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Original Research Article

PHARMACOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY *ASYSTASIA GANGETICA* (L.) T. AND.

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Abstract

The present study has been carried out to evaluate the pharmacochemical characterization and *in vitro* antibacterial activity of the whole plant extracts of *Asystasia gangetica*. Physicochemical parameters parameters (Ash value and extractive value; fluorescence analysis) and phytochemical analysis were done by using the standard methods. The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts were tested against *Bacillus thuringiensis*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Proteus vulgaris*, *Serratia marcescens* by the agar disc diffusion method. The total ash value of whole plant of *Asystasia gangetica* is 10.14%.. The extractive value of water is more than in the solvents investigated. Preliminary photochemical screening of whole plant showed the presence of alkaloids, tannins, terpenoids, xanthoprotein, and sugar in the methanol and ethanol extracts. The whole plant extracts of *Asystasia gangetica* showed potent antibacterial activity. The present investigation, whole plant of *Asystasia gangetica* revealed the high degree of antibacterial against *Salmonella paratyphi*. The pharmacochemical characterization will be helpful to study the active principles using modern techniques in the later part of this work.

Key Words: *Asystasia gangetica*, fluorescence analysis, Phytochemical, Antibacterial.

Introduction:

Plants have traditionally served as man's novel weapons against different ailments. Besides the modern medical practices existing today, about 65% of the Indian population depends on the traditional medical systems for their primary healthcare. Regions with rich biodiversity, with its traditional ethnicpeople, are the biggest source for the plant resources and its hidden knowledge[1]. The traditional knowledge of medicinal plants has been recorded in numerous literatures[2,3]. In Tamil Nadu, a lot work has been done on the ethnomedicinal plants used for various ailments by different ethnic communities[4].There is a need for documentation of research work carried out on

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traditional medicines.With this back drop it becomes extremely important to make an effort towards the standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics[5]. In the present study pharmacognostic standards of the plant is studied.The standards are utmost importance in not only finding out genuity but also in detection of adulterants in marketed drugs[6].

Asystasia gangetica also known as "Chinese Violet" is rapidly growing straggling herb mainly distributed in India, grows to 10m height at an altitude 300m. The leaves are green oval shaped with rounded base very slightly sawedged and smooth. Flowers are pale purple to violet or lime white in colour, capsules are 2.5-3.5 cm long with wide base and seeds are 5mm in diameter.

The plant *Asystasia gangetica* has been used medicinally from ancient time in Bagungo for treating different ailments. Rural peoples in Sivagangai District of Tamil Nadu,peoples of Southern part of India used entire plant juice for rheumatism. Tribal people of Marudhamalai hills Coimbatore, Tamil Nadu generally apply root paste for skin allergies. In Kwazulu-Natal, South Africa people use *Asystasia gangetica* as vegetable. Traditionally plant juice used for anthelmintic activity in swelling, rheumatism also in gonorrhea and ear disease.

It is a folk remedy for treating diabetes mellitus in parts of South India. Nigerian people claimed to be leaves of *Asystasia gangetica* are highly effective in local treatment of asthma [7].

Materials and Methods:

The whole plant of *Asystasia gangetica* was collected from Vadasery, Nagercoil, Tamil Nadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Determination of physicochemical parameters:

Determination of physicochemical parameters, such as ash and extractive values were done following the methods of Kalpanadevi *et al*[8], Mohan *et al*[9] .The behavior of the powdered leaf with different chemical reagents was studied and the fluorescence character was observed under UV light [10].

Preparation of extracts for phytochemical screening and antimicrobial activity:

Freshly collected whole plant of *Asystasia gangetica* were dried in shade, and then coarsely powdered separately in a willey mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures[11-13]. Ethanol extracts were concentrated in a rotary evaporator. The concentrated extracts were used for antibacterial activity and estimation of total free phenolics and flavonoids.

Antimicrobial study was carried out by disc diffusion method [14] against the pathogens viz *Bacillus thuringiensis, Streptococcus faecalis, Staphylococcus aureus, Salmonella paratyphi, Proteus vulgaris, Serratia marcescens.* A loopful of bacteria was taken from the stock culture and dissolved in 0.1 ml of saline. All the tests were done by placing the disc (^mm diameter) impregnated with (20 mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram

Positive and Gram Negative bacteria. Respective solvents without plant extract served as negative control. Standard antibiotic of tetracycline (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37° C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs.The inhibition zone sand antibacterial activity against the pathogenic bacteria were recorded. The experiments were repeated in triplicate and the results were documented.

Result and discussion

The result of the ash and extractive values of whole plant of *Asystasia gangetica* is depicted in table 1. The total ash content of the powdered whole plant of *Asystasia gangetica* is 10.14%. These ash values are indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. The extractive value of water is more than that in other solvents investigated in the present study. Samples have more water soluble ash than acid insoluble ash. The results of various types of ash and extractive values may provide a basis to identify the quality and purity of the drug.

The results of fluorescent analysis of whole plant of *Asystasia gangetica* is shown in table 2. The whole plant powder of *Asystasia gangetica* shows the characteristic fluorescent green colour treated with aqueous 1N NaOH, acetic acid, benzene, petroleum ether and methanol under short UV light. The powder from the whole plant of *Asystasia gangetica* emitted green under day light, brown under short UV and black in long UV light. Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [15].

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. The result of preliminary phytochemical screening of whole plant of *Asystasia gangetica* is presented in table 3. The methanol extracts of the whole plant of *Asystasia gangetica* shows the presence of catechin, flavonoid, steroid, sugar, phenol, tannin and glycoside. This could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound.

The total phenolic and flavonoid content of *Asystasia gangetica* was found to be 1.33g $100g^{-1}$ and 1.63g $100g^{-1}$ respectively. Phenolic compound, the principal antioxidant constitutes of natural plant products, are composed of phenolic acid and flavonoids [17]. These compounds are potent radical terminations by donating a hydrogen atom to the radical and preventing lipid oxidation at the initial step. The high potential of polyphenols to scavenge free radical may be because of their many phenolic hydroxyl groups. In this respect, polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer, antiproliferative, antimicrobial, wound healing and antibacterial activities including antioxidant activity[18-20].

The whole plant extracts of *Asystasia gangetica was* tested for their antibacterial activity against *Bacillus thuringiensis, Staphylococcus aureus, Streptococcus faecalis,*

Salmonella paratyphi, *Proteus vulgaris* and *Serratia marcescens* (Figure 1).

Methanol extract of *Asystasia gangetica* showed the maximum activity against *Salmonella paratyphi*. Petroleum ether extract of *Asystasia gangetica* showed the maximum activity against *Salmonella paratyphi* and *Proteus vulgaris.* Among the solvent studied, petroleum ether extracts exhibited maximum activity against the entire tested microorganism. The presence of antibacterial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, phenols, steroids, saponins etc.

Based on the present study, it is concluded that the whole plant of *Asystasia gangetica* contains various bioactive compounds with high degree of antimicrobial activity against various pathogens. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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Table 1: Ash and extractive values of the powdered whole plant of *Asystasia gangetica*[a]

Ash values		
S.No	**Type of Ash**	**% of Ash**
1.	Total ash value of powder	10.14±0.14
2.	Water soluble ash	4.24±0.03
3.	Acid insoluble ash	2.36±0.01
4.	Sulphated ash	12.58±0.27
Extractive values		
S. No	**Name of the Extract**	**Extractive value (%)**
1.	Petroleum ether	6.56±0.07
2.	Benzene	4.31±0.03
3.	Chloroform	4.38±0.01
4.	Acetone	8.36±0.11
5.	Methanol	8.74±0.16
6.	Ethanol	8.56±0.14
7.	Water	9.34±0.31

[a] All values are mean of triplicate determination ± standard error.

Table 2: Fluorescence analysis of whole plant of *Asystasia gangetica*

Treatment	Colour		
	Under Ordinary Light	Under UV light	
		245nm	365mm
Powder as such	Green	Brown	Black
Powder + 1NAqueous NaoH	Green	Fluorescent Green	Black
Powder + 1NAlcoholic NaoH	Dark Green	Dark Green	Dark Blue
Powder + 1 N HCl	Green	Dark Brown	Violet
Powder + Con. HCL	Dark Green	Brown	Black
Powder + Con.H_2So_4	Dark Green	Dark Brown	Dark Green
Powder+ 50% H_2So_4	Green	Dark Green	Blue
Powder +Con. HNO_3	Red	Dark Brown	Blue
Powder +40% NaOH + 10% Lead Acetate	Green	Dark Green	Dark Green
Powder + Acetic acid	Dark Green	Fluorescent Green	Green
Powder+ Ferric Chloride	Dark Green	Brownish yellow	Blue
Powder+ Chloroform	Dark Green	Brown	Black
Powder + Benzene	Dark Green	Fluorescent green	Black
Powder + Petroleum ether	Dark Green	Fluorescent Green	Black
Powder+ Methanol	Dark Green	Fluorescent Green	Blue
Powder+ Ethanol	Dark Green	Brownish yellow	Black
Powder+ acetone	Dark Green	Dark Brown	Dark Blue
Powder+ NH_3	Green	Dark Green	Blue
Powder+HNO_3+ NH_3	Orange	Dark Green	Blue
Powder+50% HNO_3	Dark brown	Brown	Dark Green

Table 3: Preliminary phytochemical screening of different extracts of *Asystasia gangetica*

Bioactive components	Nature of extract				
	Petroleum ether	**Benzene**	**Ethyl acetate**	**Methanol**	**Ethanol**
Alkaloids	+	+	+	-	-
Anthroquinones	+	-	-	-	+
Catechin	+	+	+	+	+
Coumarin	+	-	-	-	-
Flavonoids	-		-	-	-
Phenols	+	+	+	+	+
Quinones	-	-	-	-	-
Saponins	-	-	-	-	-
Steroids	+	+	+	+	+
Tannins	+	+	+	-	-
Terpenoids	-	-	-	-	-
Glycosides	-	+	+	+	+
Xanthoprotein	+	+	+	-	+
Sugar	-	-	-	-	-
Fixed oil	+	+	+	+	+

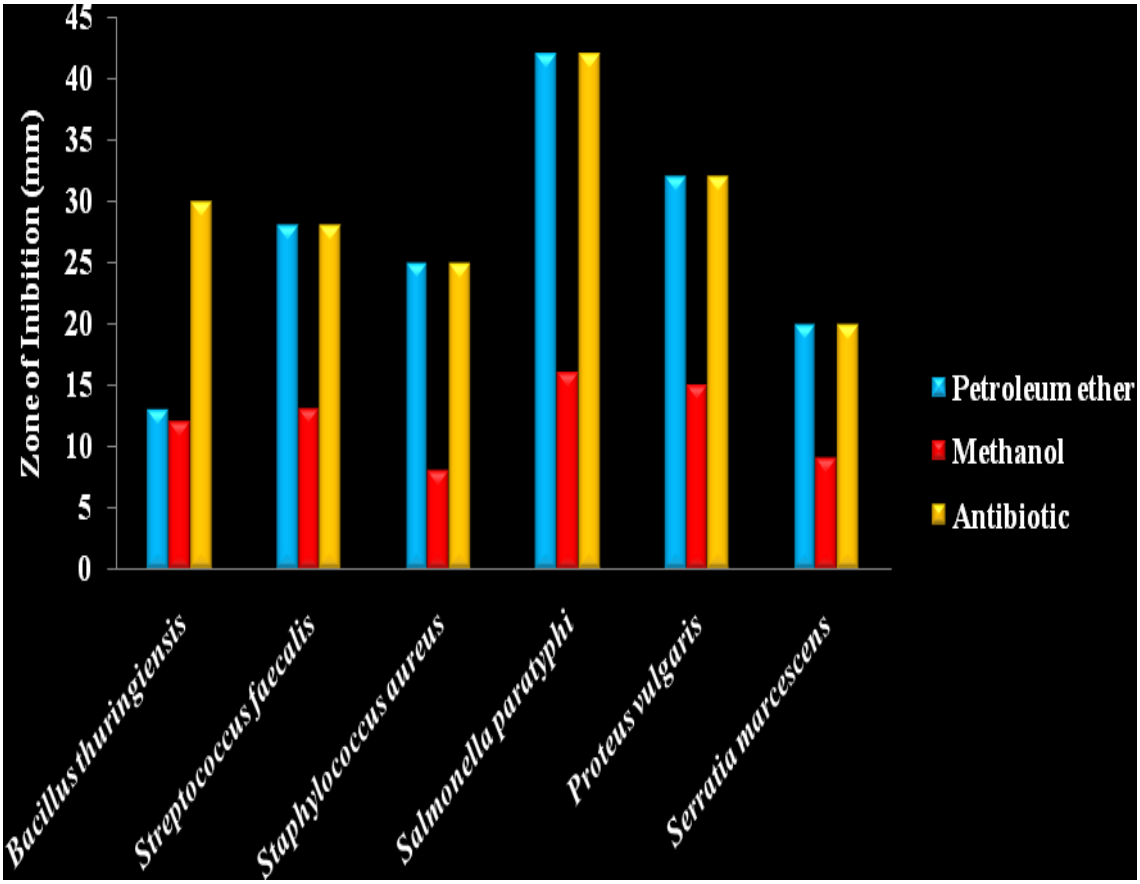

Figure 1: Antibacterial activity of two different extracts of *Asystasiagangetica*