BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **46(2)**, 191-194, 2011

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

Chemical Composition and Antimicrobial Activity of Essential Oil Collected from *Adhatoda vasica* Leaves

A. K. Sarker[a]*, J. U. Chowdhury[b] and H. R. Bhuiyan[b]

[a]Plant Protein Research Section, IFST, BCSIR, Dhaka and [b]BCSIR Laboratories, Chittagong

Abstract

Essential oil obtained from hydrodistillation of *Adhatoda vasica* (Nees.) leaves was analyzed by gas chromatograph-mass spectrometer (GC-MS). Hydrodistillation of the *Adhatoda vasica* (Nees.) leaves yielded 0.096% (v/w) of essential oil. Eleven compounds comprising about 100% of the total oil were identified. The most abundant components of essential oil were 1,2,3, trimethyl benzene (1.51 %), borneol (58.60 %), ethanonaphthalene (2.82 %), 1,1,4a trimethyl-5,6-dimethylenedecahydro naphthalene (5.28 %), 2,tert-butyl-1,4-dimethoxy benzene (6.50 %), bicyclo[jundec-4-ene,4,11-trimethyl-8-methylene (14.56 %), hexa- methyl dewar benzene (0.87 %), alpha-caryophyllene (1.95 %), cycloproplejazulene (1.48 %), caryophyllene oxide (2.35 %) and 2-naphthalenemethanol (1.46 %). The microbial activity of the oil was screened against *Bacillus subtilis, Salmonella typhimurium, Staphylococcus aureus,* and *E. coli.* It was found that all mentioned microorganisms were more or less sensitive to this essential oil.

Key Words: *Adhatoda vasica*, Essential oil, Chemical composition and Antimicrobial activity.

Introduction

Adhatoda vasica, Nees is a shrub growing in Bangladesh. The leaves vary from 10 to 15 centimeters in length, and are about 4 centimeters in width. It commonly grows in dirty or barren places. The medicinal value of this herb has been mentioned in old texts (Rajani *et al.* 1996). It is household remedy for various disorders (Mukul *et al.* 2007).

The leaves of the plant contain the alkaloid vasicine ($C_{11}H_{12}N_2O$), which is responsible for the small but persistent bronchodilatation (Nadkarni *et al*. 1954) and an essential oil which is chiefly responsible for the expectorant action (Chopra *et al.* 1982 & Sivarajan *et al.* 1994). The leaves are used, either alone or in combination with other drugs, for preparation of expectorants (Singh *et al.* 1996 and Jain *et al.* 1991).

Essential oils are also known to contain ketone, terpene, and phenolic ether, which have antitumor, antioxidant, antiaging, antimutation and sedative effects, plus the high phenolic derivative content of essential oils contributes to their antimicrobial properties (Bandini *et al.* 1981 & Wealth of India). The plant also produces a fragrant volatile oil rich in borneol, but less is known about its therapeutic contribution. Accordingly, the present study is the extraction of essential oil from the leaves of Adhatoda vasica and then investigated the chemical composition and antimicrobial activities of the extracted oil.

Materials and Methods

Plant Source

The leaves of *Adhatoda vasica* plant were collected early in the morning, noon and evening in July 2007 on BCSIR medicinal field (Baluchara, Chittagong). It was washed with cold water to remove unwanted dirt materials.

Used Plant Part

The leaves of the plant (100 g) were hydro distilled for 2.5 hours using a Clevenger-type apparatus maintaining the cooling column temperature at 4°C. The oil was extracted from the distillate and then dried over anhydrous sodium sulphate (Na_2SO_4). After filtration 0.096 ml of pure oil was obtained and kept at 4°C until analysis was performed. The essential oil has got the following physical characteristics, color: light golden yellow; flavor: fragrant odor and solubility: soluble in 80% ethanol.

* Corresponding author: E-mail: ashish10608@yahoo.com

Table: Yield of essential oil from adhatoda vasica (Nees.) leaves at different times

Sl. No.	Wt. of leaves in gm {W}	Volume of essential oil in ml {V}	Essential oil, {V/W %}	Mean, %	Mean, %
	100	0.09842	0.09842		
*1(morning)	100	0.09913	0.09913	0.09884	
	100	0.09897	0.09897		
	100	0.09284	0.09284		
*2 (noon)	100	0.09192	0.09192	0.09244	0.096
	100	0.09256	0.09256		
	100	0.09598	0.09598		
* 3 (evening)	100	0.09602	0.09602	0.09609	
	100	0.09628	0.09628		

* Three different samples were collected, analyzed and averaged.

Experimental

GC analyses of the oil were carried out on a GC HP-5890 apparatus, equipped with the split-split less injector, SPB 5 capillary column (30 m x 0.25 mm, 0.25 m film thickness) with helium as the carrier gas (1 ml/min) and fitted with FID. Operating conditions: injector temperature 250^{o}C, temperature program isothermal at 50^{o}C for 3 min, then increase of 50-250^{o}C at a rate of 5^{o}C/min and finally isothermal temperature at 250^{o} C for 15 min.

GC/MS analyses were performed on a Shimadzu apparatus, Model GC-17A gas chromatograph (Shimadzu) coupled with a mass selective detector GC-MS QP 5050A, under the same gas-chromatograph conditions.

Identification procedure

The constituents were identified by comparison of their mass spectra to those from MS library NIST-147 and NIST-27.

Chemicals

Nutrient agar was purchased from Hi-Media Laboratories Pvt. Ltd. Ampicillin was procured from Ningbo Blue Hill Chemicals Co., Ltd. China.

Bacterial cells

Both gram positive and gram negative bacteria were used for this study. Gram positive bacterial cells *Bacillus subtilis* and *Staphylococcus aureus* were obtained as a gift from the department of Botany, University of Chittagong.

Gram negative bacterial cells *E. coli* and *Salmonella typhimurium* were also obtained as a gift from the Department of Botany, University of Chittagong.

Determination of antimicrobial activity

The disc diffusion technique (Pharmacopoeia Jugoslavia, 1984) was employed for determination of antibacterial activity using nutrient agar medium with the following microorganisms: *Bacillus subtilis, Saimoneiia typhimurium, Staphylococcus aureus,* and *E. coli.* The pure culture of these micro-organisms used in this study was obtained from the stock culture maintained in the Biochemistry Section, BCSIR Laboratories Chittagong. In this method, solution of known concentration (µ l/ml) of the test samples were made by dissolving measured amount of the samples in definite volume of solvents (ethanol). Dried and sterilized filter paper disc (10 mm diameter) were then impregnated with known amount of test substance using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. The experiment was replicated two times. Standard antibiotic discs (Ampicillin) were used as a positive control for comparison of results. The plates were then incubated at 37^{o}C for 24 hours to allow maximum growth of the organism. If the test material has any antibacterial activity it will inhibit the growth of the microorganism giving a clear distinct zone called "Zone of inhibition". The antibacterial activity of the test agent was determined by measuring the zone of inhibition expressed as millimeter (mm).

Present Work

Analyses of the oil were performed using GC and GC/MS. Constituents of the oil were identified by comparison of their mass spectra to those from the NIST library (NIST-147 and NIST-27). For the purpose of the quantitative analysis the area percentage obtained by FID was used as the base without the use of correction factors.

Results and Discussion

The results of the essential oil analysis of *Adhatoda vasica leaves* can be seen in Table I. About 100 % (11 components) of the essential oil has been identified. Borneol (58.60 %) was the main compound of the oil; the other compounds with noticeable percentage in the essential oil were 1,2,3, trimethyl benzene (1.51 %); ethanonaphthalene (2.82 %); 1,1,4a trimethyl-5,6-dimethylenedecahydro naphthalene (5.28 %); 2,tert-butyl-1,4-dimethoxybenzene (6.50 %); bicyclo[jundec-4-ene,4,11-trimethyl-8-methylene (14.56 %); hexamethyl dewar benzene (0.87 %); alpha-caryophyllene (1.95 %); cycloproplejazulene (1.48 %); caryophyllene oxide (2.35 %) and 2-naphthalenemethanol (1.46 %). According to our experiment, the chemical composition of essential oil from *Adhatoda vasica* leaves is similar although collected in different times.

Table I : Percentage composition of the essential oil of *Adhatoda vasica* leaves.

Sl. No.	Name of the chemical compounds	%
1	1,2,3, trimethyl benzene	1.51
2	Borneol	58.60
3	Ethanonaphthalene	2.82
4	1,1,4a trimethyl-5,6-dimethylenedecahydro naphthalene	5.28
5	2, tert 1-butyl-1,4-dimethoxybenzene	6.50
6	Bicyclo[jundec-4-ene,4,11-trimethyl-8-methylene	14.56
7	Hexamethyl dewar benzene	0.87
8	alpha-caryophyllene	1.95
9	Cycloproplejazulene	1.48
10	Caryophyllene oxide	2.35
11	2-naphthalenemethanol	1.46

The microbial activity of extracted essential oil from *Adhatoda vasica* leaves were tested against four bacteria at a concentration of 50 µ/disc. The results of the microbial activity were determinated by measuring the inhibition zones of diameter of examined microorganisms (given in mm) are summarized in Table II. It was found that all the microorganisms were susceptible to the oil, at all dilutions of the oil, however, the oil activity declined with dilution. Microbial investigation of the essential oil of *Adhatoda vasica* leaves gave the similar results, under the same conditions (Gupta *et al.* 1977a & Grange *et al.* 1996). Escherichia coli which can cause a moderate to severe gastroenteritis in human. This essential oil shows activity against this *E. coli*. *Bacillus subtilis* is responsible for food poisoning. The extracted oil also shows activity against this *Bacillus subtilis.*

Table II : Antimicrobial activity of essential oil of *Adhatoda vasica* leaves.

Name of the contents	Dose (µg/dis)	Diam-eter of zone of inhibition in mm			
Essential oil and solvent		*E. coli*	*Saimoneii a typhi*	*Bacillus subtilis*	*Staphyl ococcus aureus*
1:1	50	6.0	2.0	-	-
2:1	50	9.0	5.0	-	1.0
3:1	50	13.0	6.5	-	3.5
5:1	50	16.0	7.5	2	6.0
Ampicillin	10	30.0	25.0	16.0	24.0

' - ' Sign indicates no activity.

Conclusion

The leaves of *Adhatoda vasica* are used, either alone or in combination with other drugs, for the treatment of common cold, gastroenteritis, food poisoning etc. Poor people of our country can not afford modern allopathic drugs due to their high cost. So, there is an urgent need for alternative treatment for different types of diseases. The results of this study reflect that potent antimicrobial phytochemicals present in extracted essential oil and the fragrant volatile oil rich in borneol, which may responsible for antimicrobial effect (Santoyo *et al*. 2005) on the treatment of the above diseases.

Acknowledgement

The authors are acknowledges thanks to M. Rafique Uddin Chowdhury, Junior Technician, BCSIR Laboratories, Chittagong for his cooperation during the investigation.

References

Bandini P. and Pacchiani M. (1981) Constituents, properties and use of Calamintha nepeta. *Essenze Deriv. Agrum.*, **51** : 325-330.

Chopra R. N. (1982). Indigenous Drugs of India, Academic Publishers, Calcutta, pp 264-266.

Dr. Nadkarni K. M. (1954). The Indian Materia Medica, **I:** 40-4.

Gupta O. P., Sharma M. L., Ray Ghatak B. J. and Atals C. K. (1977a) Pharmacological investigations of vasicine and vasicinone- the alkaloids of *Adhatoda vasica, Ind. J. Med. Res*. **66** : 680491.

Grange J. M. and Snell N. J. (1996) Activity of bromohexine and ambroxol, semi-synthetic derivatives of vasicine from the Indian shrub *Adhatoda vasica,* against Mycobacterium tuberculosis *in vitro. J. Ethnopharmacol,* **5091:** 49-53.

Jain S. K. and Defilipps R. A. (1991). Medicinal Plants of India, Vol. 1. Michigan, USA: Reference Publications Inc. pp- 92.

Mukul S. A., Belal Uddin, M. and Tito M. R. (2007). Medicinal Plants Diversity and Local Healthcare Among the people Living in and Around a Conservation Area of Northern Bangladesh. *Int. J. For. Usuf. Mngt.* **8** (2) : 50-63.

Pharmacopeia, Jugoslavia (1984.) **4**: 128

Rajani M. and Pundarikakshudu K. (1996). A note on the seasonal variation of alkaloids in *Adhatoda vasica* Nees. *International Journal of Pharmacognosy* **34**(4): 308-309.

Santoyo S, Cavero S, Jaime L, Ibanez E., Senorans F. J. and Reglero G. (2005). Chemical composition and antimicrobial activity of Rosmarinus officinalis L. essential oil obtained via supercritical fluid extraction, *J. Food Prot.* **68** (4) : 790-5.

Sivarajan V. V. and Balachandran I. (1994). Ayurvedic Drugs and their Plant sources, *Int. Science Publ.,* 503.

Singh U., Wadhwani A . M, and Johri B. M. (1996) Dictionary of Economic Plants of India. New Delhi, India: ICAR. (1985) The Wealth of India, Raw materials,**1(**A): 78.

Received : August 14, 2008
Accepted : November 25, 2010