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Phytochemical Profiling and GCMS Study of *Adhatoda vasica* Nees. an Ethnomedicinal Plant of North Western Himalaya

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ABSTRACT: The ethnic people of the Himachal Pradesh have great faith in effectiveness of medicinal herbs. The ethnomedicinal plant *Adhatoda vasica* was analysed for its phytochemical, antimicrobial and antioxidant properties. The ethanolic extract of plant root was analysed against the standard and clinical isolates of five microbial strains viz. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Pseudomonas aeruginosa.* The plant root extract showed maximum inhibition against clinical isolate of *Salmonella typhimurium* (13.3±0.5) at 200µg/ml concentration. The maximum antioxidant activity (38.55 ± 0.22) of root extract of plant was observed at 75µg/ml concentration and minimum (13.47±0.47) at 15µg/ml concentration. Standard analysis of phytochemicals in the root extract of plant showed presence of terpenoids, flavonoids, tannins, cardiac glycosides, alkaloids, reducing sugars and saponins. In GC-MS profiling the plant root extractshowed presence of important compounds possessing antimicrobial, antitumour, anti-inflammatory, anticancer and antidiabetic properties.

Keywords: *Adhatoda vasica*, north western Himalaya, antimicrobial, antioxidant, GC-MS profiling.

INTRODUCTION

India is one of the main center of the ancient human civilization in the world where wild plants have been utilized for various purposes including herbal medicines. The State of Himachal Pradesh (30°22′40″-33°12′40″ N to 75°45′55″- 79°04′20″ E) in north India, includes parts of the Trans and Northwest Himalaya and covers 55,673 km^2 which is 9% of the Indian Himalayan Region. It has a large altitudinal range (200–7109 m), with diverse habitats, species, populations, communities and ecosystems (Samant *et al*., 2007). Ethnobotany is the study of relationship between plants and people (Verma *et al*., 2012). A large number of plants are used in traditional system of medicine, which are wild in nature. Approximately, 3000 plants species are known to have medicinal properties in India (Prakasha *et al.*, 2010). Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. 60% of people in rural areas depend on the traditional medicine for the treatment of their ailments. Different plants have been used as a source of inspiration in the development of novel drugs (Killedar and More, 2011). The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in particular plant is taxonomically distinct (Wink, 1999). Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species (Narayanaswamy and Balakrishnan, 2011). The aim of the present research work was to investigate the wild plant's antimicrobial, antioxidant and phytochemical property to support its existing ethnomedicinal potential and to find its scope for future application.

MATERIAL AND METHODS

A. Plant collection

The plants of *Adhatoda vasica* used in the present study were collected from the foothills of Theog region of District Shimla, Himachal Pradesh, India, in the North Western Himalaya. The plant was identified at School of Biological Sciences, Shoolini University, Solan, India and maintained in departmental Herbarium Collection. The collected plant roots were washed with water to remove the soil and dust particles and then the roots were used to prepare the extracts for study. The roots were dried thoroughly in shaded place, grounded to fine powder form and stored in an airtight container.

B. Microbial culture

Standard culture of five bacterial strains viz. *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhimurium* (MTCC 98), *Pseudomonas aeruginosa* (MTCC 741) were procured from IMTECH Chandigarh, India.

Clinical isolates of all these microbial culture were obtained from PGIMER, Chandigarh and Indira Gandhi Medical College, Shimla, India. Plant root extract was assessed for its antimicrobial property on all standard and clinical isolates of the above bacterial strains.

C. Preparation of plant extract for antimicrobial activity

The root extract of *Adhatoda vasica* was prepared by dissolving 10g fine powder of roots in 50 ml of ethanol (Selvamohan, 2012). The contents were kept in Rotary Orbital shaker for 48h. Finally the extract was filtered and dried in hot air oven at 40°C and stored under refrigeration at 4°C for further studies.

D. Assay of Antimicrobial activity using disc diffusion method

The antimicrobial activity of the plant root extract was determined by disc diffusion method (Bauer *et al*., 1966). Sterilized Muller Hinton Agar (MHA) was poured into sterile petriplate. The turbidity of inoculum was compared with 0.5 McFarland standards, containing 1-2×108 cfu/ml, 100µl of bacterial inoculums adjusted to an optical density (OD) of 0.8 were swabbed on the respective plates. Various concentrations (80µg/ml, 120µg/ml, 160µg/ml and 200µg/ml) of plant extracts were added to the sterile Whatman No. 1 filter discs (1×1mm). These discs were placed on Muller Hinton Agar plates and the plates were incubated for overnight at 37°C. After incubation the diameter of zone of inhibition formed around each discs were measured by using HiMedia zone inhibition scale.

E. Antioxidant activity of the root extract

The antioxidant activity of the plant extracts and the standard was assessed by using radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) (Ayoola *et al*., 2008). The diluted working solutions of the test extracts (15-75µg/ml) were prepared in methanol. Ascorbic acid (1-100 µg/ml) solution prepared in double distilled water was used as standard. DPPH (0.002%) was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and OD was measured at 517 nm using Spectrophotometer. Methanol (1 ml) was used as blank for plant sample and double distilled water was used as a blank for ascorbic acid. The optical density was recorded and % inhibition was calculated using the following formula:

$$\text{Percentage (\%) inhibition of DPPH activity} = \frac{A-B}{A} X\ 100$$

Where A is OD of the blank and B is OD of the sample.

F. Phytochemical screening

The phytochemical screening of the methanolic root extract of *Adhatoda vasica* was carried out to determine the presence of reducing sugars, alkaloids, saponnin, tannins, flavonoids, steroids, terpenoids and cardiac glycosides as per the Solomon *et al*. (2013) method.

G. GC-MS analysis of the root extract

GC-MS analysis of the ethanol extract of *Adhatoda vasica* was performed using Thermo Scientific Triple Quadrupole GC-MS (Trace 1300 GC, Tsq 8000 triple quadrupole MS) equipped with TG 5MS (30m X 0.25mm, 0.25µm) column. Helium was used as the carrier gas at a flow rate of 1ml/min. using an injection volume of 1.0 µL. Injector temperature was kept at 250°C and ion source temperature was 230°C. The oven temperature was maintained at 50°C isothermal at 280°C, Mass Spectra transfer line temperature.

RESULTS AND DISCUSSION

The *Adhatoda vasica* plant is a common ethnomedicinal plant found in North Western Himalaya. It is an erect perennial herb plant of North Western Himalaya It is a large shrub, growing crowded along waste lands. Leaves are simple, opposite, ovate-lanceolate, acute and shiny. Flowers are white in colour. The leaves develop individually as tightly rolled leaf spikes. The ethanolic root extract of the plant was assessed for its antimicrobial, antioxidant, phytochemical and GCMS potential.

A. Antimicrobial activity of root extracts of Adhatoda vasica

Different concentrations of plant root extract were used for evaluating the antimicrobial property of plant and the results are shown in Table 1. Antimicrobial inhibition zone of root extracts of *Adhatoda vasica* ranged from 10±0 to 13.3±0.5 against the selected concentrations of ethanolic plant extract. The maximum antimicrobial activity was reported against clinical isolate of *S. typhimurium* (13.3±0.5) at a concentration of 200µg/ml. However, no antimicrobial activity was reported against any microbial strain at 80 µg/ml concentration.

B. Antioxidant assay of root extract

The plant root extract was evaluated for its antioxidant potential using DPPH radical scavenging activity at varied concentrations and the results of the same are shown in Table 2. The maximum antioxidant activity of alcoholic plant extract was observed at 75µg/ml (38.55± 0.22) and minimum at 15µg/ml (13.47±0.47) concentration. The root extract showed IC_{50} value of 22.5µg/ml which is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. Antioxidant activity has been examined in a number of *Adhatoda* species including *Adhatoda vasica* and *Adhatoda beddomei.* In Kerala *A. beddomei* was studied by Srinivasan *et al*. (2014) for its antioxidant property and in Sirmour district of Himachal by Kaur *et al*. (2012).

Table 1: Antimicrobial activity of root extract of *Adhatoda vasica* collected from Himachal Pradesh, India against selected standard and clinical bacterial isolates.

Bacterial strains	Inhibitory zones (in mm) of root extract measured at different concentrations		
	120 µg/ml	160 µg/ml	200 µg/ml
E. coli (S)	10.0±0.0	12.3±0.5	12.6±0.5
E. coli (CI)	10.0±0.0	10.3±0.5	10.6±0.5
K. pneumonia (S)	ND	ND	ND
K. pneumonia (CI)	10.0±0.0	11.0±0.0	11.6±0.5
S. typhimurium (S)	ND	10.0±0.0	13.3±0.5
S. typhimurium (CI)	10.0±0.0	10.3±0.5	10.6±0.5
S. aureus (S)	ND	ND	11.0±0.0
S. aureus (CI)	ND	ND	10.3±0.5
P. aeruginosa (S)	ND	10.0±0.0	12.0±0.0
P. aeruginosa (CI)	10.0±0.0	10.3±0.5	12.3±0.5

S-Standard, CI-Clinical isolate, ND-Not Determined
Values are expressed as mean± Standard deviation (n=3)

Table 2: Antioxidant effect of root extracts of *Adhatoda vasica* using DPPH radical scavenging (as inhibition percentage).

Plant	Different concentrations of the plant extract (µg/ml)				
	15	25	50	75	IC50*
Adhatoda vasica	13.47 ± 0.47	22.51 ± 0.37	29.16 ± 0.18	38.55 ± 0.22	29.44

*IC50 is a value at which 50% of DPPH is scavenged

The extracts from *Adhatoda beddomei* showed 80% inhibition of free radicals at 100µg/ml concentration (Srinivasan *et al*., 2014).

C. Analysis of root extract of Adhatoda vasica for phytocomponents

Assessment of the various phytochemicals in selected medicinal plant was done on the ethanolic extracts using standard procedures as described by Solomon *et al*. (2013). Preliminary analysis of phytochemicals in the plant was determined by employing different tests for terpenoids, flavonoids, tannins, cardiac glycosides, alkaloids, reducing sugars and saponins by standard phytochemical tests. All these phytochemicals were found present in the *A. vasica* root extracts (Table 3). It has been shown that *in vitro* screening of plant root extract provides the needed preliminary data necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. Medicinal plant *Adhatoda vasica* have great potential for curing asthma, bronchitis, cough and rheumatism (Rana and Masoodi, 2014) because of the various phytochemicals present in the plant.

Table 3: Phytochemical screening of *Adhatoda vasica* collected from Shimla district in Himachal Pradesh, India.

Phytochemicals	Tests	Inference
Terpenoids	Salkowki's test	+ve
Flavonoids	Alkaline reagent test	+ve
Tannins	Braymer's test	+ve
Cardiac Glycosides	Keller Kelliani's test	+ve
Alkaloids	Wagner's reagent	+ve
Reducing sugars	Fehling's test	+ve
Saponins	Foam test	+ve

+ve = Present, -ve = absent

D. GC-MS analysis of Adhatoda vasica root extract

The volatile phytochemical components present in the ethanol root extract of *A. vasica* were further identified by GC-MS analysis (Fig. 1). The chemical profile of *Adhatoda vasica* extract and amount (%) of the individual components obtained and gas chromatographic-mass spectra data is summarized in Table 4. Sixteen different major compounds were identified in ethanolic roots extract of *A. vasica* at significant peaks of the chromatogram of GC-MS. The detected compounds were further identified by searching online library databaseand cited literature.

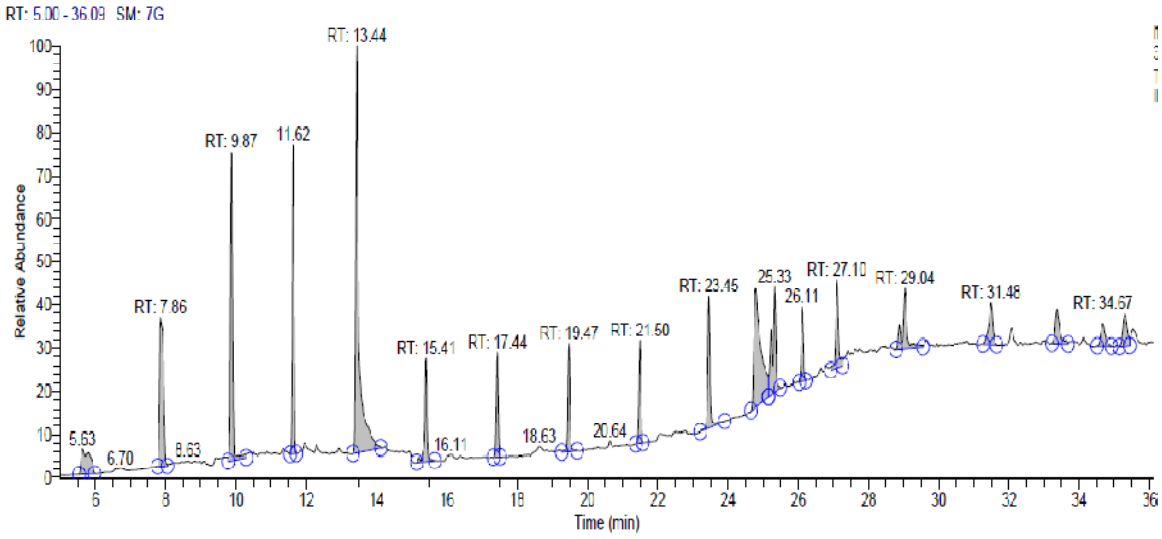

Fig. 1. Gas chromatography-mass spectrometry spectrum of *Adhatoda vasica.*

Table 4: Some major volatile compounds detected in the root extract of *Adhatoda vasica* collected from Shimla district in Himachal Pradesh, India using GC-MS analysis.

S.No.	Retention Time	%Area	Chemical Compound	Chemical Formula	Mol. Wt.
1.	7.86	8.58	Ethanethioic acid, S-[2dimethylamino)ethyl] ester	$C_6H_{13}NOS$	147
2.	7.86	8.58	2-Amininonadecane	$C_{19}H_{41}N$	283
3.	9.87	9.64	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224
4.	11.62	7.16	3-Eicosyne	$C_{20}H_{38}$	278
5.	11.62	7.16	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224
6.	11.62	7.16	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl ester)	$C_{16}H_{22}O_4$	278
7.	13.44	19.44	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224
8.	15.41	3.42	trans-Z-à-Bisabolene epoxide	$C_{15}H_{24}O$	220
9.	17.44	2.42	2-Cyclopentene-1-undecanoicacid	$C_{16}H_{28}O_2$	252
10.	24.78	12.00	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224
11.	25.33	5.62	Vitamin E	$C_{29}H_{50}O_2$	430
12.	25.33	5.62	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-,[S-(Z)]-	$C_{15}H_{26}O$	222
13.	25.33	5.62	Pyrrolo[2,1-b]quinazolin-9(1H)-one, 3-[2-(dimethylamino)phenyl]-2,3-dihydro-	$C_{19}H_{19}N_3O_3$	351
14.	33.36	2.06	β-Sitosterol	$C_{29}H_{50}O$	414
15.	33.36	2.06	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one,3-	$C_{22}H_{32}O_2$	328
16.	34.67	1.46	Lupeol	$C_{30}H_{50}O$	426

From the peaks *Adhatoda vasica* root extract in GC-MS chromatogram eleven different medicinally important phytochemicals were identified as mentioned in Table 5. Different identified phytochemicals from the GC-MS analysis possess antimicrobial, antioxidant, anti-inflammatory, anticancer, hepatoprotective, antiasthma and diuretic properties. GC-MS technique has been widely applied in medical, biological and food research for the determination of different bioactive compounds or for the analysis of secondary metabolites in the test samples (Ribeiro *et al*., 2007).

The active principles with their retention time (RT), % age area, compound name, chemical formula, and molecular weight are given in the Table 4. The chemical compound, its nature, activity and reference is mentioned in Table 5. The GCMS chromatogram of ethanolic extract is given in Fig. 1.

Table 5: Important phytochemicals identified in the ethanol root extract of *A. vasica.*

S.No.	Compound Name	Nature	Activity	Reference
1.	Ethanethioic acid, S-[2dimethylamino)ethyl] ester	Ester compound	Antimicrobial	Srinivasan,2014
2.	2-Aminononadecane	Amino Compound	Antimicrobial	
3.	3-Eicosyne	Alcoholic Compound	Antimicrobial	
4.	trans-Z-à-Bisabolene epoxide	Sesquiterpene Oxide	Anti-tumor, Antiinflammatory	
5.	Pyrrolo[2,1-b]quinazolin-9(1H)-one, 3-[2-(dimethylamino)phenyl]-2,3-dihydro-	Alkaloid	Antimicrobial, Anti-inflammatory	
6.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl ester)	Ester compound	Anticancer	Krishnan, 2014
7.	Vitamin E	Vitamin Compound	Antioxidant	Mokrosnop, 2014
8.	β-Sitosterol	Sterol compound	Antioxidant, Anthelmintic andAnti-mutagenic, Antidiabetic, Hypocholesterolemic and anti-inflammatory	Soodabeh, 2014
9.	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one,3-hydroxy-,(3á,17á)-	Steroid Compound	Antiarthritic, Hepatoprotective, Antiasthma, Diuretic, Anti-inflammatory, Cancer preventive	Ruba and Mohan, 2014
10.	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-,[S-(Z)]-	Sesquiterpene alcohol	Anti-tumor, Fungicide, Analgesic, Sedative, Antibacteria, Antiinflammatory,	
11.	Lupeol	Triterpene	Anti-inflammatory and Anti-cancer	Mohammad, 2009

CONCLUSION

In conclusion, *Adhatoda vasica* is an important plant of north western Himalayas and is presently used for the treatment of asthma in the study area by the traditional practitioners. The scientific investigation of the plant included antimicrobial, antioxidant and preliminary phytochemical studies which suggests the presence of important phytochemical constituents in the plant extracts and which was further justified by the GC-MS analysis of the plant. The presence of various bioactive compounds in the *Adhatoda vasica* justifies the use of plant for various ailments by the ethnic people of north western Himalayas. From the results of the present study, it could be concluded that *Adhatoda vasica* contains various bioactive compounds of potential medicinal use and is a plant of pharmaceutical importance.

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REFERENCES

Ayoola G.A., Folawewo A.D., Adesegun S.A., Abioro O.O., Adepoju-Bello A.A. and Coker H.B. (2008). Phytochemical and antioxidant screening of some plants of Apocynaceae from south west Nigeria. *African Journal of Plant Science,* **2**: 124-128.

Bauer A.W., Kirby W.M.M., Sherris J.C. and Truck M. (1966). Antimicrobial susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,***45**: 493-496.

Kaur I., Chauhan P.K., Jaryal M., Saxena S. and Kanishak (2012). Antioxidant and antimicrobial activity of leaf extract of *Adhatoda vasica* against the bacteria isolated from the sputum samples of asthmatic patients. *International Journal Drug Research and Technology,* **2**: 273-278.

Killedar S.G. and More H.N. (2011). Screening of antimicrobial potential and phyto-constituents for Different Extracts of *Memecylon umbellatum* Burm Inflorescences. *Asian Journal of Pharmaceutical Research*, **1**: 114-118.

Krishnan K., Mani A. and Jasmine S. (2014). Cytotoxic Activity of Bioactive Compound 1, 2- Benzene Dicarboxylic Acid, Mono 2- Ethylhexyl Ester Extracted from a Marine Derived *Streptomyces* sp.VITSJK8. *International Journal of molecular and cellular medicine,* **3**: 246-54.

Mohammad S. (2009). Lupeol, A Novel Anti-inflammatory and Anti-cancer Dietary Triterpene. *Cancer Letter*, **285**: 109-115.

Mokrosnop V.M. (2014). Functions of tocopherols in the cells of plants and other photosynthetic organisms. *Ukrainian Biochemical Journal,* **86**: 26-36.

Narayanaswamy N. and Balakrishnan K.P. (2011). Evaluation of some medicinal plants for their antioxidant properties. *International Journal of PharmTech Research,* **3**: 381-385.

Prakasha H. M., Krishnappa M., Krishnamurthy Y. L. and Poornima S.V. (2010). Folk medicine of NR Pura Taluk in Chikamaglur district of Karnatka. *Indian Journal of Traditional Knowledge*, **9**: 55-60.

Rana D. and Masoodi R. U. H. (2014). Ethno-botanical survey for wild plants in fringe villages around Shimla Water Catchment Sanctuary, Himachal Pradesh, India. *Journal of Applied and Natural Science,* **6**: 720-724.

Ribeiro B., Valentao P., Baptista P., Seabra R.M. and Andrade P.B. (2007). Phenolic compounds, organic acids profiles and antioxidative properties of beefsteak fungus (*Fistulina hepatica*). *Food and Chemical Toxicology,***45**: 1805-1813.

Ruba A.A. and Mohan V.R. (2014). GC-MS Analysis of bioactive compounds present in the whole plant of *Andrographis echioides* (l) Nees. (Acanthaeceae). *European Journal of Biomedical and Pharmaceutical sciences*, **1**: 443-452.

Samant S. S., Pant S., Singh M., Lal M., Singh A., Sharma A. and Bhandari S. (2007). Medicinal plants in Himachal Pradesh, north western Himalaya, *Indian International Journal of Biodiversity Science and Management* **3**: 234-251.

Selvamohan T., Ramadas V.S. and Kishore S.S. (2012). Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Advances in Applied Science Research,* **3**: 3374-3381.

Solomon C. U., Arukwe U., Onuoha I. (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. *Asian Journal of Plant Science and Research,* **3**: 10-13.

Soodabeh S., Azadeh M., Ahmad R.G. and Mohammad A. (2014). The Story of Beta-sitosterol- A Review. *European Journal of Medicinal Plants,* **4**: 590-609.

Srinivasan M., Padmaja B. and Nair S. (2014). GC-MS profiling and *in vitro* radical scavenging effect of *Adhatoda beddomei. Journal of Pharmacognosy and Phytochemistry,* **2**: 55-59.

Verma R., Parkash V. and Kumar D. (2012). Ethnomedicinal uses of some plants of Kanag Hill in Shimla, Himachal Pradesh, India, *International Journal of Research in Ayurveda and Pharmacy,* **3**: 319-322.

Wink M. (1999). Biochemistry of plant secondary metabolism. *Annual of Plant Reviews,* **2**: 374.