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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ASSAYS OF CRUDE EXTRACT OF ADATHODA VASICA LIN. FROM GANGAPUR TALUKA, AURANGABAD DISTRICT, MAHARASHTRA STATE, INDIA

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ABSTRACT

Preliminary phytochemical and antibacterial investigations were carried out of the crude extracts obtained from the leaves of *Adhatoda vasica*, using solvents of varied polarity. The presence of phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars was indicated by the tests conducted. The effect of ethanol, petroleum ether and water extracts were tested on *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klesiella aerogens*. The minimum inhibitory concentration of the crude extracts was determined for various organisms.

KEY WORDS: *Adhatoda vasica*, Antibacterial Activity, Phytochemical Screening.

INTRODUCTION

Adhatoda vasica is an evergreen dense and bushy shrub with long opposite ascending branches, it showed broadly elliptic leaves. The flowers showed off white or purple colour. It was in dense axilliary pedunculate and bracteates spikes on it. It grows widely in Bangladesh, Myanmar, Pakisthan and also in India. The leaves of the plant showing expectorant, bronchodilator, antispasmodic, hypotensive, cardiac depressant, respiratory stimulant, uterotonic, antimicrobial and hypoglycemic properties. The roots and barks are expectorant, antispasmodic and antiseptic. Its vernacular names are in Bengali name-Basakpata, English name is Malabar nut, having Family- Acanthaceae.

Phytochemical investigations of *Adhatoda vasica* led to the isolation of steroid, alkaloids, saponin, phenols. The vasicinone, vasicolinone, vasicoline, adhatodine, vasicolinine, vasicinine, vasicinol, anisotine, 3-hydroxyl anisotine, betaine, visicine, adhatodic acid, essential oil, fats, resins, sitosterol, vasicine, vasicionol, essential oil, indole alkaloid, galactoside, Dgalactose, deoxy vasicinone, vasicinine, kaempferol, quercetin, tritriacontane, fatty oil consisting of arachidic, behenic, lignoceric,cerotic,oleic and linoleic acids (Ghani A. 2003). The plant AV and its component vasicine and its derivatives are extensively being used for bronchodilatory/mucolytic cough syrup preparations since ancient history of Ayurveda and till date.

Plant Description-

Family- Acanthaceae.

It is an evergreen shrub of 6-8 feet in height along with lot of long and opposite branches. The leaves were large and lance-shaped. Stem is herbaceous and woody in nature. The leaves are opposite and exstipulate, Flower are off white in colour with spikes or panicles, small in size irregular zygomorphic, hypogynous and bisexual (Shinwari *et al.*, 1995). It has capsular four seeded fruits. The flowers are sometime purple in colour. It's local name is Vasaka is as per Sanskritname **(**Shete *et al.*, 1993). Inflorescences in axillary spicate cymes, densely flowered; peduncles short; bracts broadly ovate, foliaceous. The leaves, flowers, fruit and roots are extensively used for treatingcold cough, whooping cough, chronic bronchitis and asthma, as sedative, expectorant and antispasmodic (Pandita *et al.*, 1993).

Figure- 1) Showing entire plant of *Adathoda vasica* Linn.

Phytochemistry-

The vast variety of pharmacological uses of Adhatoda is believed to be the result of its rich concentration of alkaloids. The main alkaloid found in Adhatoda vasica leaves was vasicine **.** It is also containsome other alkaloids l-vasicinone, deoxyvasicine, maiontone, vasicinolone and vasicinol. Several research proved that these chemicals are responsible for Adhatoda's bronchodilatory effect.

Anti-asthmatic and bronchodilator activity-

Adhatoda has been used in traditional medicine to treat respiratory disorders. Both vasicine and vasicinone the primary alkaloid constituents of Adhatoda are well established as therapeutical respiratory agents. Extracts of Adhatoda's leaves and roots are useful in treating bronchitis, and other lung and bronchiole disorders, as well as common coughs and colds. A decoction of the leaves of Adhatoda has a soothing effect on irritation in the throat.

Insecticidal activity-

Adhatoda vasica has been used for centuries in India as an insecticide. Its leaves have been shown to control insect pests in oil seeds, in both laboratory and warehouse conditions **(**Srivastava *et al.,*1965). Research has shown Adhatoda's alkaloid, vasicinol, to have an antifertility effect against several insect species by causing blockage of the oviduct. Research has also proven Adhatoda's effectiveness as an insect repellent (Saxena *et al.,* 1986).

Anti-bacterial activity-

A leaf extract was investigated for antibacterial activity using the paper disc and dilution methods. *In-vitro* screening showed a strong activity of Adhatoda's alkaloids against the bacteria. *Pseudomonas aeruginosa*. Significant antibacterial activity against the Gram-positive bacteria strains *Streptococcus faecalis, Staphylococcus aureus, Staph epidermidis* and the gram-negative *E. coli* were also noted (Patel *et al*., 1984).

Anti-tubercular activity-

A chemical constituent of Adhatoda alkaloids, vasicine, produces bromhexine and ambroxol – two widely-used mucolytics. All these chemicals have a pH-dependent growth inhibitory effect on *Mycobacterium tuberculosis*. Indirect effects of Adhatoda on tuberculosis include increased lysozyme and rifampicin levels in bronchial secretions, lung tissue and sputum, suggesting that it may play an important curing role in the treatment of tuberculosis (Narimaian *et al.,* 2005).

Antifertility Effect-

Research has shown Adhatoda's alkaloid, vasicinol, to have an antifertility affect against several insect species which causing blockage of the oviduct. Research has also proven Adhatoda's effectiveness as an insect repellent (Saxena *et al.,* 1986).

Hepatoprotective Activity-

The hepatoprotective activity of Ethyl acetate extract of *Adhatoda vasica* was investigated against CCl4 induced liver damage in Swiss albino rats. At the dose of 1ml/kg, CCl4 induced liver damage in rats as manifested by statistically significant increase in serum Alanine aminotransferase, (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and also in serum Bilirubin. Pre-treatment of rats with the ethyl acetate

Extract of *Adhatoda vasica* (100mg/kg and 200mg/kg) prior to the CCl4 dose at 1ml/kg statistically lowered the three serum level enzymes and also Bilirubin. Histopathological observations also coincided with the above results, however 200mg/kg dose was found to be more active. Current results suggest that Ethyl acetate extract of *Adhatoda vasica* has potent hepatoprotective effect against CCl4 - induced liver damage (Ahmed *et al.*, 2013).

Anti-bacterial Activity-

It is reported that the leaves of *Pongamia pinnata* show antibacterial effect. It is clear that the extracts have great potential as antibacterial compounds against enteric pathogens and that they can be used in the treatment of enteric infectious. This plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. It is hoped that this study would leadto the establishment of some compounds that used to formulate new and more potent antimicrobial drugs of natural origin (Shastri K.A. 2001).

Anti-oxidant and Anti-hyperammonemic-

It has been observed that effect of Pongamia pinnata leaf extract on circulatory lipid peroxidation and antioxidant status was evaluated in ammonium chloride-induced hyperamm.

Gangapur is located on west side of Aurangabad-Ahmednagar Highway, 38 km from Aurangabad (Coordinates 19.6991°N 75.0086°E). Gangapur is Taluka place in Historical District Aurangabad. It is situated near backwater of Jayakwadi dam so most of the area is irrigated. The collection of samples was done from different pre-selected site of Gangapur taluka region, viz. Antapur, Saidapur, Bhendala, Ganeshwadi, Amalner, Lakhmapur, Ager wadgoan, Wazar, Mahalaxmi kheda. This places located near Godavari river sites.

METHOD AND MATERIAL

Collection of samples-

Collection of samples was done from different pre-selected site of Gangapur taluka region, viz. Antapur, Saidapur, Bhendala, Ganeshwadi, Amalner, Lakhmapur, Ager wadgoan, Wazar, Mahalaxmi kheda. This places located near Godavari river sites. The leaves was collected by an eco-friendly. They were identified by taxonomist. All the parts of the plants were washed with tap water and sterilized by $HgCl_2$.They get dried and crushed in mixer grinder and the grinding was performed in a hygienic condition.

Preparation of Crude Extract-

The coarsely powdered parts were soaked in methanol, ethanol and Aqueous and ethyl acetate solvents in a conical flask and left for 24 hours. The extracts were taken out and filtered using sterile filter paper and concentrated using water bath.

Working Crude Extract solution-

Working concentration of 25mg/ ml were prepared by dissolving respective amount of extract in one ml of methanol, ethanol and aqueous and ethyl acetate solvents in separate test tubes. Then extract was filtered through Whatmann paper No. 1 and solvent was removed by rotary vacuum evaporator (Buchi type-Superfit, Bangalore) under reduced pressure so as to get the crude extract. The concentrated extract was used for further study.

Phytochemical screening-

It is the process to know the presence or absence of number of chemical. Plant material is subject to preliminary phytochemical screening for the detection of various plant constituents.

Tests for detection of Alkaloids:-

Mayer reagent (potassium Mercuric iodide Solution)- Test solution produce cream colour precipitate with Mayer reagent which indicates the presence of alkaloids.

Wagner reagent (Iodine Potassium Iodide solution)- Test solution produce reddish brown Precipitate with Wagner reagent which indicates the presence of alkaloids.

Hager's reagent (Saturated solution of Picric acid)- Test solution produce yellow precipitate with hager reagent which indicates the presence of alkaloids.

Tests for detection of Carbohydrates

Molish's Test- To prepare this reagent, 10 gm of -napthol was dissolved in 100 ml of 95% ethanol [18]. The reagent was added to aqueous and alcoholic extract as such as to hydrolyzed extract (heated with dil HCl on a water bath). Purple colour was obtained indicating the presence of carbohydrates.

Test for Proteins and Amino acids

Millon's test- To the test solution add about 2ml of million reagents white precipitate was not observe it indicates absence of amino acid.

Biuret test- To the alcoholic extract of the powdered drug 1 ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added. Violet color was not obtained indicating the absence of proteins.

Test for Glycosides

General test- (Test A) 200 mg of the powdered drug was extracted with 5 ml of dilute sulphuric acid by warming on a water bath, filtered and neutralised with 5% sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it becomes alkaline and heated on a water bath for 2 minutes.

(Test B) 200 mg of the powdered drug was extracted with 5 ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it becomes alkaline and heated on a water bath for 2 minutes. The quantity of red precipitate formed in test A is greater than in test B indicating the presence of glycosides.

Test for Anthraquinones glycosides:

Borntrager's test- The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The inorganic layer was separated and ammonia solution was added slowly. No red color is observe in ammonical layer indicating the absence of anthracene derived glycosides.

Modified Borntrager's test- About 0.1 gm of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well. Colour was not observed in ammonical layer indicating the not of anthracene derived glycosides.

Test for cyanogenetic glycosides

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place [19]. No Change in the colour of the sodium picrate paper was observed indicating the absence of cyanogenetic glycosides.

Test for cardiac glycosides

Keller Killiani test- About 1 gm of the powdered leaf was boiled with 10 ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10 ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid containing a trace of ferric chloride. To this 3 ml of concentrated sulphuric acid was added to the sides of the test tube carefully. Reddish brown layer acquiring bluish green colour after standing was observed indicating the presence of deoxy sugars of cardiac glycosides.

Raymond Test - To the alcoholic extract of the leaf, hot methanolic alkali was added. Violet color was produced indicating the presence of cardiac glycosides.

Legal's Test -To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro prusside solution were added. Red colour was formed indicating the presence of cardiac glycosides.

Coumarin glycosides

A small amount of powdered drug was placed in test tube and covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes. Then the paper was removed and exposed to UV light. Green fluorescence was not observed indicating the absence of coumarin glycosides.

Test for Steroid and Triterpenoids

Salkowski Test- Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside. The chloroform layer of the solution was not turned red in color indicating the absence of sterols.

Libermann-Burchard's Test- To the chloroform solution few drops of acetic anhydride was added and mixed well. 1 ml of concentrated suiphuric acid was added through the sides of the test tube and set aside for a while. A brown ring was not formed at the junction indicating the absence of sterols.

Test for Saponins-

About 0.5 gm of the powdered drug was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously. Frothing occurred indicating the presence of saponins.

Test for Tannins-

To the aqueous of the powdered drug, few drops of ferric chloride solution were added. Bluish black color was produced, indicating the presence of tannins.

Test for Flavonoids

Shinoda Test- A little amount of the powdered drug was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for 5 minutes. Purple color was obtained indicating the presence of flavonoids.

Alkaline reagent test- To the alcoholic extract of the powdered drug, few drop of sodium hydroxide solution was added. Yellow color formed, turning to colorless on addition of few drops of dilute acid indicating the presence of flavonoids.

Zinc Hydrochloride Test- To the alcoholic extract, mixture of zinc dust and concentrated hydrochloric acid was added. Formation of red color indicating the presence of flavonoid. Qualitative phytochemical analysis of total extracts was carried out using standard procedures todetect flavonoids, alkaloids, triterpinoids, glycosides, steroids, saponins, fixed oils and fats,proteins, tannins, phenolic compounds (Khandelwal K.R.1999).

Test Pathogenic Organisms-

Organisms such as *B. subtilis* (MTCC 441), *K. aerogens* (ATCC5139), *E. coli* (ATCC 25922), *S. aureus* ATCC 5021) were used for the study. The ATCC culture was procured from National Chemical Laboratory, Pune. And were maintained by serial sub culturing every month on nutrient agar slantsand incubating at 37°C for 18–24 hours. The cultures were stored under refrigerated condition (Myer, 1982).

Antimicrobial activity-

The antibacterial activity was tested against selected bacterial cultures were by using Well Diffusion Method and Disc Diffusion Method. The results were considered according to highest inhibition zone between them. The ability of the extracts to inhibit growth of bacteria was determined using the agar disc diffusion method (Tepe *et al.,* 2005) extract was tested for antimicrobial activity in four different solvents against the selected test organisms. Extract was compared with standard drug ampicillin (10 µg disc). The nutrient agar was made and poured in every petri plates about 25ml per plates. The 10 µg standard disc were transferred into 6 mm diameter discs and all were labeled. The test solutions were allowed to diffuse in discs for 2 h at room temperature. The petri plates were incubated for 24 h at 37ºC temperature. The stringent aseptic conditions were maintained during microbial culture. The zone of inhibition measured by using scale and noted as their antibacterial effectiveness.

RESULT AND DISCUSSION

The crude methanol, ethanol, aqueous and ethyl acetate extracts of *Adathoda vasica* Linn were used to investigate the preliminary phytochemical screening. The table 1 shows the results of preliminary phytochemical activity of crude extract of sponge of *Adathoda vasica;* the methanol and ethyl acetate crude extract contains alkaloids, tannins, flavonoids and proteins and amino acids, steroids, carbohydrates, fats and fixed oils; as well as ethanol extract contains alkaloids, flavonoids, sterol and terpenoids, carbohydrates, fats and fixed oils. But in aqueous extract contains only few biologically active compounds like, alkaloids, terpenoids and carbohydrates.

Table- 1) Showing phytochemical analysis of *A. vasica* leaves.

Phytochemicals	**ME**	**EE**	**AQ**	**EA**
Alkaloids a.Mayer's test b.Wagner's test c.Hagers test	 + + +	 + + +	 + + +	 + + +
Carbohydrates a.Molish test	-	-	-	-
Test for protein and amino acids Millons test Biuret test	 + +	 + +	 + +	 + +
Test for glycosides	+	+	+	+
Test for anthroquanine glycosides. a. Borntragers test b. Modified borntragers test.	 + +	 + +	 + +	 + +
Cynogenetic glycosides	+	-	-	+
Cardiac glycosides a.Raymond test b. Legals test	 - -	 - -	 - -	 - -
Coumarin glycosides	+	+	+	+
Steroids and triterpenoids	–	+	-	-
Libermann-Burchard test	–	-	-	-
Test for saponin	-	-	-	-
Test for tannins a.shinoda test b.alkaline reagent test	 + +	 + +	 + +	 + +
Zinc hydrochloric test.	–	–	–	–

The phytochemical activity of *A. vasica* proved to be presence of variety of phytochemicals. The alkaloids, proteins, glycosides were present and saponins, triterpenoids were absent. Due to presence of main phytochemicals antibacterial activity might be showed. The crude methanol, ethanol, aqueous and ethyl acetate extracts of *Adathoda vasica* Linn were used to investigate the antimicrobial activity against four pathogenic bacteria; and the preliminary phytochemical screening. Table 1 shows result of in vitro testing of crude extracts against pathogenic bacteria.

Table -2) Showing Zone of Inhibition of *Adathoda vasica*

Sr. No.	Name of Bacteria	Zone of Inhibition				Standard	DMSO
		ME	EE	AQ	EA		
1	*B. subtilis*	12	NA	10	12	15	NA
2	*S. aureus*	NA	11	NA	NA	12	NA
3	*E. coli*	13	12	NA	15	12	NA
4	*K. aerogens*	10	NA	NA	15	12	NA

Inhibition zones of extracts against the specific test organisms were measured in mm. The extract restricted the growth of pathogens on the media around wells. The inhibition zone shows of crude methanol, ethanol, aqueous and ethyl acetate extracts of *Adathoda vasica* in the order of ethyl acetate> methanol> ethanol> aqueous against all test microorganisms. The maximum inhibition zone (12-10 mm) was observed in ethyl acetate crude extract against *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Fusarium* spp. as well as *Escherichia coli, K. aerogens, Bacillus subtilis, Staphylococcus aureus*.

Figure -1) Showing zone of Inhibition of crude extract of *A. vasica.*

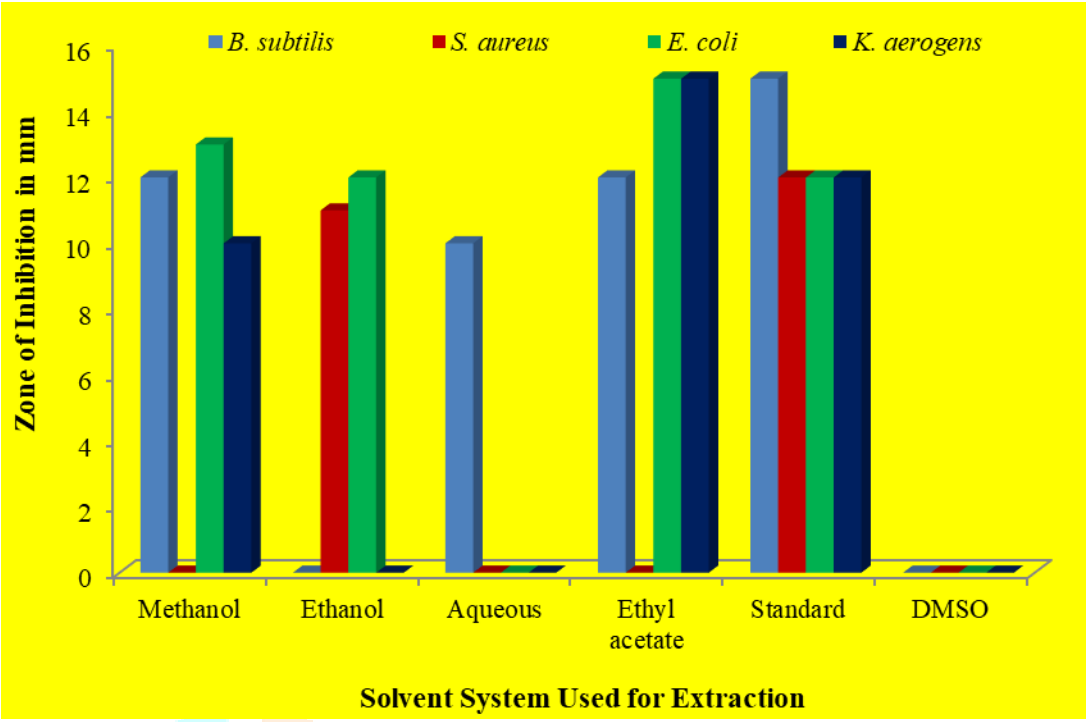

Figure -2) Showing antibacterial activity of crude extract of *A. vasica.*

The *A. vasica* Ethyl acetate extract proved to be effective against selected bacteria. But the all tested extract not that much effective as compared to standard Gentamycin. The negative control used as DMSO which does not showed any activity. Methanol and Ethyl acetate showed almost similar antibacterial activity against *B.subtilis.* The ethanol extract does not show any activity. The standard used was also effective it showed inhibition zone of 15mm against selected *B. subtilis*. The ethanol extract was proved to be potent against *S. aureus* bacteria.

CONCLUSION

The broad spectrum antibacterial activity of crude methanol, ethanol, aqueous and ethyl acetate leaves extracts of *Adathoda vasica* Linn seemed to be due to the presence of alkaloids, tannins, flavonoids and proteins and amino acids, steroids detected in the bioactive fractions. This work confirms the hypothesis on the richness as well as chemical gradient of leaves extracts of *Adathoda vasica* and probably is the first report on the antibacterial activity of leaves extracts of *Adathoda vasica* from Gangapur region, Maharashtra, India, to the best of our knowledge. Further research also needs to purify and characterize the secondary metabolites from the leaves extracts of *Adathoda vasica* for the valuable source of novel substances for future discoveries in pharmaceutical science.

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