Anti-bacterial and Essential Oil Analysis of the Medicinal Plant *Adhatoda vasica* leaves

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Abstract: The present study was undertaken to analyze the chemical constituents of the essential oil of the leaves of *Adhatoda vasica* using GC-MS spectrophotometer. From the GC-MS analysis of the leaves of *A. vasica* 11 different compounds were identified belonging to various functional groups. The concentration of five main volatile oil chemical components obtained from the leaves of *A. vasica* using GC-MS were o-Cymene 52.8%, Sabinene 23%, alpha-Citral 4%, beta-Pinene 3.97%, Cineole 3.4%. The rest of the analytes were 1% to2.95%. The anti-bacterial activity of essential oil extracted from *A. vasica* leaves were tested against four bacterial strains at a concentration of 10µL, 25µL and 50µL. High activity 13.7 mm and 9.5 mm of the essential oil was found against the *E. coli* thus indicating that the oil can be used for different health purposes by comparing with the standard. A relatively low zone of inhibition was recorded against *B. cereus*. The present study was therefore carried out to explore the chemical constituents and anti bacterial activity which may play a key role in the pharmaceutical industry and for the herbal practioners. [Riaz Ullah, Iqbal Hussain, Jameel A. Khader, Naser M. AbdEIslam, Shabir Ahmad, Sadar Jan, Kamin Khan; Anti-bacterial and Essential Oil Analysis of the Medicinal Plant *Adhatoda vasica. Life Sci J* 2013;10(2):787-790] (ISSN:1097-8135). http://www.lifesciencesite.com. 111

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1. Introduction

Economically important medicinal plants not only providing raw materials for pharmaceuticals, perfumery, flavor and cosmetic industries but also protecting and curing human against certain diseases. The use of plant materials to prevent and treat infection diseases successfully over the years has attracted the attention of scientists worldwide (Afolayan 2003). The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes. The selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds (Kasamota et al 1995). Such screening of various plant extracts has been previously studied by many workers (Parek *et al* 2006, Jain and Defilipps 1991). Essential oils and plants extracts have been screened for their potential uses as alternative remedies for the treatment of many infection diseases, (Kasamota 1995]. Essential oils have been shown to possess anti-bacterial, anti-fungal, antiviral, insecticidal and anti-oxidant properties (Parek *et al.,* 2006, Jain and Defilipps 1991).

Adhatoda vasica belong to the family *Acanthaceae*. It is a shrub found in most part of the Khyber Pakhtunkhwa. The plant is used extensively in the treatment of asthma, cough, bronchitis and tuberculosis, joint pain, lumber pain, sprains, eczema, malaria, rheumatism, swellings, venereal diseases, as an anti–hyperglycemic, anti–diarrhoeal, anti–convulsant, cytotoxic (Jain and Defilipps 1991; Nadakarni, 1976, Gupta and Chopra 1954, Kirtikar and Basu, 1935; Chopra *et al*., 1982, Rastogi et al., 1994).

The leaves, roots and flowers of the plant contain the alkaloid vasicine, which is responsible for the persistent bronchodilatation (Nadkarni *et al*. 1954) and an essential oil which is the main agent responsible for the expectorant action (Chopra *et al.,* 1982, Sivarajan *et al.* 1994). The leaves alone or in combination with other active constituents are used for preparation of expectorants (Singh *et al.,* 1996 and Jain and Defilipps 1991).

Keeping in view the wide applications of *A. vasica* against different human diseases, the present study was therefore carried out to explore the chemical constituents and anti bacterial activity and to provide a scientific data base which will be very helpful for pharmaceutical consumers and for the local practitioners.

2. Material and Methods

The leaves of *Adhatoda vasica* were collected from Landi Kotal area of Khyber agency, of Khyber Pakhtunkhwa. The identity was checked by plant taxonomist Mr. Shahid Farooq, Senior Scientific Officer, at Pakistan Council for Scientific and

Industrial Research Laboratories Peshawar. A voucher specimen was deposited at the Herbarium of Medicinal Botanic Centre PCSIR Peshawar.

Isolation of essential oil

The essential oil from the leaves of *Adhatoda vasica* was obtained by hydro-distillation for 2 h. The oil obtained was dried over anhydrous Na_2CO_3, filtered and stored at + 4 °C until analysis.

Gas Chromatography-Mass Spectrometry

Reagents: Dichloromethane, HPLC grade

GC/MS analysis was performed using a Shimadzu Model QP 2010 plus, Injector temperature: 240 °C, Ion source temperature (EI): 240 °C, Interface Temperature: 240 °C, Pressure: 80 KPa, Carrier gas: helium, Split ratio: 1:50

Column oven programming:

Rate (°C/min)	Temperature	Hold (minutes)
-	40	0
3	90	0
10	240	15

GC programme time:

46.67 minutes total, Solvent cut time: 2.5 minutes, MS start time: 3 minutes, MS end time: 46 minutes, Acquisition mode: Scan M/Z: 40 – 500, Volume injected: 1 µl.

Column Specifications

Length: 30 m, id: 0.25 mm, thickness: 0.25 µm, (95% Dimethyl-5% diphenyl polysilphenylene; DB-5MS, Agilent technologies, USA).

Sample preparation:

Dilute approximately 40 mg of oil samples, weighed accurately up to 0.1 mg, with 2 mL of dichloromethane and filtered through 0.45µm - membrane filter and injected 1µl to GC-MS using auto injection system. The compounds were identified by comparison of their retention time with their retention indices (RI) (Martindale 2009), retention times (RT) and mass spectra with those of authentic samples and /or the NIST/NBS, NIST02, Wiley 575 spectra library and published literature.

Microorganisms and their growth conditions

Microbial strains including *Salmonella typhi, Escherichia coil ATCC 739, Staphylococcus aureus ATCC6538, Bacillus cereus,* were obtained from Food Microbiology Laboratory PCSIR Laboratories Complex Jamrud Road Peshawar Pakistan. All bacterial strains were cultivated in Nutrient Agar and Potato Dextrose Agar for 48 h at 37^{o}C (bacteria) following refrigeration storage at 4°C until use.

Antimicrobial Activity of Essential Oil;

Antibacterial Susceptibility Assay;

The anti-bacterial activity of essential oil was determined by agar well diffusion method. Pure isolate of each bacterium was first sub-cultured in nutrient broth at 37°C for 24 h. One hundred microlitres (100 µL) of standardized (10^6CFU/mL; 0.5 Mac-Farland) of each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton Agar plate. The plates were allowed to dry and a sterile cork borer (6.0 mm diameter) was used to bore wells in the agar. Subsequently, 10µL, 25 µL and 50µL volume of the essential oil was introduced in triplicate wells of the agar plates. Ciprofloxacin was used as a standard drug. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37°C for 24h. The zone of inhibition was recorded to the nearest size in mm.

3. Results and discussion

From the GC-MS analysis of the leaves of *A. vasica* 11 different compounds were identified belonging to various groups including alkenes (alpha-Pinene, Sabinene, beta-Pinene, o-Cymene, D-Limonene, alpha-Curcumene), alcohol (Cineole, 1-Terpene-4-ol), aldehyde (alpha-Citral), ketone (3-Thujanone), oxide (Limonene oxide, cis). The concentration of five main volatile oil chemical components obtained from the leaves of *A. vasica* using GC-MS were o-Cymene 52.8%, Sabinene 23%, alpha-Citral 4%, beta-Pinene 3.97%, Cineole 3.4%. The rest of the analytes were 1% to2.95%.

The results obtained from the GC-MS analysis of the leaves essential oil is depicted in **Table-1** and the graphical representation is shown in Figure-1.The GC-MS analysis of the essential oil from *Adhatoda vasica* was also carried out by Sarker et al (2011), and found 11 chemical constituents including 1,2,3, trimethyl benzene, Borneol, Ethanonaphthalene, 1,1,4a trimethyl-5,6- trimethylenedecahydro-naphthalene, 2, *tert* 1-butyl-1,4-dimethoxybenzene, Bicyclo[jundec-4-ene,4,11-trimethyl-8-methylene], Hexamethyl dewar benzene, alpha-caryophyllene, Cycloproplejazulene, Caryophyllene oxide, 2-naphthalenemethanol, which is quite different from the results we obtained **Table-1**.

The chemical composition of the essential oil has been noted to depend upon different geo-environmental conditions including climatic conditions, Seasonal, geographical, soil, irrigation, harvesting time and scientific distillation techniques

(Jazbi et al., 1999, Cook and Hokard 1966) supporting the above results of the essential oil obtained from the hydro-distillation of the leaves of *A. vasica*.

The anti-bacterial activity of essential oil extracted from *A. vasica* leaves were tested against four bacterial strains at a concentration of 10µL, 25µL and 50µL. As can be seen from the above **Table-2**, that the results obtained are very promising as the concentration increases there is an increase in measuring the zone of inhibition of the applied essential oil. High activity 13.7 mm and 9.5 mm of the essential oil was found against the *E. coli* thus indicating that the oil can be used for different health purposes by comparing with the standard. A relatively low zone of inhibition was recorded against *B. cereus*, however still acceptable for its applications against certain infectious diseases. Bandini *et al.,* 1981, reported that the essential oils are also known to contain various functional groups including ketone, terpene, and phenolic ether, have antitumor, antioxidant, antiaging, antimutation and sedative effects, and the rich phenolic essential oils are the main agent responsible for antibacterial activity.

Table-1. Quantitative results of the GC-MS of essential oil from the leaves of *A. vasica*

Peak No	Name	Area	Conc.%	R. Time
2	alpha-Pinene	1423	1.155	8.63
4	Sabinene	28703	23. 294	10.31
5	beta-Pinene	4895	3.973	10.49
10	o-Cymene	65072	52.810	12.69
11	D-Limonene	2067	1.677	12.88
13	Cineole	4214	3.420	13.04
21	3-Thujanone	3155	2.560	17.17
26	1-Terpene-4-ol	1682	1.365	19.38
40	alpha-Citral	5142	4.173	21.86
63	alpha-Curcumene	3632	2.948	25.27
72	Limonene oxide, cis	3235	2.625	26.41

Table-2. Anti-bacterial activity of *A. vasica* leaves.

Tested Microorganism	**Used volume of Essential Oil**			
	10 µL	**25 µL**	**50 µL**	
	Zone of Inhibition in mm			Standards Ciprofloxacin
Salmonella typhi	03	6.5	9.8	25
Escherichia coil ATCC 739	07	9.5	13.7	30
Staphylococcus aureus ATCC6538	03	5.6	7.2	20
Bacillus cereus	2	4.2	5.5	16

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