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Assesment on Biological Activity of Adhatoda Vesica

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ABSTRACT:
In healthcare system the medicinal plants are most commonly used in a whole world. Themetabolites that is present in the extract of herbal plants that contain different chemicalsubstance that may produce different therapeutic effects. The Adhatoda Vesica herbal plant that is most commonly used is a southeast region for the treatment of different microbialdiseases. The methanolic extract of leaf and synthesis of AgNPs is able for the curing ofdifferent skin diseases and wound healing purpose. The Adhatoda Vesica plant contains anumber of secondary metabolites such as alkaloids. saponins, amino acids, carbohydrate,flavonoids and proteins etc. A prolonged intense look of literature data revealed someimportant biological activities like antibacterial, anti-inflammatory, antifertility, anti-ulcer,anti-tissue, anti-typhoid and anti oxidant, Immunomodulatory, hepatoprotective, andwound healing. The pharmacological and phytochemical review of AdhatodaVesica realizedthe importance of the medicinal plants.

Keywords: AdhatodaVesica, Microbiological activity, Phytochemistry and Metabolites.

INTRODUCTION :

[1]Plants are the natural source of drugs that are used for the treatment and curing of variousdiseases generated by microorganisms such as bacteria and virus in the body of living organisms The natural products are used to control the complication in the body of living beings. [2] The braches that used herbal medicinesfor the treatment of various pharmacological diseases arecommonly called Ayurvadia, Unani, Homeopathy, Siddha etc. The whole principle of these ancients branches depend upon the availability of herbal plants. Different plants are rich source of chemical substance that is helpful for the analgesic and anti-inflammatory treatments. The AdhatodaVesica and many others species contain high quantity of secondary metabolites, especially Vitamin C, used against for the treatment of various biological activity includes anti-pyretic, anti-diabetic and oxytoxic. About 40% of medicines that are use for the curing of dangerous diseases have plant origins. In Southeast Asia the AdhatodaVesicaspecie parts are used against throatirritatious and skin disorders. AdhatodaVesicacontain large amount of alkaloids. Theleaf and root extract of AdhatodaVesicaused as medicines in form of tablets. The herbal medicine is slow reactive natural chemical with respect to synthetic drugs. The old medicinal system and modern studies suggested that the use of natural herbs as a medicine that improves the body resistance mechanism against the microorganism effect without anyside effects.

METHADOLOGY :[3,4]

A)Plant material :

[3,4] The green plant materials, mainly leaves and stems, were collected from medicinal plantgarden, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, bangladesh in the month ofJune, 2012. the taxonomical identification of the plant was confirmed by National Herbarium,Bangladesh and the accession number was DACB-37880. The plant material was dried undershade, blended to powder and stored in air tight container.

B)Chemicals and Instruments :

All solvents and reagents were of analytical grade and were used without furtherpurification.HPLC grade methanol (Sigma Aldrich, Germany) and water purified by Milliporepurified system were used for analysis. Reference standard of vasicine (potency100%) wassupplied as donation by Square Herbal and Nutraceuticals Limited, Pabna, Bangladesh. Silicawith mesh size 400-200 was used for column chromatography. TLC plate (Aluminium foilprecoated with silica 60F254), UV-Vis spectrophotometer (Shimadzu, Japan) FT-IR(Shimadzu,Japan) and LCMS/MS (Shimadzu, Japan) were used for identification of vasicine. HPLC(Shimadzu, Japan) equipped with a manual

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injector, a vacuum degasser, a multiple-wavelengthUV/Visible detector (Shimadzu SPD 20A, Japan) and an ODS column namelyCapcell Pak (150mm×4.6 mm i.d., 5 µm particle size) was used for quantitative estimation ofpurified vasicine

.Extraction of plant material :

The dried powdered material of A.vasica (250 gm) was placed in a soxhlet apparatus andextracted with methanol (500 mL×3) at ambient temperature for 72 hrs. The combinedmethanolic extract was concentrated under reduced pressure in a rotary evaporator. The extractwas then treated with aqueous solution of citric acid (1%) and was stirred at ambienttemperaturefor 3 hrs. It was filtered and the clear solution thus obtained was extracted with chloroform (200mL×3). The aqueous acidic layer was collected and then basified with ammonia solution to pH9.5 and further extracted with chloroform (200 mL×3). The organic layers were collected andcombined, dehydrated by anhydrous sodium sulphate (Na2SO4) and filtered. the chloroformextract was then evaporated to dryness in a rotary evaporator at a temperature not exceeding450C and the residue was triturated with a mixture of acetone and petroleum ether (1:1; 40 mL)and filtered. The filtrate was subjected to dryness at room temperature and an amorphous residuewas obtained

Preliminary screening for vasicine alkaloid :

The amorphous residue was primarily screened for the presence of alkaloid with thealkaloidal reagents like Mayer's reagents, Dragendroff's reagents, wager's reagent and Hager's reagent.

Preparation of reagent Vasaka (Adhatodavasica) leaf:[5]:

[5]Juice from A. vasica leaf was prepared by different methods. Firstly traditional bolusmethod (modified Put Pak Vidhi in which 100 g of fresh leaves of A. vasica were crushed usingmortar and pestle, made into a bolus and it was covered with fresh leaves of Syzigiumcumini. Itwas then covered with a layer (approximately 1½ inch thick) of paste of wheat flour, followed bya layer of clay paste and the ball (bolus) so obtained was dried at room temperature. The driedbolus was subjected to heat in a muffle furnace at 450°. During heating it was checkedperiodically and when the outer layer of the bolus became red hot and aroma of the wheat flourbeing baked emanated (it takes approximately 15-20 min of heating), it was taken out. The boluswas opened while hot and the leaf paste was squeezed through 4 folds of muslin cloth to obtainjuice. The volume of the juice obtained was measured. This sample was coded as S-1. In thetraditional method, the bolus is subjected to laghu puta (heat), using cow dung cakes. Wemodified the method slightly by heating the bolus in a muffle furnace.

The second methodemployed was steaming, which was carried out using two different methods. In the first method,100 g of fresh leaves were crushed using mortar and pestle and placed in a steel vessel (withoutadding any water to the leaves) and heated at 121° (15 lb pressure) for 30 min. The crushedleaves were taken in 4 layers of muslin cloth and squeezed in order to obtain juice out of it. Thejuice obtained was measured. This sample was coded as S-2. In the second method, 100 g of fresh leaves were crushed using mortar and pestle and 100 ml of distilled water was added to itand it was subjected to heat at 121° (15 lb pressure) for 30 min. The steamed material was takenin a 4-layered muslin cloth and squeezed in order to obtain juice out of it.

The juice obtained was measured. This sample was coded as S-3.VasikaSwarasa (manual) was the third method employed in which, 100 g of fresh leaves were triturated to a fine paste in a stone motor. It wastaken in 4 layers of muslin cloth and squeezed by hand to take out the juice. This sample wascoded as S-4. The fourth method used was VasikaSwarasa (using a grinder). One hundred gramsof fresh leaves were ground in a mixer/juicer with 100 ml of water and filtered through 4 layersof muslin cloth and squeezed by hand to take out the juice. This sample was coded as S-5.Finally, the juice was prepared from dry leaf powder15, where to 100 g of dry leaf powder, 200ml of water was added and macerated for 24 h at room temperature. The above mixture wastaken in 4 layered muslin cloth and squeezed to take out the juice. The juice obtained wasmeasured. This sample was coded as S-6.

Preparation of the extract [6]:

[6]The leaves of Adusa were oven dried at 45 0C. The dried leaves were powdered using a grinder.The powder thus obtained, was extracted in hydro alcoholic solution. For the extraction, 40g ofsample powdered drug with 320 ml hydro alcohol solvent was used at different concentration's ofethanol, temperature as per the experimental plan(Table.1)

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Table 1: Boundaries of the experimental domain and spacing of the Compositional variable levels for Adhatoda leaf

Independent variables	Symbol code	Low variables	High variables
Temperature (0C)	A	60	80
Concentration of ethanol	B	30	70

Experimental design for extraction of Adhatodaleaf:
The Box-Behnken Design from RSM was used for designing the experimentalcombinations. The variables used were temperature (0C), concentration of ethanol (%), (% of alcohol in hydro-alcoholic solvent) and time (h).The tables were generated using Minitab version 18 which were followed to run the extracts with specified conditions.

FOR EXTRACTION:
All the extractions were carried out according to randomized design generatedthrough RSM by Minitab 18 for yield response with temperature and concentration variation. The extract was cooled, filtered through Whatman filter paper No 1. After that water bath was use to concentrate the extract then calculated yield in grams (g) for every extraction Plantextract yield (EY): The yield of the extract was calculated from the equation$W1/W2$X 100 where, W1 is the weight of extract after evaporation of solvent and W2 is the dry weight of the plant sample.

9.Plant Description:
AdhatodavasicaNees. Belongs to the medicinal family Acanthaceae.

Fig: 1 whole plant

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Be1-3 feet in height with many long opposite branches. Leaves are large and lance-shaped. Stem herbaceous above and woody below. Leaves opposite and exstipulate. Flower spikesOrpanicles, small irregular zygomorphic, bisexual, and hypogynous. It has capsular fourseeded fruits. The flowers are either white or purple in colour. Its trade name Vasaka isbased on Sanskrit name. [7] Inflorescences in axillary spicate cymes, densely flowered;peduncles short; ovate,

Fig2:fresh leaves fig 3:dried leaves

10.Phytochemistry:

The vast variety of pharmacological uses of Adhatoda is believed to be the result of its richconcentration of alkaloids . The prominent alkaloid found in Adhatoda leaves is thequinazoline alkaloid known as vasicine. In addition to vasicine, the leaves and rootsofAdhatodacontainthealkaloidsl-vasicinone, deoxyvasicine,maiontone,vasicinoloneandvasicinol . Research indicates that these chemicals are responsible for Adhatoda'sbronchodilatoryeffect .

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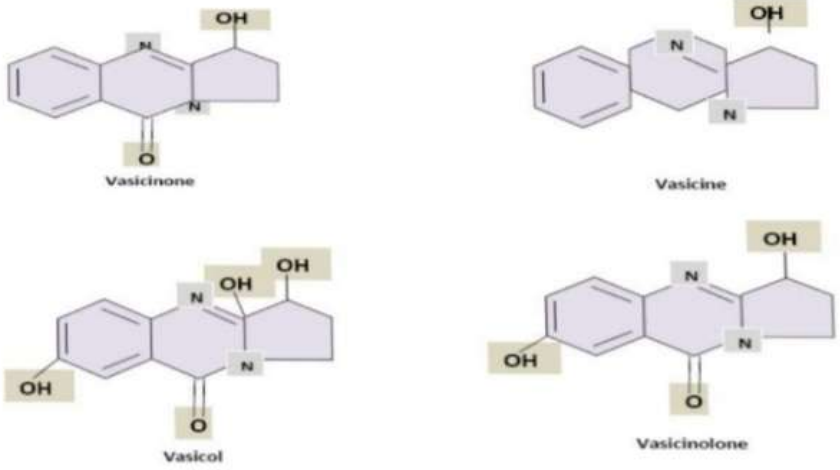

11.ACTIVITY:

Anti-asthmatic and bronchodilator activity Adhatoda has been used in traditional medicine to treat respiratory disorders. Both vasicine and vasicinone the primary alkaloidconstituents of Adhatoda are well established as therapeutical respiratory agents. Extracts ofAdhatoda'sleaves and roots are useful in treating bronchitis, and other lung and bronchioledisorders, as well as common coughs and colds. A decoction of the leaves of Adhatoda has asoothing effect on irritation in the throat, and acts as an expectorant to loosen phlegm in therespiratorypassages. To evaluate the antitussive activities of Adhatoda extract in anesthetizedguinea pigsand rabbits and in unanesthetized guinea pigs showed the plant to have a goodantitussive activity. Recent investigations using vasicine showed bronchodilatory activityboth in vitro and in vivo.

A).Wound healing activity:

For the purposes of the study, wounds were created along thevertebral columns ofbuffalo calves, and alcoholic and chloroform extracts of Adhatoda in a powdered form wereapplied. As compared to control animals, the calves treated with Adhatodavasica showedsignificantly improved healing. Vasica improved breaking strength, tensile strength, absorptionandextensibility in the wound repair tissue. In addition, the levels of elastin, collagen,hydroxyproline, hexosamine and zinc were greatly increased in the animals treated withAdhatoda. The alcoholic extract of the herb was found to be the most effective.

B).Anti-ulcer activity:

Adhatodavasica was studied for its anti-ulcerogenic activity against ulcers induced byethanol, pylorus, and aspirin. Adhatoda leaf powder showed a considerable degree of antiulceractivity in experimental rats when compared with controls. The highest degree ofactivity wasobserved in the ethanol-induced ulceration model .These results suggest that in addition to itsclassically established pharmacological activities, Adhatodavasica hasimmense potential as ananti-ulcer agent. Further research showed that a syrup of Adhatoda improved symptoms ofdyspepsia.

C).Tubercular activity:

A chemical constituent of Adhatoda alkaloids, vasicine, produces bromhexine and ambroxoltwo widely-used mucolytics. Both of these chemicals have a pH-dependent growth inhibitoryeffect on Mycobacterium tuberculosis. Indirect effects of Adhatoda on tuberculosis includeincreased lysozyme and rifampicin levels in bronchial secretions, lung tissue and sputum,suggesting that it may play an important adjunctive role in the treatment of tuberculosis.

D).Cholagogue activity:

In laboratory experiments on cats and dogs, Adhatodavasica was found to increase

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bileactivity when the animals were given an intravenous dose of 5 mg/kg. In dogs, the amount ofexcreted bile increased by 40-100%. The animals also showed an increase in bilirubin excretion

12.Chemical composition [8]:

[8]The principle constituents of Vasaka are its several alkaloids, the chief one being vasicine.The leaves contain two major alkaloids called vasicineandvasicinone2,,3.Thepharmacologicalacti vities of vasicine and vasicinone are well known. Recent investigations on vasicine showedbronchdilatory activity comparable to theophylline , both in vitro and in vivo. Both the alkaloids in combination showed pronounced bronchodilatory Vasicine also exhibits strongrespiratorystimulant activity. There has also been a report of thrombopoetic4 (platelet increasing) activitywith vasicine. Uterine stimulant activity and moderathrombopoetic4 activity of the alkaloidshave been observed. The leaves of Vasaka are rich in vitamin C, carotene and an essential oil. A study showed that Mycobacterium tuberculosis was inhibited by the essential oil (at a specific concentration)

13. Health benefits of Adhatoda:

AdhatodaVasica has been used in traditional Indian medicine for thousands of years to treat respiratory disorders. AdhatodaVasica is useful in treating bronchitis, tuberculosis and otherlung and bronchiole disorders. A decoction of the leaves can be used as an herbaltreatmentfor cough and other symptoms of colds. The soothing action helps irritation in t hethroat and theexpectorant will help loosen phlegm deposits in the airway which makes ad heptode a goodremedy for sore throat. AdhatodaVasica has been used to control both internal and external bleeding such as peptic ulcers, piles and bleeding gums. A poultice of the leaves may be appliedto wounds f or their antibacterial and anti-inflammatory properties. The poultice is also helpful in relieving rheumatic symptoms when applied to joints. This herb exhibits antispasmodic, expectorant and blood purifying qualities. AdhatodaVasica has also been used to speed delivery during childbirth.

14.Side Effects and Possible Interactions:

Adhatoda is considered safe in recommended usage and dosing. The safety of this herb has not been tested in children and should be avoided, unless directed by a medical professional. Use of this supplement is not recommended during pregnancy (except at birth, and then only underthedirection of a medical practitioner.) Care should b e exercised when taking this herb with other drugs or supplements that exhibit expectorant or antisplasmodic effects.

15.Medicinal Applications of Adhatoda [9]:

[9] Healing Power and Curative Properties the leaves, roots and the flowers are extensively used in indigenous medicine as a remedy for cold, cough, bronchitis and asthma.

A)Bronchitis and Asthma:

In acute stages of bronchitis it gives unfailing relief. especially where the sputum is thick and sticky. It liquefies tilt sputum so that it is brought up more easily. For relief in asthma, the dried leaves should be smoked.

b)Tuberculosis:

In Ayurveda, a preparation made from vasakaflowers , known as gulkand is used to treat tuberculosis. A few fresh petals of vasaka flowers should be bruised and put in a pot of chilly clay. Some sugar crystals are added and the jar kept in the sun. It should be stirred every morning and evening. The preserve is ready for use in about a month. Even the juice from its leaves is useful in treating tuberculosis. About 30 ml of the juice is taken thrice a day with honey. It relieves the table cough by its soothing action on the nerve and by liquefying the sputum, which makes expectoration easier Coughs For coughs, 7 leaves of the plant are boiled in water, strained and mixed with 24 grams of honey. This decoction provides relief. Similarly a confection ofvasaka flowers eaten in doses of 12 grams twice daily relieves cough. About 60 grams of flowers and 180 grams of jaggery should be mixed for preparing this confection.

c) Intestinal Warms:

Its leaves, bark, the root-bark, the fruit and flowers are useful in the removal of intestinalparasites.The decoction of its root and bark in doses of 30 grams twice or thrice a day for 3 dayscan be given for this purpose. The juice of it s fresh leaves can also be used in doses of ateaspoon thrice a day for 3 days.

d)Diarrhoea and Dysentery:

The juice from its leaves should be given in doses of 2 to 4 grams in treating diarrhoea dysentery.

16.Siddha Home Remides of Adhatodavasaka:[10]

[10] Cough, cold and asthma contain preparations made using Adhatoda. Along with thippili(piper lignum), chukka (dry ginger), pepper, adathoda, thoothuvalai (trilobatum)are the

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Common herbs used in treating all conditions relate d to lungs, airway passage, throat etc. Of this, adathoda holds a vital position in treating asthmatic conditions. Adathodaikudineer andmanappagu are the two time tested Siddha remedies used in treating asthma. Kudineer is Tamilword for decoction. Adhatodakudineer is prepared by boiling adathoda leaves in water and thencooling down the water for consumption. Now for the ingredients needed to prepare thekudineer or decoction. Adathodai leaves, chukku or dry ginger, and pepper are taken. They arecrushed and put in 1 litre of water and allowed to boil.

Once it boils for 3-5 minutes, remove fromthe stove and allow it to cool. When it reaches a lukewarm state, drink this. This is done threetimes a day for 3-4 days. This is found to be helpful in clearing cough , cold and phlegm in chest.

One other preparation for treating cough is to follow the above-mentioned process usingadathoda root and kandankathiri root. Piper longum powder is added to this decoction. This is taken three times a day after food. In case of chronic wheeze, the following time-teste preparations can be up. Prepare a decoction using adathoda leaves, terminalia chebula (kadukkai),and grapes. Add honey and palm candy to this decoction and take it three time s a day after food.

17.Morphological features:

It is a dense shrub having height of 1.2-2.4 bark is yellowish in colour .leaves are 10-13cm long Leaves are of ovate lanceolate-shaped and light- green in colour. It have characteristicodour.The taste is bitter. The apex of leaf is acuminate , margin slightly crenate to entire.Thebase of leaf is symmetric and venation is pinnate. The transverse section of leaf show palisade, epidermis, spongy mesophyll, trichrome phloem, xylem, collenchyma andcytolith. Leaves are oppositely arranged, smooth-edged and borne on short petioles. On drying they have dull brownish –green colour. The trunk of this plant is long ,opposite, having ascending brancolourm.The flowers are of white in colour. Inflorescence represent large ,dense, axillary spikes, Fruits are pubescent and have club- shaped capsules. This plant is perennial, evergreen and highly branched.

18.Chemical constituents/Phytochemicals:

The chemical constituents of vasica are alkaloids, tannins, flavinoids, terpenes, sugar and glucosides. The major chemical constituents of vasaca are its several alkaloids, and the chief one is vasicine. Leaves composed of major constituents which are vasicine andvasicinone. Alsothe leaves of vasaca contain vitamin C in large amount. They also have carotene and essential oil inlarge amount. The roots of this plant contain vasicinone, vasicol, peganine, sitosterol

They have beta – glucoside glalctose and deoxyvasicine and 2- hydroxyl – 4 – glucosyloxychalcone in roots The flowers of this plant contain b-sitosterol-D- glucoside, kaempferol.Itsglycosides and quercetin minor alkaloids include adhatinine and vasinol.

19. Adulterants:

Vasaca show adulteration with the leaf of araluka which is Ailanthus excels Use.

20.Medicinal Uses:

It is used in the treatment of various disease because of its ability of formation of secondarymetabolites such as tannins, alkaloid, saponins, flvanoids ,reducing sugar and anthraquinoneswhich have ability to restore health and heal many disease. The leaves of vasaca are used totreat cough, asthma, fever, tuberculosis, piles, jaundice, bleeding gum. It is also used as an expectorant. It has ability of bronchodilator. Its decoction has ability to treat cold andrheumatism. The extract of leaves, bark and flower is used to treat bronchial, asthmatic and pulmonary affection.

CONCLUSIONS:

It is concluded that a vesica roots do possess anthelmintic activity. It is, however, suggestedthat further research on large scale be carried out on large number of animals on higher dosesthan those used in the current study, identification of active principles, and standardization ofdose and toxicity studies for drug development.

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