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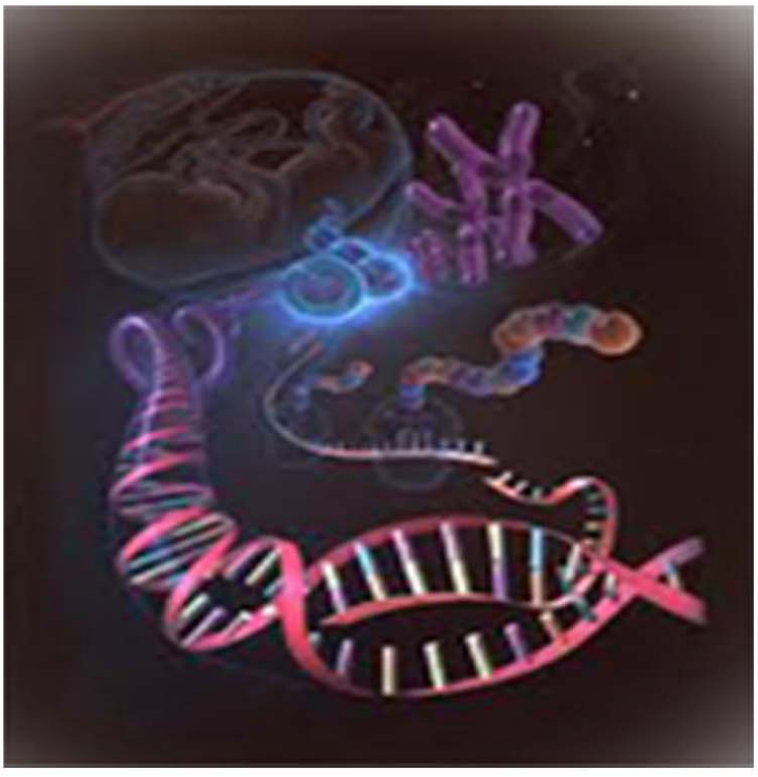

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Research Paper

INFLUENCE OF DRYING TEMPERATURE ON QUALITY PARAMETERS OF *ADHATODA VASICA* LEAVES

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Adhatoda vasica (AV), an official drug in the Indian Pharmacopoeia has been used for the treatment of cold, cough, bronchitis and asthma. However, no conclusive formulation study of its leaves has been performed yet. In present investigation, optimization of drying conditions of AV leaves based on qualitative analysis and the retention of phenols, antioxidants and alkaloids was carried out to lay down the standards which could be useful in future experimental studies. The ethanol and water shows the highest extractive values. The presences of alkaloids, steroids, flavonoids and saponins were confirmed during preliminary phytochemical screening. A significant difference was observed in phenolic content and antioxidant activity of 40 ºC dried leaves than the others. The alkaloid content retention is more in all the three different temperature. The suitable drying condition and extraction solvent for a therapeutic formulation preparation based on the retention of active compounds is 40 ºC and ethanol. In conclusion, this study would serve as a useful gauge in standardization and development of functional food, isolation of medicinally important phytoconstituents, performing biotechnological developments and ensuring quality formulations.

Keywords:*Adhatoda vasica*, Polyphenols, Antioxidants, Alkaloids, Asthma

INTRODUCTION

Adhatoda vasica (AV) belong to the family *Acanthaceae*. AV has been used in traditional Indian medicine for thousands of years to treat respiratory disorders (Shabir *et al.,* 2013). 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 and cytotoxic (Jain *et al.,* 1991; Nadkarni, 1954; Gupta nad Chopra, 1954; Kirtikar, 1935; Chopra, 1982; Sampath *et al.,* 2010). Quinazoline alkaloids present in the leaves are established as active principles. In the indigenous food preparations, AV leaves were made into a decoction with pepper and dried ginger. But the modern medicine searched its active ingredients and found out that vasicine, oxyvasicine and vasicinone are the alkaloids present in vasaka, the active ingredients for expelling sputum from

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the body (Sampath *et al.,* 2010). Bromhexine, a synthetic derivative of the alkaloid vasicine found the market in the treatment of respiratory disorders. Some of the herbal preparations containing AV leaves used in the treatment of asthma disorder worldwide are *kada, fermiforte, salus tuss, kan jang* and *spirote* (Sandeep *et al.,* 2011). The leaves, roots and young plants of AV contain the quinazoline alkaloids vasicine, 7-hydroxyvasicine, vasicinolone, 3-deoxyvasicine, vasicol, vasicoline, vasicolinone, adhatodine, anisotine) betaine, steroids carbohydrate and alkanes. In the flowers triterpines (a-amirine), and flavonoids (Apigenin, astragalin, kaempferol, quercetin, vitexin) have been found (Lahiri and Pradhan, 1964; Joshi *et al.,* 1994).

The aim of this study was to investigate the retention of phenols, antioxidants and alkaloids in three different drying temperatures which paves the way for the development of several treatment regimens.

MATERIALS AND METHODS

Authentification of Plant Material

The leaves of AV (*Acanthaceae)* 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.

Drying at Different Temperature

The fresh AV leaves was divided into three batches. The leaves were immediately dried at 40, 50 and 60°C (room temperature 25°C and relative humidity 60%) by placing in a thin single layer on the drying tray of the conventional hot air dryer. The sample weight was kept constant at 100 g for all runs. The moisture loss was recorded at 1 h intervals during drying to achieve the equilibrium moisture content (12%). The dried samples were powdered prior to evaluation studies.

Extract Preparation and Phytochemical screening

A weighed quantity (100 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with ethanol, methanol, chloroform, ethyl acetate and hexane individually and the residual marc was collected. The extract was filtered through a Whatman filter paper (no. 1). The extract was evaporated under hot air oven at a low temperature (40-60^{o}C) until all the solvent had been removed. The ethanol and water extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, flavonoids, phytosterols, fixed oils, fat and phenolic compounds as major active constituents by suitable chemical tests. Phytochemical screening of plant extracts was done following the standard procedure (Trease and Evans, 1989)

Estimation of Total Phenolic Content

The amount of total phenolics in extracts was determined according to the Folin-ciocalteu procedure. Gallic acid was used as a standard and the total phenolics were expressed as mg/g Gallic Acid Equivalents (GAE). Samples (200 µl) were introduced into test tubes. One milliliter of Folin ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as GAE in milligrams per gram as calculated from standard gallic acid graph (Maurya and Singh, 2010).

Antioxidant Assay

Air dried and powdered samples (1 g) were extracted with 10 ml of methanol which is soaked overnight in a shaking incubator at room temperature and temperature not exceeding the boiling point of the solvent. The methanolic extracts were filtered using what man filter paper. From that extract, 0.1, 0.2, 0.3 ml of concentration taken for analysis. Standard Ascorbic acid prepared in the 0.1 (10 mg), 0.2 (20 mg), 0.3 (30 mg) concentration for analysis. To all the tubes, 6 ml of 0.004% of DPPH in 80% methanol was added. The solution mix was kept at incubation for 30 min at 25ºC. The absorbance was read against a blank at 517 nm (Koleva *et al.,* 2002). Mean value of triplicate was plotted in graph in order to calculate the concentration required for 50% reduction (50% inhibition concentration, IC_{50}) of DPPH radical scavenging activity in the following way.

$$AA\% = [(A_B - A_S) / A_S] \times 100$$

Where, AA% is the antioxidant activity, A_B is the absorbance of the DPPH reagent and A_S is the absorbance of the sample proving 50% inhibition [IC_{50})]. BHT and Ascorbic acid was used for the comparison to IC_{50} of the extracts.

Estimation of Alkaloids

The alkaloid determination was done by following previously published work (Harbone, 1984). 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 4 h, 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.

STATISTICAL ANALYSIS

In all the experiments, sample analysis assay were carried out in triplicate. The results were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

The results of the AV leaves extraction at different temperature and solvents were given in Table 1. The methanol (18.4%) and ethanol (16.3%) extracts showed a high extraction value at 40ºC. In both the ethanol and water extracts, the phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols and phytosterols in 40ºC and 50ºC ethanol extract (Table 2). The fixed oils and fat were found absent in both the extracts. Comparing the extraction solvents in multiple test, alkaloids, phenols and flavonoids were present in ethanol than water extracts. It is well known that, in the traditional healing water is the primary source for the preparation of plant extracts, but for the instance we found ethanol to be as the most appropriate solvent for the active components of the plant. This might have resulted from the lack of solubility of active compounds in the water. This study received pronounced importance, as the results implies the effect of drying temperature on AV leaves and confirms the presence of varied composition of secondary metabolites in lieu with the earlier studies[16].

The DPPH radical scavenging activity of the extract was decreased with the increasing temperature, the reducing power of extract was

Table 1: Percentage Yield of AV Leaf Powder in Different Drying Temperature and Solvent

Extract	Colour	Nature of the Extract	Percentage Yield (%w/w)		
			40°C	50°C	60°C
Methanol	Dark Green	Semisolid	18.4	17.6	12.8
Ethanol	Dark Green	Semisolid	16.3	14.9	10.7
Ethyl acetate	Dark Green	Semisolid	13.6	11.2	9.2
Choloroform	Dark Green	Semisolid	8.9	6.8	3.4
Hexane	Dark Green	Semisolid	8.1	5.1	3.9

Table 2: Qualitative Analysis of AV Leaf Powder in Different Drying Temperature

Secondary Metabolites	Reagents	40°C		50°C		60°C	
		Ethanol	Water	Ethanol	Water	Ethanol	Water
Alkaloids	a) Mayer 's reagent	+	+	+	+	+	+
	b) Dragendroff's reagent	+	+	+	-	+	-
	c) Hager 's reagent	+	-	+	-	+	-
	d) Wagner 's reagent	+	+	+	+	+	-
Polyphenols	a) with Ferric Chloride Solution	+	+	+	-	-	-
	b) with Gelatin Solution	+	-	-	+	-	-
	c) with Lead acetate Solution	+	+	+	-	-	-
	d) with Aqueous bromine Solution	+	+	+	-	+	-
Flavanoids	a) with Aq.NaOH	+	+	+	+	-	-
	b) with Con.H2SO4	+	-	+	+	-	-
	c) with Mg.+ HCl	+	+	+	-	-	-
Fixed oil and Fats	a) Spot Test	-	-	-	-	-	-
	b) Saponification Test	-	-	-	-	-	-
Phytosterols	a) Libermann's Sterol Test	+	-	+	-	+	-
	b) Libermann – Burchard Test	+	-	+	-	+	-

Note: + : Positive - presence of the compounds, - : Negative.

Table 3: Total Phenolics, Antioxidants and Alkaloid Content of AV in Different Drying Temperature

S. No.	Drying Temperature	Total Phenolics(mg/g)	Total Antioxidant(%)	Alkaloids (mg/mg)
1	40°C	280.25 ± 1.05	76 ± 0.10	15.03 ± 0.05
2	50°C	238.22 ± 0.75	68 ± 0.75	14.09 ± 0.06
3	60°C	168.25 ± 2.05	42 ± 1.05	13.99 ± 0.10

Note: The values are the average of three determinations and are expressed as mean ± Standard deviations.

carried out with BHT and ascorbic acid as standard reducing agents. The DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by extract either by transfer of hydrogen or of an electron (Shimada *et al.,* 1992). AV showed high DPPH radical scavenging activity (76±0.10%) in 40ºC dried leaf powder and standard antioxidants, BHT and ascorbic acid showed 78.30±1.42% and 76.71±1.83% of inhibition respectively (Table 3). Total Phenolics were studied by comparing with standard Gallic acid. The total phenol content of AV leaves found to be higher in 280.25±1.05 mg/g in 40ºC compared to 238.22 ± 0.75 and 168.25 ± 2.05 mg/g in 50ºC and 60ºC respectively. 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. The alkaloid content of AV leaves in 40ºC is 15.03±0.05 mg/g where as 14.09 ± 0.06 mg/g and 13.99 ± 0.10 mg/g in 50 and 60ºC respectively (Manoj Kumar *et al.,* 2013).

CONCLUSION

The influence of drying temperature on the retention of phenolic compounds, alkaloid and antioxidant activity in AV leaves was studied. On the basis of the results obtained in the present investigation, it is possible to conclude that the extraction value is more in polar than the non polar solvents. The preliminary phytochemical screening result is in line with the quantitative analysis observations. There is a significant difference in phenolic content and antioxidant activity in 40ºC dried leaves than the others. The alkaloid content retention is more in all the three different temperature. From the observations, it can be concluded that the AV leaves can be dried at 40ºC effectively so as to avoid losing active compounds.

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