International Journal of Agriculture and Plant Science
Online ISSN: 2664-7664; Print ISSN: 2664-7656; Impact Factor: RJIF 5.24
Received: 03-09-2020; Accepted: 18-09-2020; Published: 08-10-2020
www.agriculturejournal.in
Volume 2; Issue 4; 2020; Page No. 41-44

Qualitative and quantitative analysis of *Adhatoda vasica* collected from Paschim Medinipur, West Bengal, India

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Abstract
The qualitative phytochemical studies were carried out in the solvents *viz*. Methanol, Chloroform and n-Butanol. The Methanol, Chloroform and n-Butanol tuber and leaves extract of *Adhatoda vasica* shows the presence of alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols but in tuber of *Adhatoda vasica* high intensity of phytochemicals than that of leaves showed in table no.1 by twice and tannin absence in methanolic and n-butanol extract of leaves. Also, the quantitative studies were carried out in the same solvent mentioned above, Alkaloid content in tuber of *Adhatoda vasica.* that was 2.921, 2.546 and 3.045 µg/ml respectively, and total flavonoids in tuber, 0.845, 0.641 and 0.978 µg/ml respectively and also followed by total content of phenols that was 1.284, 0.652 and 1.361 µg/ml.
Total content of Alkaloid, flavonoids and phenols in the Methanol, 1.926, 0.434 and 1.641 µg/ml. respectively, in the Chloroform 1.554**,** 0.391 and 0.856 µg/ml. and followed by in the n-Butanol leaves extract of *Adhatoda vasica* that was 2.045, 0.423 and 0.426 µg/ml.

Keywords: *Adhatoda vasica*, phytochemicals, solvents, qantitative and leaves extract etc

Introduction
Adhatoda vasica in west Bengal Basak (Bengali), is a small, evergreen, perennial shrub, which reaches an average height of three meters. Its branches are opposite and ascending. The broad, leathery leaves, which are sometimes used as an insecticide, measure from 10 to 15 centimeters in length, and are about 4 centimeters in width. They are pubescent; light green on top and darker green beneath. The leaves grow in an opposite formation, and are entirely lanceolate, and shortly petiolate, tapering towards both apex and base. The leaves become brownishgreen when dry and taste bitter with a smell similar to strong tea. Its stem is soft and makes a good charcoal. The flowers are large, dense, terminal spikes with large, attractive white petals, streaked with purple on the lower lip. The fruit is a small, clavate, longitudinally channeled capsule, containing four globular seeds. *Adhatoda vasica* is useful in treating bronchitis, tuberculosis and other lung and bronchiole disorders. A decoction of the leaves of Vasaka may be used to help with cough and other symptoms of colds. The soothing action helps irritation in the throat and the expectorant will help loosen phlegm deposits in the airway. A poultice of the leaves of Vasaka may be applied to wounds for their antibacterial and anti-inflammatory properties. The poultice is also helpful in relieving rheumatic symptoms when applied to joints. Vasaka has been used to control both internal and external bleeding such as peptic ulcers, piles and bleeding gums. Vasaka exhibits antispasmodic, expectorant and blood purifying qualities. It is a very well-known remedy. It is considered an essential herbal plant of Indian system of medicine (ISM) and has been used in the treatment of fever, urinary problem, dysentery, skin diseases leprosy, diabetes, and many more diseases. The plant reported containing chemical compound including Alkaloids, Terpenoids, Lignans, Steroids and others that establish the phytochemistry and pharmacological activity of *Adhatoda vasica*. The present review highlights the pharmacological importance *viz* antioxidant activity, antimicrobial activity, antibacterial activity, antifungal activity, anti-diabetic activity, antistress activity, hypolipidaemic effect, hepatic disorder, anticancer anti-HIV potential, antiosteoporotic effects, antitoxic effects, wound healing, anticomplementary activity, and immunomodulating activity, systemic infection and Parkinson's disease and “Ethical Phytomedicines”, which are standardized toxicologically and clinically define crude drugs, are seen as a promising low-cost alternative in primary health care. The field also has benefited in recent years from the interaction of the study of traditional ethnobotanical knowledge and the application of modern phytochemical analysis and biological activity studies to medicinal plants. A wide range of chemical compounds including Aporphine alkaloids, clerodane diterpenes, berberine, palmatine, tembertarine, magniflorine, choline etc. have been isolated from this plant. The trace element studies on the aqueous extract of these medicinal plants have been carried out using particle-induced X-ray emission technique for their medicinal uses. The very high concentrations of Cl, K, and Ca in all the leaf samples, appreciable levels of Mn and high Zn content in *Adhatoda vasica*.
So, I have selected present study on qualitative and quantitative analysis of Basok is a medicinal plant with various properties of therapeutic importance. It belongs to the family *Acanthaceae* and scientifically it is called *Adhatoda vasica.*
The plant has heart-shaped leaves with greenish-yellow flowers. It grows in tropical and sub-tropical regions of the globe, of these the African and Asian continents are abundant with *Adhatoda vasica*. In traditional medicine, the

extracts from all the parts of the plant are used including the root, stem, and leaf (aerial part) of the plant.

Material and Methods

Collection of Plant parts

Collections of *Adhatoda vasica* plant parts were collected from in the area Medinipur Town & Midnapore sadar block, Paschim Medinipur district for gathering medicinal value of different plants used by the peoples in their daily life. further works conducted in the Department of Botany, Sabang Sajanikanta Mahavidyalaya, Lutinia, West Bengal, India Plant part Stem and leaves cleaned soil dust with tap water then dried under shade and prepared fine powder and kept it in airtight bottle. The plant materials were identified by using standard floras like Cook 1907, Dhore 2005, Naik 1989, Yadav and Sardesai 2002.

Preparation of Plant Part Extract

Methanol, Chloroform and n-Butanol, extract was prepared by using Soxhlet extractor. 30 gm of each plant part powder was placed in a thimble, which was placed in chamber of the Soxhlet apparatus. 300 ml solvent in the flask and the temperature was maintained at 55 ^{0} c. for 72 hours. Then the extracts were filtered through Whatman filters paper No 1. Solvent was evaporated at 40-50 ^{0}C by using Rotary evaporator. The collected powder was weight and dissolved in Dimethyl sulfoxide (DMSO) with 10% concentration. The extracts were used for Qualitative and quantitative evaluation of phytochemical. (Handa *et al*., 2008., Subramanian *et al*., 2011) [9].

Qualitative analysis of *Adhatoda vasica* plant parts

The qualitative screening test were performed for the presence of following secondary metabolites such as alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols (Harborne, 1973) [5] and Sofowara (2005).

Alkaloids test

The plant extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. Formation of turbidity or yellow precipitation showed the presence of alkaloid.

Glycosides

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (Glycone or Genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish-brown coloration at the junction of two layers and the bluish green colour in the upper layer.

Terpenoids and steroids

Four milligrams of extract were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids.

Flavonoids

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5 – 6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.

Saponins

0.5 g of extracts was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Phenols

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds. 3

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

Quantitative analysis of *Adhatoda vasica*

Preparation of plant extracts for quantitative determination of alkaloids 5 gm of powdered plant material was taken into 20 ml of n-butanol and vigorously stirred. The content was transferred into a reagent bottle. The slurry was kept overnight at room temperature. Then it was centrifuged at 6000 rpm for 10 min and the supernatant was made up to 50 ml with n-butanol

Estimation of total alkaloids by titrimetric methods used by Plummer., 2013 and Debnath *et al.*2015 [2].

Obtained supernatant of the plant sample was used for the estimation of total alkaloids by titrimetric methods. 10 ml of the supernatant was taken into a100 ml separating funnel. 10 ml of 0.1 (N) HCl was added and shaken thoroughly for 2-3 min. This results in the solubility of alkaloids. The lower layer contains alkaloids neutralized with 0.1 (N) HCl and the upper layer contains n-butanol. 10 ml HCL portion was collected in a beaker and 2-3 drops methyl red was added to it, that turns the solution into slightly reddish colour. The contents of beaker were titrated against 0.1 (N) NaOH, till colour change changed from red to pale yellow. The neutralization point was determined. Same procedure was repeated triplicate. The total amount of alkaloids was calculated by considering the following equivalent:

1 ml 0.1N HCl ≡ 0.0162 g alkaloid

Estimation of Total Phenolic Content

Total phenol content of *Adhatoda vasica* was assayed by modified Dewanto *et al.*, 2002 [13] and Jothi *et al.*, 2019 [12] procedure. The different concentrations of 10µg, 20µg, 40µg, 60µg, 80µg, and 100µg were using an aliquot of diluted extract and added to 0.25 ml of Folin Ciocalteu reagent. The elucidation was adjusted with distilled water to a final volume of 3ml and shaken thoroughly. The solution was incubated and kept in the dark placed and read at 760 nm was read against prepared blank. The total phenol content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight. The total sample was analyzed in three replicates.

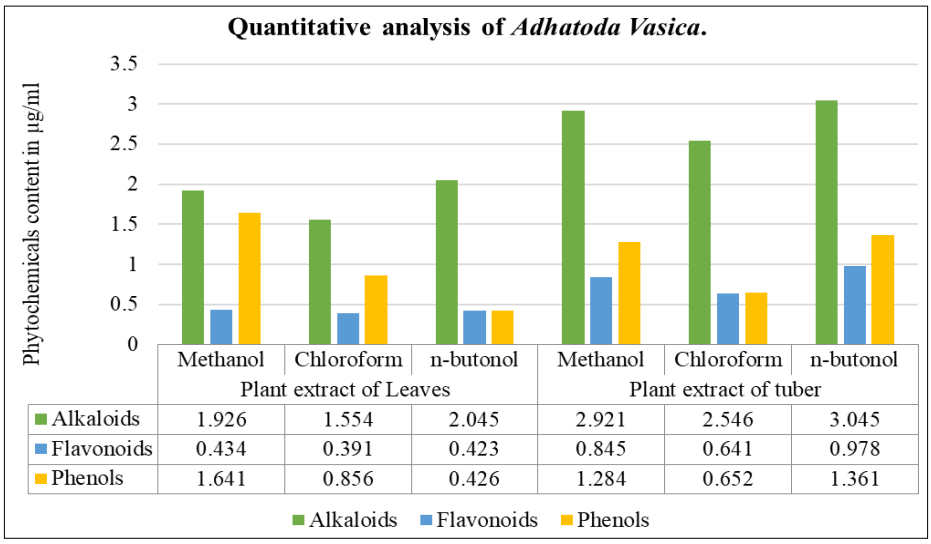
Estimation Total Flavonoid Content

Total Flavonoid content in *Adhatoda vasica*. whole plant extract was analyzed by the aluminium chloride colorimetric system M.M Mervat, *et al* 2009 [7] and Jothi *et al*., 2019 [12]. 0.5ml of plant part extract of at different concentrations like 10µg, 20µg, 40 µg, 60 µg, 80 µg, and 100 µg were taken and the final volume was made up to 3ml with methanol. After that, 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8ml of distilled water were added continuously and test solution was vigorously shaken. After 30 minutes for the incubation periods, absorbance was recorded at 415 nm. The concentration of flavonoids in test samples was calculated and expressed as the equivalent of quercetin (QE) / g of sample. The entire sample was analyzed in three replicates.

Results and Discussion

Table 1: Qualitative analysis of *Adhatoda vasica* stem and leaves

Sr. No.	Phytochemical	Plant extract of stem			Plant extract of Leaves		
		Methanol	Chloroform	n-Butanol	Methanol	Chloro-form	n-Butanol
1	Alkaloids	++	++	++	++	+	+
2	Glycosides	++	+	++	+	+	+
3	Terpenoids	+	++	+	+	+	+
4	Steroids	++	+	++	++	+	+
5	Flavonoids	++	++	++	++	+	+
6	Saponins	++	++	++	+	++	++
7	Phenols	++	+	++	++	+	-
8	Tannins	++	++	++	-	+	-



Graph 2: Quantitative analysis of *Adhatoda vasica*. plant parts, (leaves and Stem µg/ml)

Results taken average of triplicates for different concentration of plant extract.

The qualitative phytochemical studies were carried out in the solvents *viz*. Methanol, Chloroform and n-Butanol. The Methanol, Chloroform and n-Butanol tuber and leaves extract of *Adhatoda vasica*.. shows the presence of alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols but in tuber of *Adhatoda vasica*.. high intensity of phytochemicals than that of leaves showed in table no.1 by twice and tannin absence in methanolic and n-butanol extract of leaves. Also, the quantitative studies were carried out in the same solvent mentioned above, Alkaloid content in tuber of *Adhatoda vasica*.. that was 2.921, 2.546 and 3.045 µg/ml respectively, and total flavonoids in tuber, 0.845, 0.641 and 0.978 µg/ml respectively and also followed by total content of phenols that was 1.284, 0.652 and 1.361 µg/ml.

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Conclusion

Adhatoda vasica is the rich source of phytochemicals, alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols. Its extraction in n-butanol solvent shows highest intensity and content of phytochemicals followed by methanolic extract of stems of *Adhatoda vasica*. and it shows antimicrobial activities. Sagbo *et al*, 2005 and Jothi.2019 [12], reported that Polyphenols and phenols found in plants are two secondary metabolites considered as natural antioxidants. Jothi, 2019 [12], and Trease *et al*,1983 [10] showed that *Adhatoda vasica*. is an alkaloid plant, which contains alkaloid components which are mostly used in pharmaceutical formulation for drug. Noroozi *et al*, 1998 and Al-Humaid *et al*, 2010 [1] reported Flavonoids are ketonic compounds that can induce anti-inflammatory activity and inhibits the oxygen compounds, enzyme cyclo-oxygenase dependent pro inflammation activity. Furthermore, flavonoids have a powerful anti-inflammation activity as they inhibit prostaglandin synthesis. Flavonoids in higher plants are inseparable with cardiovascular diseases and antioxidant potentials that can treat cancer disease. Pietta, 2000 and T.

Sivakumar, 2017 reported that Flavonoids and antioxidants origin of vitamins A, C, E and plant source diets.

So, *Adhatoda vasica* of plant presence different phytochemical compounds useful for Further purification, identification and characterization of the active compounds of would be our priority in future studies.

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