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E-mail: bjsir07@gmail.com

Characterization of an Expectorant Herbal Basak Tea Prepared with *Adhatoda vasica* Leaves.

Ashish K. Sarker[a]*, Kabir Ahamed[b], Jasim Uddin Chowdhury[b] and Jarifa Begum[b]

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

Abstract

A herbal tea for an expectorant action was prepared with *Adhatoda vasica* leaves. Analytical, pharmacological, microbiological and animal toxicity studies were carried out to characterize the herbal tea. The analytical data indicates that the alcohol extract from herbal basak tea contains 0.67% crude alkaloids and the isolated tracheal chain experiment with this extract showed small relaxation effect compare to the standard histamine drug. The crude alkaloids and the other extracts (petroleum ether extract, alcohol extract and hot water extract) showed mild inhibition in different degrees against different microorganisms. The animal toxicity studies on rats reveled no mortality after 24 hours and also no abnormal delayed effect indicates no toxicity of prepared tea at all. Based on the above results, the prepared herbal basak tea is proposed as a good expectorant. Herbal tea prepared with *Adhatoda vasica* leaves collected in May to September showed better efficacy than those of other times.

Key wards: Herbal basak tea, Crude alkaloids, Relaxation effect.

Introduction

Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contributions towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Ayurvedic and Unanii medicine system or it can be the base for the development of a medicine, a natural blueprint for the development of new drugs. *Adhatoda vasica*, a well known medicinal plant commonly known as Basak in Bengali is indigenous to Bangladesh. It is 1.2 - 6.0 m in height. Leaves are 5 - 30 cm long and light green in color. It grows even as a mixed crop in the rubber garden as well as in the tea gardens. The farmers on the roadside and fallen land can also cultivate Basak. It possesses a wide spectrum of medicinal properties (Dorsch *et al.* 1991; Chakraborty *et al.* 2001& Shrivastava *et al.* 2006). The alkaloids of an *Adhatoda vasica* were mainly reported for their effect on respiratory system (Cambridge *et al.* 1962 Amin *et al.* 1963).

Detail pharmacological investigation (Gupta *et al.* 1977a) were carried out in regional research laboratory, Jammu on the alkaloid vasicine reveal that besides the bronchodilator activity, it is also possessed marked uterine stimulant and abortifacient activities possibly mediated through the release of prostaglandin. *Claeson et al.* (2000) studied a critical review of ethno pharmacological and toxicological data on *Adhatoda vasica*. Some investigators also studied antibacterial (Gautam *et al.* 2007) and phytochemical (Juneja *et al.* 2007) screening of *Adhatoda vasica* leaves extract.

Ayurvedic and Unani companies produce herbal medicine in combination of *Adhatoda vasica* leaves and other selected herbs without any analytical criteria for quality control. Local people extract the juice by crushing and pressing the leaves that contains very small amount of alkaloids with large amount of leaves pigment. Therefore, we made an attempt to prepare a good expectorant herbal tea with single herb leaves like *Adhatoda vasica* leaves that will give required vasicine alkaloids and less pigment in hot water extraction for the treatment of common cold.

Materials and Methods

Preparation of herbal basak tea.

2.0 kg fresh leaves of *Adhatoda vasica* (basak) were collected, cleaned and cut into small pieces. It was then ruptured by blending. The blended leaves were allowed to ferment in a thick layer for 18 hours at a temperature of 30 - 32^{O}C. After fermentation, the material was sprayed on trays and dried by air circulation for 4 - 6 hours. The partially dried tea was again crushed into fine particles to increase the surface area and dried in hot air dryer at 65-70^{O}C for 4 hours. The yield was 712 g.

*Corresponding Author, Email:

Extracts for microbiological and pharmacological test.

Water extract: The tea (100 g) was crushed in powder form and soaked in hot water (80 - 85^{O}C) for 5 minutes. The extraction was carried out three times. The combined extracts were filtered and concentrated on water bath and then in a vacuum oven to a dry mass. The yield was 28.0 g.

Alcohol extract: Powdered tea (100 g) was soaked in alcohol and extracted after 24 hours. Three times extractions were carried out and filtered. The combined extract was evaporated in a rotary vacuum evaporator and finally dried in a vacuum dryer. The yield was 33.87 g.

Petroleum ether extract: 100 g tea was extracted with petroleum ether (40 - 60^{O}C) in a Soxlet apparatus for two hours and after removing solvent on hot water bath, the residue was dried in a vacuum oven for 5 hours at 60^{O}C. The yield was 9.14 g.

Crude alkaloids: 100 g tea were finely powdered and extracted with dilute hydrochloric (2 N) in warming (40-45^{O}C) condition. The water-soluble salts of alkaloids were thus removed in solution, leaving the insoluble materials behind. The acids extracts were then made basic with dilute ammonia solution and extracted two times with chloroform. The chloroform layer separated and washed with water to free alkali. After removing chloroform, the crude alkaloids were obtained and purified by column chromatography using chloroform solvent. The yield was 0.67 %.

Determination of antibacterial activity.

The disc diffusion technique was employed for determination of antibacterial activity using nutrient agar medium. In this method, solution of known concentration (µl/ml) of the test samples were made by dissolving measured amount of the samples in definite volume of solvents. 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 positive control for comparison of results. These plates were kept at low temperature (4^{O}C) for 24 hours to allow maximum diffusion. During this time disc absorb water from the surrounding medium and then test materials dissolved and diffused out of the media. 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.

Pharmacological study

Test on isolated tracheal chain

The effect of test samples on tracheal chain was investigated following the method of Castillo and De-Beer with some modification. Male guinea pigs (300-500 g) were killed by a blow on the neck and trachea removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle and sutured together to form a tracheal chain. Tissue was then suspended in a 10 ml organ bath (Kent, UK) containing Krebs-Henseleit solution of the follow composition (mM) : NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, and dextrose 11. The Krebs solution was maintained at 37^{O}C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1h while it was washed with Krebs solution every 20 minutes.

The effects of the test samples were observed on guinea pig ileum following the method of Magnus. The strip of ileum was mounted in an aerated bath (20 ml) containing tyroid solution and maintained at 32^{O}C. Doses of crude extract were used in the bath. Responses of the tissue with test sample and interaction with histamine were recorded on chart paper using GIMINI two channels Recorder 7070 (UGO Basile, Italy).

Acute toxicity test on rat.

The water extract of the basak tea and the fresh juice of basak leaves were dosed orally at the rate of 2 g / kg body weight on 10 white strain rats weighing about 150 - 160 g (5 male and 5 female) and observed for 24 hours for their mortality and next 10 days for any delayed effects.

Assay of basak tea.

A sample of basak tea was analyzed for its chemical composition. Applying the technique of Thin Layer Chromato-graphy (TLC), alkaloid vasicine was identified. The prepared silica gel plate was used in this method. The sulfuric acid was sprayed on the TLC plate to make the spot visible. The other parameters like total ash, acid soluble ash, fat, nitrogen, fiber, tannin, minerals and carbohydrate were analyzed by applying techniques following "Official method of analysis", Association of the Official Agricultural Chemist, (Ed. 1971). BFS -228 -010G automatic balance - moisture analyzer was used for estimation of moisture.

Results and Discussion

The effect of crude alkaloid extract on tracheal chain preparation was observed before and after histamine application. The histamine induced contracted tracheal chain relaxes

when test sample was added to the bath. The results are shown in Table I.

Table I. Tracheal chain relaxation effect with crude alkaloid extract of herbal basak tea.

Dose(ml)	Concentration (mg/ml)	Relaxation (cm)	% Relaxation
15	100	1.13	27.53
30	100	2.21	53.67
45	100	3.20	78.04
60	100	4.10	100

With the crude alkaloid extract of herbal basak tea, smooth muscles of guinea pig are relaxed. It reduces the contraction of histamine. The action of histamine is blocked by the crude alkaloid extract but the hot water extract showed very little response because of low concentration of alkaloid in water extract compare to that of crude alkaloid extract. Vasicine, at low concentrations induced bronchodilation and relaxation of the tracheal muscle (Chopra *et al.* 1925).

The antibacterial activities of three extracts (petroleum ether extract, alcohol extract and water extract) and crude alkaloids of herbal basak tea have been evaluated against seven bacteria and the test results are summarized in Table II.

Among the three extracts, alcohol extract and water extract exhibited mild inhibition in different degrees against four microorganisms. The crude alkaloid of basak tea showedinhibition in different degrees against five microorganisms. From the above experiments it is seen that the efficiency of different extracts depends mainly on the concentration of alkaloid. Since water extract contains less alkaloid than alcohol extract and crude alkaloid, its inhibitory effect is also little.

Acute toxicity test for water extract of basak tea on rats revealed no mortality after 24 hours and also no abnormal delayed effect. Similar observation was also found with the fresh juice of basak leaves. Mortality of the white strain rats has been shown in Table III.

Table III. Effect of water extract of basak tea and water extract of basak fresh leaves on the mortality of white strain rats (both male and female).

Extracts	White strain rats	White strain rats	Number of death after 24 hours	Number of death after 20 days
Water extract of basak tea dosed orally at the rate of 2 g / kg body weight	5 male	5 female	Nil	Nil
Water extract of fresh leaves dosed orally at the rate of 2 g / kg body weight	5 male	5 female	Nil	Nil

It is concluded that the basak tea as prepared has no gross toxicity on white strain rats.

Herbal basak tea thus prepared has needed to chemical analysis for its quality control. The proximate composition of herbal basak tea is shown in Table IV.

From it chemical analysis, it is seen that herbal basak tea contains mainly vasicine alkaloid. This alkaloid present in tea possesses respiratory stimulant activity (Amin et al. 1959). The above subsequent studies support the herbal basak tea as a good expectorant. Seasonal variation of the

Table II. Antibacterial activity of different extracts (petroleum ether extract, alcohol extract and hot water extract) and crude alkaloids of herbal basak tea.

Name of the extracts/ alkaloids	Dose (µg/dis)	Diameter of zone of inhibition in mm						
		Bacillus cereus	*Bacillus subtilis*	*Bacillus megaterium*	*Saimoneiia typhi*	*Staphylo-coccus aureus*	*Vibrio cholerae*	*E. Coli*
I. Petroleum ether extract	1832	-	11	-	-	-	15	-
II. Alcohol extract	1500	6	11	-	-	-	13	5
III. Hot water extract	1700	3	8	-	-	-	7	2
IV. Crude alkaloids	333	11	17	13	-	-	16	6
V. Ampicillin	10	-	16	-	25	24	28	30

' - ' Sign indicates no activity.

active alkaloids occurs with the highest concentration occurring in the May to September when the leaves begin to change color and become fresh. Due to higher alkaloid content the herbal tea gives better efficacy.

Table IV. Chemical analysis of herbal basak tea for quality control.

General information	Comments
Appearance	Free flowing fine particle
Color of herbal tea	Iron gray
Odour	Leafy
Taste	Slight astringence
Physico-chemical constants	
Loss in weight on drying at $105^{O}C$	6.41 %
Solid content	93.59 %
Extractive values	
Water soluble extracts	28.0 %
Petroleum soluble extracts	9.14 %
Alcohol soluble extracts	33.87 %
Crude fiber	19.86 %
pH value	
pH of 1.0 % aqueous solution	6.31
Specification	
Bulk density	0.99- 1.003
Minerals	11.12 %
Acid insoluble minerals	0.81 %
Arsenic (as As_2O_3)	1.57 p. p. m.
Total heavy metals (as Pb)	3.41 p. p. m.
Crude alkaloids content	
(mainly vasicine)	0.67 %
Tannin	0.016 %
Total nitrogen	2.57 %
Microbiological analysis	
Total bacterial count	Nil
Total fungal count	Nil
E. Coli	Absent
Salmonella	Absent
Pathogens	Absent

Conclusion

Herbal drugs now manufactured in tea form are based on combination of several herbs which is very difficult to set up the analytical criteria for quality control. New thoughts for dispensing the single herb in crude but active form are introduced. All the results taken together show that water extract of herbal basak tea prepared with Adhatoda vasica leaves contains pharmacologically active vasicine alkaloids with antibacterial properties. During the fermentation process, the enzymatic action on Adhatoda vasica leaves proceeds and the aqueous insoluble alkaloids in leaves are converted into aqueous soluble alkaloids. The cough is relived and sputum is liquefied by the action of vasicine alkaloid so that it is brought out easily. Thus, we presume that herbal basak tea can be developed as a good expectorant for the treatment of asthma.

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