Volume 3 (6), 2015, Page-874-879
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

A Comparative Study: The Impact of Solvent Extraction on Phytochemical Profiling of *Adhatoda Vasica*

K Srinivasan[1, *], S Kumaravel [2]

[1] Senior Research Fellow, Department of Food Safety & Quality Testing, Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu, India.

[2] Assistant Professor and Head, Department of Food Safety & Quality Testing, Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu, India.

ARTICLE INFO

Received: 30 Aug 2015
Accepted: 28 Oct 2015

ABSTRACT

In Ayurveda, the leaf juice of *Adhatoda vasica*, a shrub native to Asia is incorporated in many traditional herbal formulations. However, suitable solvent and a suitable extraction method for phytochemical profiling are not well established, and there is no published mass spectra structural interpretation of the identified compounds. This has caused a few problems in herbal formulation research due to the bias derived from different extraction methods. Therefore, this study used polar and non polar extraction for phytochemical analysis on *Adhatoda vasica*, aiming to assess the potential impact of different solvents. This study included extractive value, total phenol and alkaloid content of the leaves in different preparations. Gas Chromatography coupled with Mass Spectrometry (GC-MS) was used to study the phytochemical profile of different solvents. Significant differences were observed in all the parameters such as extract yield, total phenol, total alkaloid and phytochemical composition. The ethanol extract stood out most for effective extraction of phytochemicals, especially for the alkaloids. The results highlight the necessity for comparative analyses of chemical composition in different solvent extractions and careful choice and validation of analytical methodology in herbal formulation research.

Keywords: *Adhatoda vasica,* Extraction, GC-MS, Phenol, Alkaloids

1. INTRODUCTION

Even today plants are the exclusive source of drugs for the majority of the world's population. Indian health care consists of medicinal pluralism and Ayurveda still remains dominant compared to modern medicine particularly for treatment of a variety of chronic disease conditions[1]. The leaves of *Adhatoda vasica* has

Corresponding author *
K. Srinivasan
Research Scholar, Department of Environmental & Herbal Sciences
Tamil University, Thanjavur – 613 005.
Mobile No. 9842639565
Email: srinigene@gmail.com

been used for centuries to treat asthma where it works as a bronchodilator and mild expectorant *Adhatoda* also works by decreasing the viscosity of mucous to assist with expectoration. Ayurvedic system of medicine describes the use of this plant for the treatment of respiratory ailments, particularly for the treatment of cough, bronchitis, asthma and tuberculosis, it is also claimed that it causes thinning of sputum and phlegm and asthma. The various preparation of leaves are used for curing bleeding, haemorrahge, skin diseases, wounds, head- ache and leprosy in Southeast Asia[2-4]. The bruised fresh leaves are used for snake-bites in India and SriLanka[4]. Usually, yellow leaves are exploited for cough[5]. and smoke from leaves is used for asthma[6]. The plant leaves are used for checking postpartum haemorrhage and urinary trouble[7]. It is found that 70% of the pregnant women in the Gora village of Lucknow (Uttar Pradesh, India) use the leaves of *J. Adhatoda* to induce abortion[8]. Moreover, it is observed that the Neterhat people in Bihar (India) used a decoction of the leaves to stimulate and heal before and after delivery[9]. The leaf powder boiled in sesame oil is used to stop bleeding, earaches as well as pus from ears and jaundice[10].

Not only in Ayurveda but also in modern allopathy medicine, the *Adhatoda vasica* plant leaves have been used for several active ingredient preparations. There is no report in the literature on the suitability of solvent for the extraction of phytochemical constitutes scientifically. Thus, the present study is the investigation of suitability of extraction solvent through extraction value, phenol and alkaloid content and phytochemical profiling through GC-MS study.

2. MATERIALS AND METHODS

2.1 Collection & Authentication of plant material

The leaves of *Adhatoda vasica* were collected from the Herbal garden, Tamil University, Thanjavur, India and authenticated by Professor Jagadeesan, Head, Department of Herbal and Environmental Sciences, where a voucher specimen was submitted.

2.2 Determination of extractive-values & yield Percentage

The leaves of *Adhatoda vasica* were dried at 40°C in the conventional hot air dryer and powder was used for the extraction process. The yield percentage of the solvent extracts of the plant was calculated from the product that was obtained after evaporation[11]. Five gm of the *Adhatoda vasica* leaf powder was macerated with 50 ml of ethanol, methanol, hexane and chloroform (1:10, w/v) in a closed flask for 24 hrs. The flask was shaken intermittently during 6 hrs and allowed to stand for 18 hrs. The extract was filtered rapidly, taking precautions against loss of solvent. The filtrate was evaporated to dryness in a porcelain dish and dried at 105ºC, to a constant weight. The percentage of methanol-soluble extractive value was calculated with reference to the air dried powder.

2.3 Determination of total phenolic content

The total phenolic content was determined with Folin—Ciocalteau reagent. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium produce blue colored complex (molybdenum blue). The sample of 1.0 g was weighed and grind with a pestle and mortar in volume of 80% methanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was saved and the re extracted with five times the volume of 80% ethanol. Centrifuge and pool the supernatants. The supernatant was evaporated to dryness. The residue was dissolved in a 5ml of distilled water. Different aliquots like 0.2, 0.4 and 0.6 ml were taken in the test tubes and make upto 3 ml with distilled water. Folin-ciocalteau reagent of 0.5 ml was added to the tubes. After three minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube. The homogenate was mixed thoroughly and placed in

boiling water for exactly one minute, cooled and measured the absorbance at 650 nm against a reagent blank. A standard curve was prepared using different concentrations of gallic acid. The concentration of total phenolic compounds in the plant extract was determined and expressed as microgram of gallic acid equivalent. Analysis was done in triplicate for each sample and each concentration of standard [12].

2.4 Estimation of alkaloids

The alkaloid determination was done by following previously published work[13]. The sample was weighed in to a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4hr. then it was filtered and the extract was concentrated on water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to stand till settlement of precipitate. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. Alkaloid was collected as residue and weighed after complete dryness and percentage was calculated and expressed in mg/g of plant extracts.

2.5 Phyto-chemical components identification through GC-MS

The *Adhatoda vasica* leaves (25 grams) powder was soaked in 40 ml of ethanol, methanol and hexane and kept for overnight soaking. The samples were filtered and concentrated through nitrogen flushing. 2 µl of prepared sample was injected into the GC-MS instrument. GC Clarus 500 Perkin Elmer, Carrier gas: 1ml per min, Split: 10:1, Detector: Mass detector Turbo mass gold-Perkin Elmer,Software: Turbomass 5.2, Sample injected: 2µl, Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25mm x 0.25µm df , Oven temperature Programme: 110° C with 2 min hold ,Up to 200° C at the rate of 10 ° C/min without hold, Up to 280 ° C at the rate of 5° C / min with 9 min hold, Injector temperature 250° C, Total GC running time 36 min, Inlet line temperature 200°C, Source temperature 200°C Electron energy:70 eV, Mass scan (m/z): 45-450,Solvent Delay: 0-2 min, Total MS running time: 36 min. In the MS Programme, NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components of the *Adhatoda vasica* leaves. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

3. RESULTS & DISCUSSION

The influences of nature of solvents and total time of extraction on the percentage extraction yield of the *Adhatoda vasica* leaves are shown in figure 1. The different yields of extracts with ethanol, methanol, n-hexane and chloroform might be influenced by the polarities of solvents[14&15]. The methanol (18.4%) and ethanol (16.3%) extracts showed a high extraction value. The percentage extraction yield for leaves with methanol was better than n-hexane. The average extracted yield by methanol is almost 50% more than average extracted yields by n-hexane. This was also reported by Klejdus et al. that ethanol and methanol are better solvent during their research on comparing the different extraction conditions including solvents and techniques for extraction of isoflavones soybeans samples[16].

The total phenolic content was studied by comparing with standard gallic acid. The total phenol content of *Adhatoda Vasica* leaves found to be higher 279.25±0.05 mg/g in ethanol extract compared to 228.22 ± 0.25 in methanol, 89.28 ± 0.09 mg/g hexane and 105.25 ± 1.05 mg/g in chloroform extracts. Comparing the alkaloid content in different drying temperature, little difference has been observed as most

of the alkaloids boiling point is above the drying temperature.

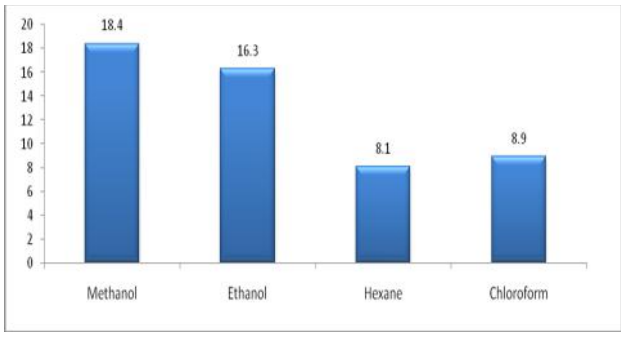

Fig 1: Extraction Yield (%) of *Adhatoda vasica* leaves in different solvents

The alkaloid content of leaves in ethanolic extract is 14.99 ± 0.05 mg/g where as 14.52 ± 0.26 mg/g in methanol (figure. 2). The alkaloid content of hexane and chloroform extracts is lesser than the polar solvent extractions.

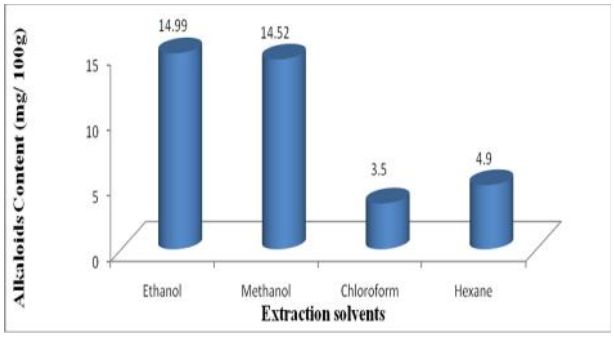

Fig 2: Alkaloid content (%) of *Adhatoda vasica* leaves in different solvents

The GC-MS spectral studies and comparison of results with library successfully enabled the identification of the eighteen compounds belonging to the various groups like Aminoacids, Ester, Phenol, Terpene Alcohol, Diterpene, Linolenic acid, Alkaloid, Vitamin, Steroid, Sesquiterpene oxide and nitrogen compounds. The active principles with their retention time (RT), compound name, molecular formula, activity are given in the Table 1. The GC-MS chromatogram of ethanol, methanol and hexane extracts were given in figure 3 to 6.The result clearly highlights that more compounds were identified in the ethanolic extract.

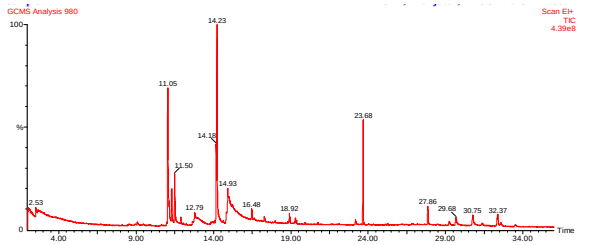

Fig 3: GC-MS Chromatogram of ethanol extract of *Adhatoda vasica* leaf

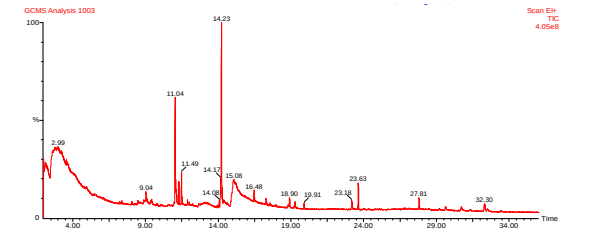

Fig 4: GC-MS Chromatogram of methanol extract of *Adhatoda vasica* leaf

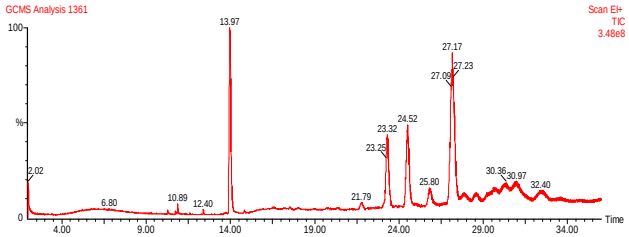

Fig 5: GC-MS Chromatogram of hexane extract of *Adhatoda vasica* leaf

4. CONCLUSION

Through comparison of extraction methods, this study highlights the bias on phytochemical compounds, demonstrated by clear differences in GC-MS analyses. As outlined in previous studies, the extraction yield is highly dependent on solvent selection in addition to method suitability. In the present comparison, this study would highly recommend the ethanol extraction for phytochemicals. Meanwhile, this study also can serve as model for how such studies would be conducted across careful selection of solvent for active ingredients preparation. From a commercial perspective, a techno-economic assessment is needed and should ideally be carried out for large-scale extraction where costs are likely to be very different compared to the presents laboratory-based study.

Table 1: Components identified in the *Adhatoda vasica* ethanol extract sample through GC - MS study

No.	RT	Name of the compound	Peak Area %		
			Ethanol	Methanol	Hexane
1.	6.13[a],7.23[b], 6.80[c]	Benzenemethanol, à-[1-(methylamino)ethyl]-	1.84	0.15	0.60
2.	7.38[b]	Cyclopentaneethanamine, N,à-dimethyl-	-	0.33	-
3.	8.53[a], 8.49[b]	Pseudoephedrine, (+)-	0.43	0.58	-
4.	9.08[a], 9.04[b]	2,3-O-Benzal-d-mannosan	0.67	2.27	-
5.	9.44[b]	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	-	1.05	-
6.	10.30[c]	2-Aminononadecane	-	-	0.11
7.	11.05[a], 11.04[b]	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	10.73	10.10	-
8.	11.30[a]	2-Tridecen-1-ol, (E)-	1.94	-	-
9.	11.49[b]	9-Tetradecen-1-ol, acetate, (E)-	-	2.65	-
10.	11.50[a]	3-Eicosyne	3.61		-
11.	11.90[b]	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	-	0.50	-
12.	11.91[a]	Bicyclo[2.2.1]heptane, 2,2,3-trimethyl-	0.31	-	-
13.	12.40[c]	2,4,6,8-Tetramethyl-1-undecene	-	-	0.22
14.	12.79[a]	n-Hexadecanoic acid	6.88	-	-
15.	13.97[c]	9,12-Octadecadienoic acid (Z,Z)-	-	-	22.23
16.	14.18[a]	1-Eicosanol	4.32	-	-
17.	14.23[a&b]	Phytol	14.62	15.75	-
18.	14.93[a], 15.08[b]	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	30.93	48.30	-
19.	16.48[a&b]	3,trsns-(1,1-dimethylethyl)-4,trans- ethoxycyclohexanol	1.89	3.19	-
20.	16.61[a]	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	1.97	-	-
21.	17.28[a], 17.29[b]	9-Oxabicyclo[6.1.0]nonane, 1-methyl-, cis-	1.08	1.90	-
22.	17.98[a]	Z,Z-2,5-Pentadecadien-1-ol	0.41	-	-
23.	18.81[a]	2-Cyclopentene-1-undecanoic acid, (+)-	0.13	-	-
24.	18.92[a], 18.90[b]	Ethanethioic acid, S-[2-(dimethylamino)ethyl] ester	0.80	1.30	-
25.	23.18[a&b]	Pyrrolo[2,1-b]quinazolin-9(1H)-one, 3-[2-(dimethylamino)phenyl]-2,3-dihydro-	0.58	1.29	-
26.	23.68[a], 23.63[b], 23.32[c]	Squalene	7.40	2.76	13.95
27.	24.52[c]	Hexadecane, 3-methyl	-	-	16.84
28.	25.80[c]	Octadecane, 6-methyl-	-	-	1.65
29.	27.17[c]	Heptacosane	-	-	39.68
30.	27.86[a], 27.81[b]	Vitamin E	1.74	1.47	-
31.	29.19[b]	Z,Z,Z-4,6,9-Nonadecatriene	-	0.43	-
32.	29.24[a]	1-Naphthalenepropanol, à-ethyldecahydro-5-(hydroxymethyl)-à,5,8a-trimethyl-2-methylene-, [1S-[1à(S*),4aá,5à,8aà]]-	0.75	-	-
33.	29.68[a]	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	1.59	-	-
34.	30.36[c]	Octadecane, 1-(ethenyloxy)-	-	-	1.92
35.	30.75[a], 30.72[b], 30.97[c]	trans-Z-à-Bisabolene epoxide	2.05	1.12	1.50
36.	31.37[a], 31.31[b]	5à-Androstan-16-one, cyclic ethylene mercaptole	0.45	0.44	-
37.	32.37[a], 32.30[b]	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	1.95	1.82	-
38.	32.57[a]	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	0.74	-	-
39.	33.51[a]	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	5.75	-	-

5. REFERENCES

1. Wijesekera RB. Plant derived medicines and their role in global health. The Medicinal Plant Industry. CRC Press; 1991; 256
2. Adnan M, Hussain J, Shah MT, Ullah F, Shinwari JK, Bahadar A, Khan AL. Proximate and nutrient Composition of Medicinal Plants of Humid and Sub-humid regions in Northwest Pakistan. J. Med. Plant Res 2010; 4: 339-345.
3. Atta-Ur-Rahman, Said HM, Ahmad VU. Pakistan Encyclopaedia Planta Medica. Hamdard Foundation Press, Karachi 1986; 1: 181-187.
4. Roberts E. Vegetable materia medica of India and Ceylon. Plate Limited, Colombo 1931: 16-17.
5. Lal SD, Yadav BK. Folk medicine of Kurukshetra district (Haryana), India. Econ. Bot 1983; 37: 299-305.
6. Shah NC, Joshi MC. Ethnobotanical study of the Kumaon region of India. Econ Bot 1971; 25: 414-422.
7. Pushpangadan P, Nyman U, George V. Glimpses of Indian Ethnopharmacology. Tropical Botanic Garden and Research Institute, Kerala 1995; 309-383.
8. Nath D, Sethi N, Srivastava S, Jain AK, Srivastava R. Survey on indigenous medicinal plants used for abortion in some districts of Utter Pradesh. Fitoterapia 1997; 68: 223-225.
9. Jain SP, Singh SC, Puri HS. Medicinal plants of Neterhat, Bihar, India. India. J ethnopharmacol 1994; 32: 44-50.
10. Reddy MB, Reddy KR, Reddy MN. A survey of medicinal Plants of Chenchu tribes of Andhra Pradesh, India. Ind. Int. J. Crude Drug Res 1988; 26: 189-196.
11. Roy Saumendu, Karmakar Prithivi Raj, Dash Suvakanta, Chakraborty Jashabir, Das Biswajit. Hair growth stimulating effect and phytochemical evaluation of hydro-alcoholic extract of Glycyrrhiza glabra, Global J Res. Med. Plants & Indigen. Med 2014; 3(2): 40–47.
12. Folin O. Tyrosine and tryptophan determinations in proteins. J. Sci. Food Agri 1927; 73:672-649.
13. Harbone J B. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Co. New York, Third Edition 1984; 1: 289.
14. Luque de Castro, M. D., Valcarcel, M. & Tena, M. T. Analytical Supercritical Fluid Extraction, Butterworth Publishers, Boston 1994.
15. Romdhane, M. & Gourdou, C. Investigation in solid-liquid extraction influence of ultrasound. Chem Eng J 2002; 87: 11-19.
16. Klejdus B, Mikelova R, Adam V, Zehnalek J, Vacek J, & Kizek R. Liquid chromatographic-mass spectrophotometric determination of genistin and daidzin in soybean food samples after accelerated solvent extraction with modified content of extraction cell. Analytica Chimica Acta 2004; 517: 1-11.

Conflict of Interest: None

Source of Funding Nil