds.

Antimicrobial activity

Microorganisms

The microorganisms employed in the current study were procured from Institute of Microbial Technology, Chandigarh (India) which includes clinical isolates of Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Pseudomonas auerigenosa.

Media

Nutrient broth (Hi Media M002) containing Peptic digest of animal tissue (5g/L), yeast extract (1.50g/L), Beef extract (1.50g/L) was used for the growth of the bacterial cultures. Antibiotic assay media No:11 (Hi Media MM004) containing Peptic digest of animal tissue (6g/L), Casein enzymic hydrosylate (4g/L), yeast extract (1.50g/L), Beef extract (1.50g/L), Dextrose (1.00g/L), Agar (15.00g/L) was used for testing of Anti-bacterial activity.

Agar well diffusion bioassay

The antimicrobial activity of the essential oil was determined by using the agar well diffusion technique. Nutrient agar plates were each seeded with 0.5 ml of an overnight culture of each bacteria. The 24 hrs broth culture of each bacterium were used to seed sterile molten nutrient agar at 45^{0}C, allowed to set and well made by sterile cork borer and 100 µL (50, 25, 10 and 4 µg/mL) solution of essential oil added in to in each well . Then bacterial plates were incubated at 37^{0}C for 24 hrs after which diameter of zones of inhibition was measured. Oxytetracyclin was used a standard drug while DMSO was negative control [2].

Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined through the broth dilution method. Bacteria were grown in nutrient broth (brain heart infusion liquid medium) for 6 h. After this, 20 µL of 106 cells/mL were inoculated in tubes with nutrient broth supplemented with four different concentrations (50, 25, 10 and µg/mL) of the oils. After 24 h at 37 ºC, the MIC of each sample was measured through optical

density in the spectrophotometer (620nm), through the comparison of the sample readout with the non-inoculated nutrient broth (15). All determinations were performed in duplicate [3, 4].

Results and Discussion

Water distillation of aerial parts of *Desmostachya bipinnata* yielded 0.42% v/w of the yellow coloured essential oil (calculated on dry weight basis). About 16 constituents were identified by means of GC-MS analysis of the plant essential oil representing 99.97%. Mainly monoterpenes and sesquiterpenes compound such as: camphene (16.79%), isobornyl acetate (9.92%), tricyclene (4.30%), (+,-) trans-2,6-gamma-Irone (2.21%), Caryophyllene diepoxide (12.29%) , β-eudesmol (11.16%) Eseroline (25.15%) and Calarene (3.48%) represent the most abundant compounds in *Desmostachya bipinnata*.These compounds are listed in Table 1, along with their retention time and relative content, while Table 2. lists out the reported pharmacological activities of major constituents of the essential oil. The spread of drug resistant pathogens is one of the serious threats to successful treatment of microbial diseases. Since ages, essential oils and extracts from plants are being screened for potential uses in treating many infectious diseases. Plants also protect themselves from microbial attack by the secondary metabolites produced by them. It is well established that several secondary metabolites synthesized by plant play central role in plant defense against microbial attack. Thus, plant secondary metabolites offer molecules with potential antimicrobial activity [6].

The essential oil of *Desmostachya bipinnata* showed strong antibacterial activity against S.epidermidis, S. aureus, E.coli and P. aeruginosa. Disc diffusion is one of the most common assays used in the evaluation of antibacterial activity of essential oils. Table 3 shows the in vitro antibacterial property of the essential oil of Desmostachya bipinnata on four bacterial strains. Antibacterial activity by disc diffusion method showed that the oil of *Desmostachya bipinnata* was most active against S.epidermidis followed by E.coli, P.auerigenosa and S. aureus. The essential oil at all dilutions showed potent inhibitory activity against the tested microorganisms.

Table 1. Chemical composition of the essential oil from the ariel parts of *Desmostachya bipinnata* determined by gas chromatography-mass spectrometry

Peak	Name	Retention time	Area%	Area	Similarity
1	Tricyclene	5.932	191038	4.303	100
2	Diphenyliodinium bromide	6.160	87571	1.8475	100
3	Camphene	6.714	796090	16.7948	99
4	1.limenone	9.032	76043	1.6043	98
5	Endoborneol	13.179	132454	2.7943	97
6	2-cyclohexene-1-one	15.099	40458	0.8535	99
7	Isobornyl acetate	16.108	470259	9.9209	99
8	(-)Caryophyllene oxide	22.722	72623	1.5321	99
9	(+,-) trans-2,6-gamma-Irone	22.856	104855	2.2121	99
10	Caryophyllene oxide	22.988	582606	12.2910	99
11	8-nitro-12-tridecanolide	23.519	130610	2.7554	99
12	Caryophyllene oxide	23.712	73550	1.5517	99
13	Caryophyllene diepoxide	24.254	95377	2.0121	99
14	β-eudesmol	24.462	529242	11.1652	99
15	Calarene	26.514	165180	3.4847	99
16	Eseroline	27.316	1192131	25.1500	99

Table 2. Major constituents of the essential oil and its reported pharmacological activity [5].

S.No	Constituent	Pharmacological Activity	Functional group
1.	Eseroline	It produces potent analgesic effects mediated through the μ-opioid receptor. It produces weak and reversible anti-cholinesterase activity. It induces neuronal cell death or depression of neurons. It also has specific anti-nociceptive action.	Sesquiterpene alcohol
2.	Camphene	Allelopathic, Antilithic, Hypocholesterolemic, Insectifuge Artificial flavoring agent, expectorant property, anti- oxidant activity.	Monoterpene hydrocarbon
3.	Caryophyllene oxide	Artificial flavoring agent. It has shown significant central and peripheral analgesic and anti-inflammatory activity. Also reported to possess anti-microbial and local anesthetic, anticarcinogenic properties. It is used as fragrance. Antiedemic, Antifeedant, Antiinflammatory, Antitumor, Insecticide	Sesquiterpene hydrocarbon
4.	L-limonene	Immunomodulatory Activity, spasmolytic activity, Antinociceptive, AChE-Inhibitor, Acaricide, Antifeedant, Antiinflammatory, Antimutagenic, Antiobesity, Antiseptic, Antitumor, Antitumor (Pancreas), Apoptotic, Chemopreventive, Fungiphilic, Histaminic, Insecticide, Insectifuge, Lipolytic, Myorelaxant, Nematicide, Ornithine-Decarboxylase-Inhibitor, Ozone-Scavenger, Sedative, Transdermal	Monoterpene
5.	Beta-Eudesmol	Antimutagenic, Antipeptic, Antisalmonella, Antitumor-Promoter, Hepatoprotective, Sedative	Sesquiterpene alcohol

The diameter of the inhibition zone of oil of *Desmostachya bipinnata* varied from 15.2 to 56.2 mm. The largest zone of inhibition was obtained for S.epidermidis (56.2mm) with undiluted sample of oil of *Desmostachya bipinnata* and the lowest for S. aureus (33.9mm). The growths of tested bacteria in high concentrations of oil were highly inhibited, where it was considered that these organisms were sensitive to the oil. At low concentrations 4 μg/mL, a very limited inhibitory effect was observed on the growth of micro-organisms in comparison with standard and higher concentrations. The Table 2 shows with an increasing dose of essential oil of *Desmostachya bipinnata*, the resulting diameter of the zone of inhibition increased for all the organisms. The gram-positive bacterial strains (S. aureus and S. epidermidis) were more susceptible to the oil as compared to the gram-negative bacteria (E. coli and P.auerigenosa). This is in agreement with previous reports that plant essential oils are more active against gram-positive than gram-negative bacteria [7-11].

Similarly, when the minimal inhibitory concentration was evaluated using the oil of *Desmostachya bipinnata* against gram-negative and positive bacteria, similar results to the agar well diffusion method was produced. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the test samples where the absence of growth was

recorded [12]. The results are presented in Table 4.

Table 3. Antimicrobial activity of essential oil of the ariel parts of *Desmostachya bipinnata* linn.

Micro-organism	Standard (1000µg/mL)	Sample drug concentration(µg/mL)					
		Undiluted sample	50	25	10	4	DMSO
S. aureus,	38.0	38.9	31.2	29.9	28.4	23.2	-
S.epidermidis	40.0	56.2	29.9	28.5	24.5	22.9	-
E.coli,	33.2	36.3	26.3	22.3	17.1	15.2	-
P.auerigenosa	36.1	36.1	27.0	22.6	17.3	15.4	-

Table 4 The minimum inhibitory concentration (MIC, µg/ml) of *Desmostachya bipinnata* linn. essential oil of aerial parts against bacteria.

Micro-organism	Standard (1000µg/mL)	Sample drug concentration(µg/mL)				
		Pure	50	25	10	4
S. aureus,	-	-	-	-	-	+
S.epidermidis	-	-	-	-	-	+
E.coli,	-	-	-	-	-	+
P.auerigenosa	-	-	-	-	-	+

+ indicates growth - indicates absence of bacterial growth

The MIC of the essential oil of *Desmostachya bipinnata* was tested at a concentration ranging from undiluted sample to 4 µg/ml. The minimum inhibitory concentration (MIC) for the oil of *Desmostachya bipinnata* for all the tested organisms was > 4 µg/ml. Gram-negative bacteria were more resistant to the essential oils and can be attributed in part to the great complexity of the double membrane-containing cell envelope in contrast to the single membrane structure of gram-positive bacteria [13].

The results of the study revealed that essential oil of *Desmostachya bipinnata* have antibacterial activity against gram-positive as well as gram-negative bacteria. Some authors have reported that gram-positive micro-organisms are slightly more sensitive to essential oils when compared to gram-negative [14, 15]. This lower sensitivity of gram-negative organisms has been related to the presence of an outer membrane surrounding their cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolyssacharide covering [16].

Essential oils rich in monoterpenes and sesquiterpenes are widely reported to possess high levels of antimicrobial activity [17-22]. On the other hand, it should be noted that the three major volatile constituents, Caryophyllene oxide, isobornyl acetate and Limonene contained in the *Desmostachya bipinnata* are compounds with interesting antibacterial activity.

Conclusion

To the best of our knowledge, the antibacterial activity of the essential oil of *Desmostachya bipinnata* has not been reported before. Some of main constituents identified in study such as limonene, Caryophyllene oxide and beta eudesmol are reported to have antibacterial property. Therefore, antibacterial constituents from *Desmostachya bipinnata* essential oil could hold promise for future application in therapy.

References

1. http://www.botanical.com/site/column_poudhia/116_janjgir.html
2. Burt SA. Essential oils: their antibacterial properties and potential applications in Foods: a review. Inter J Food Microbiolgy. 2004;94: 223-25
3. Prudent D, Perineau F, Bessiere JM, Michel GM, Baccou JC. Analysis of the essential oil of wild oregano from Martinique (Coleus aromaticus Benth.)- evaluation of its bacteriostatic and fungistatic properties. J Essen Oil Res.1995;7:165-173.
4. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Inter J Food Microbiol.2002; 74: 10-109.
5. http://www.cosmicjoy.com/product_phytochemistry.html
6. Darokar MP, Mathur A, Dwivedi S, Bhalla R, Khanuja SPS, Kumar S. Detection of antibacterial activity in the floral petals of some higher plants. Curr Sci 1998; 75: 187.
7. Iauk L, Ragusa S, Rapisarda A, Franco S, Nicolosi VM. In vitro antimicrobial activity of *Pistacia lentiscus* L. extracts: Preliminary report. J. Chemother.1996; 8: 207–209.
8. Koutsoudaki C, Krsek M, Rodger A. Chemical Composition and Antibacterial Activity of the Essential Oil and the Gum of *Pistacia lentiscus* Var. chia J. Agric. Food Chem. 2005; 53(20): 7681–7685.
9. Ozçelik B, Aslan M, Orhan I, Karaoglu T. Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of Pistacia vera. Microbiol. Res.2005; 160:159–164
10. Kamrani YY, Amanlou M, Esmaeelian B, Bidhendi MS, SahebJamei M. Inhibitory Effects of a Flavonoid-Rich Extract of *Pistacia vera* Hull on Growth and Acid Production of Bacteria Involved in Dental Plaque. Int. J.Pharmacol.2007;3: 219–226.
11. Benhammou N, Bekkara AF, Panovska KT. Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts .Afri. J. Pharm. Pharmacol. 2008; 2:22–28.
12. Glowniak P, Łos R, Skalicka-Wozniak K, Widelski J, Burczyk J, Malm A. Activity of Crithmum maritimum L. (Apiaceae) against Gram-positive bacteria. XIX 2006 (2)17. Annales universitatis Mariae Curie - Skłodowska . Polonia.
13. Bagamboula CF, Uyttendaele M, Candan F, Daferera D, Unli GV, Polissiou M, Sokmen A. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of S. cryptanha and S. multicaulis (Vahl.). Food Chem.2004; 84:519–525.
14. Canillac N, Mourey A. Antimicrobial activity of the essential oil of Picea excelsa on Listeria, Staphylococcus aureus and coliform bacteria. Food Microbiol.2000; 18: 261–268.
15. Dermetzos C, Perdetzoglou DK. Composition and antimicrobial studies of the essential oils of Origanum calcaratum Juss. and O.scabrum Boiss. J. Essent. Oil Res. 2001; 3:460–462.
16. Vaara M. Agents that increases the permeability of the outer membrane. Microbiol. Rev. 1992; 56:395–411.
17. Malekzadeh F. An antimicrobial compound in two Pistacia species. Mycopathologia. 1974; 54: 73–77.
18. Yalpani M, Tyman JHP.Long-chain phenols. The phenolic acids of Pistacia vera. Phytochemisrty.1983; 22:2263–2266.
19. Conner DE, Beuchat LR. Sensitivity of heat-stressed yeasts to essential oils of Plants. Appl. Environ. Microbiol. 1984; 47:229–233.
20. Marner FJ, Freyer A, Lex J. Triterpenoids from gum mastic, the resin of *Pistacia lentiscus*. Phytochemistry1991;30:3709–3712.
21. Kubo I, Muroi H, Kubo A. Antimicrobial activity of long-chain alcohol's against Streptococcus mutants. J. Agric. Food. Chem. 1993; 48: 2143–2145.
22. Ben Douissa F, Hayder N, Chekir-Ghedira L, Hammami M, Ghedira K, Mariotte AM, Dijoux-Franca MG. New study of the essential oil from leaves of *Pistacia lentiscus* L. (Anacardiaceae) from Tunisia. Flavour Fragrance J. 2005; 20: 410–414.