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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LEAF OF *ATALANTIA MONOPHYLLA* (L.) CORR (RUTACEAE)

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ABSTRACT

Preliminary phytochemical analysis and antibacterial activities of Ethanol, Chloroform and Ethyl acetate extract obtained from the leaves of Atalantia monophylla were investigated in an attempt to evaluate its medicinal potentials. Antibacterial activity was determined against three gram positive bacteria Staphylococcus aureus,Streptococcus epidermidis, Bacillus subtilius and six Gram negative bacteria Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Vibrio cholerae, Proteus vulgaris was carried in well diffusion method. The results obtained showed that Ethanol, Chloroform and Ethyl acetate extracts of Atalantia monophylla had inhibitory effect on the growth of isolates. The effect exhibited by Ethanolic extract of leaves was significantly greater than the Chloroform and Ethyl acetate leaves extracts. Gram negative bacteria Escherichia coli and the Gram positive bacteria Staphylococcus aureus showed the highest zone of inhibition in all the three ethanol, chloroform and Ethyl acetate extracts.The phytochemical screening revealed the presence Carbohydrates, alkaloids, flavonoids, Cardiac glycosides,Protein and Phenolic compounds found in Atalantia monophylla ethanolic leaves extracts.

KEY WORDS

Atalantia monophylla, inhibitory effect, antibacterial activities, phytochemical screening

INTRODUCTION

World over the medicinal plants are used as a main source of traditional and orthodox medicines. The attention has been made towards developing the new antibiotics that reduce the increasing resistance among the microorganisms (Anonymous, 1962). The Rutaceae family consists of 140 genera and about 1300 species. The plant Atalantia monophylla Correa is a shrub, belongs to family Rutaceae. It is found in Kolli hills, Namakkal district of Tamil Nadu, India. Kolli hills are also called as Sathuragiri or Square hill. Kolli hills is located at the west of Pachaimalais, in Namakkal district of Tamil Nadu, India. It comprises a compact block of hills with high rising peaks and ravines and total area of 490 square kilometer and altitude ranging from 1000 to 1300 m above MSL, latitudinal and longitudinal range of Kollihills are 11°10′- 11°30′N latitude 75°10′- 75°30′E longitude respectively. The leaves of Atalantia monophylla Linn are used for the treatment of snakebite by local Malayali tribes of kolli hills. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. The need of microbial resistance for the use of antimicrobial drugs is growing day by day,so studies has to be developed to synthesis new drugs from plants and offer appropriate and efficient antimicrobial drugs to the patient. In recent years, multiple drug/ chemical resistance in both
human and plant pathogenic microorganisms has been developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects (Prusti et al., 2008). There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the

bioassay-guided fractionation of them to yield active principles (Zgoda-Pols et al., 2002). In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Schenkel et al., 2000). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases and also with less side effects. The present study is aimed at preliminary phytochemical screening of the leaves extracts of Atalantia monophylla Linn and evaluation of the same for potential antimicrobial activity using known microbial pathogens as test organisms.

MATERIALS AND METHODS

Source of plant materials

Plant material Atalantia monophylla (Rutaceae) were collected from Kolli hill in the Namakkal district of Tamil Nadu state. Collected plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India.

Solvents selected for extraction methods

The collected plants leaves was washed with tape water, shade dried at room temperature and powdered using electric blender.The powdered material was soaked in solvents such as Ethanol, Chloroform and Ethyl acetate. After 48 hours the extract was filtered using No.1 Whatmann filter paper and the solvents was condensed using rotary vacuum evaporator at 45°C. The residue were collected, weighed and stored at 4°C for subsequent bioassay against selected bacteria.

Preliminary phytochemical Screening:

The stock solution was used for preliminary screening of phytochemical such as carbohydrates (Molisch's test), Protein (Biuret test), alkaloid (Wagner and dragendroff's test), flavonoids (10% HCl&5% NaOH test and Alkaline test) , tannins5% ($FeCl_3$ test), (Steroids Libermann - Burchard's test), saponins(Foam test), cardiac glycosides (Keller killanitet) and terpenoinds (Salkowsi test), Phenols (Ferric chloride test).

Antimicrobial assay

The antimicrobial activity of selected plants extracts was evaluated by diffusion method (Chung et al., 1990). By plant extracts the growth of inhibition of bacteria was determined by using well diffusion method. The nutrient agar (Muller Hilton) plates were prepared and seeded with the test organisms. Antibacterial activity was carried out by using Baver et al., (1966) with minor modification of the method. Extracts of different concentration 30 µl, 60 µl, 90µl mg/ml and streptomycin were used as control. Plates were placed in incubator set to 35°Cwithin 15 minutes. After 1 hour, plates were inverted and again placed in incubator for about 18 - 24 hrs. The plates were examined for evidence of zone of inhibition which appear as a clear area around the holes. The diameter of such zone of inhibition was measured by vernier caliper and the value was recorded and expressed to the nearest millimeter.

RESULTS

Detailed phytochemical analysis of Chloroform, ethyl acetate, Ethanol extracts of Atalantia monophylla deciphered the presence of a variety of plant secondary metabolites as it is evidenced from the Tables 1. Perusal of the data clearly indicates that among the Chloroform, ethyl acetate, Ethanol extracts of Atalantia monophylla showed presence of majority of secondary metabolites such as Carbohydrates, Alkaloids, Flavonoids,Protein Cardiac glycosides and Phenols. In the present study effort have been made to find the anti-bacteria effect of Ethanol, Chloroform and Ethyl acetate extract of Atalantia monophylla were assessed for anti-bacterial activity against therefore pathogenic bacteria such as Gram positive (Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilius) and Gram negative (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae , Proteus vulgaris). The activity was measured in terms of zone of growth inhibition in mm. These included animal and plant pathogens, food spoisoning and spoilage bacteria. The Ethanol extract of A. monophylla show the strong growth inhibiting activity was observed in the deferent Concentrations at (30 µl, 60 µl, 90µl and Control) fund in (21mm, 27mm, 31mm and 33mm) was inhibiting the gram-positive bacteria Staphylococcus aureus and moderate growth inhibition was found in (15mm, 17mm, 21mm, 30mm was inhibiting the Streptococcus epidermidis); (9mm, 11mm, 17mm, 25mm was inhibiting the Bacillus subtilius).

Table 1: Preliminary phytochemical analysis of Ethanol, Chloroform and Ethyl acetate leaf extracts of Atalantia monophylla Linn

S. No	Compounds	Tests	Results Ethanol	Chloroform	Ethyl acetate
1	Carbohydrates	Molisch's test	+	+	_
2	Alkaloids	Dragendroff's test	+	+	+
		Wagner's test	+	_	+
3	Flavonoids	10% HCl & 5% NaOH test	+	+	+
		Alkaline test	+	+	+
4	Tannins	5% $FeCl_3$ test	_	_	_
5	Steroids	Libermann - Burchard's test	–	–	–
6	Saponins	Foam test	_	_	_
7	Cardiac glycosides	Keller killanitet	+	_	+
8	Terpenoinds	Salkowsi test	_	_	_
9	Phenols	Ferric chloride	+	_	_
10	Protein	Biuret test	+	_	+

+ Denotes Presence of compound *- Denotes Absence of compound*

Table 2: Antibacterial activity of Ethanol, Chloroform and Ethyl acetate extract of Atalantia monophylla Linn tested against the important selected human pathogenic bacteria

Solvents used	*Type*	*Pathogens tested*	*Concentrations tested*			
			30 µl	*60 µl*	*90µl*	*Control*
			Zone of inhibition (mm)			
Ethanol	*Gram positive*	*Staphylococcus aureus*	*21*	*27*	*31*	*33*
		Streptococcus epidermidis	*15*	*17*	*21*	*30*
		Bacillus subtilius	*9*	*11*	*17*	*25*
	Gram negative	*Escherichia coli*	*21*	*29*	*33*	*36*
		Klebsiella pneumoniae	*13*	*18*	*24*	*30*
		Pseudomonas aeruginosa	*13*	*17*	*21*	*30*
		Salmonella typhi	*9*	*16*	*20*	*24*
		Vibrio cholerae	*11*	*13*	*19*	*22*
		Proteus vulgaris	*13*	*15*	*17*	*25*
Chloroform	*Gram positive*	*Staphylococcus aureus*	*13*	*17*	*25*	*30*
		Streptococcus epidermidis	*10*	*15*	*19*	*25*
		Bacillus subtilius	*7*	*9*	*13*	*24*
	Gram negative	*Escherichia coli*	*19*	*25*	*29*	*35*
		Klebsiella pneumoniae	*11*	*13*	*19*	*30*
		Pseudomonas aeruginosa	*11*	*15*	*17*	*25*
		Salmonella typhi	*7*	*11*	*13*	*25*
		Vibrio cholerae	*7*	*9*	*13*	*24*
		Proteus vulgaris	*9*	*11*	*15*	*25*
Ethyl acetate	*Gram positive*	*Staphylococcus aureus*	*11*	*17*	*21*	*30*
		Streptococcus epidermidis	*9*	*11*	*15*	*25*
		Bacillus subtilius	*9*	*13*	*17*	*25*
	Gram negative	*Escherichia coli*	*17*	*19*	*25*	*26*
		Klebsiella pneumoniae	*7*	*11*	*17*	*25*
		Pseudomonas aeruginosa	*13*	*17*	*19*	*30*
		Salmonella typhi	*7*	*9*	*15*	*25*
		Vibrio cholerae	*9*	*15*	*17*	*25*
		Proteus vulgaris	*11*	*13*	*20*	*24*

Values showing in the table are zone of inhibition obtained through disc diffusion method; Control = commercially available chemical drug: Streptomycin.

The Ethanol extract of A. monophylla show the strong growth inhibiting activity was observed in the deferent Concentrations at (30 µl, 60 µl, 90µl and Control) fund in (21mm, 29mm, 33mm and 36mm) was inhibiting the gram negative bacteria Escherichia coli and moderate growth inhibition was found in (13mm, 18mm, 24mm and 30mm was inhibiting the Klebsiella pneumoniae); (13mm, 17mm, 21mm, 30mm was inhibiting the pseudomonas aeruginosa); (9mm, 16mm, 20mm and 24mm was inhibiting the salmonella typhi); (11mm, 13mm, 19mm, 22mm was inhibiting the vibrio cholerae); (13mm, 15mm, 17mm and 25mm was inhibiting the prteus vulgaris) respectively.

The Chloroform of A. monophylla show the strong growth inhibiting activity was observed in the deferent Concentrations at (30 µl, 60 µl, 90µl and Control) found in (13mm, 17mm, 25mm and 30mm) was inhibiting the gram-positive bacteria Staphylococcus aureus and moderate growth inhibition was found in (10mm, 15mm, 19mm, 25mm was inhibiting the Streptococcus epidermidis); (7mm, 9mm, 13mm, 24mm was inhibiting the Bacillus subtilius). The Chloroform of A. monophylla show the strong growth inhibiting activity was observed in the deferent Concentrations at (30 µl, 60 µl, 90µl and Control) fund in (19mm, 25mm, 29mm and 35mm) was inhibiting the gram negative bacteria Escherichia coli and moderate growth inhibition was found in (11mm, 13mm, 19mm and 30mm was inhibiting the Klebsiella pneumoniae); (11mm, 15mm, 17mm, 25mm was inhibiting the pseudomonas aeruginosa); (7mm, 11mm, 13mm and 25mm was inhibiting the salmonella typhi); (7mm, 9mm, 13mm, 24mm was inhibiting the vibrio cholerae); (9mm,11mm, 15mm and 25mm was inhibiting the prteus vulgaris) respectively.

The Ethyl acetate of A. monophylla show the strong growth inhibiting activity was observed in the deferent Concentrations at (30 µl, 60 µl, 90µl and Control) fund in (11mm, 17mm, 21mm and 30mm) was inhibiting the gram-positive bacteria Staphylococcus aureus and moderate growth inhibition was found in (9mm, 11mm, 15mm, 25mm was inhibiting the Streptococcus epidermidis); (9mm, 13mm, 17mm, 25mm was inhibiting the Bacillus subtilius). The Ethyl acetate of A. monophylla show the strong growth inhibiting activity was observed in the deferent Concentrations at (30 µl, 60 µl, 90µl and Control) fund in (17mm, 19mm, 25mm and 26mm) was inhibiting the gram negative bacteria Escherichia coli and moderate growth inhibition was found in (7mm, 11mm, 17mm and 25mm was inhibiting the Klebsiella pneumoniae); (13mm, 17mm, 19mm, 30mm was inhibiting the pseudomonas aeruginosa); (7mm, 9mm, 15mm and 25mm was inhibiting the salmonella typhi); (9mm, 15mm, 17mm, 25mm was inhibiting the vibrio cholerae); (11mm,13mm, 20mm and 24mm was inhibiting the prteus vulgaris) respectively.

DISCUSSION

The presence of phytochemicals in the plant extracts highly correlated to the biological activity [Lavanya et al, 2016]. Nowdays, number researcher evaluated the antimicrobial compounds from plant material, they act as lesser side effect in the human body.The results indicate that Ethanol, Chloroform and Ethyl acetate extracts of Atalantia monophylla Linn, had inhibitory effect on the growth of isolates. The effect exhibited by Ethanolic extract of leaves was significantly greater than the Chloroform and Ethyl acetate leaves extracts. Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores [Chatterjee et al 2011; Magana-Arachchi et al 2001 and Purkayastha et al 2005].

MounyrBalouiri et al. (2016) reported that there has been a rising interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Several bioassays such as disk-diffusion, well diffusion and broth or agar dilution are well known and commonly used, nut others such as flow cytofluorometric and bioluminescent methods are not widely used because they requires specified equipment and further evaluation for reproducibility and standardization, even if they can provide rapid results of the antimicrobial agent's effects and a better understanding of their impact on the viability and cell damage inflicted to the tested microorganism.

The present study was aimed to analysis and antibacterial activities and Preliminary phytochemical of Ethanol, Chloroform and Ethyl acetate extract obtained

from the leaves of Atalantia monophylla Linn for its medicinal potentials. The experimental data clearly revealed that the effect exhibited by Ethanolic extract of leaves was significantly greater than the Chloroform and Ethyl acetate leaves extracts.

Previous studies showed that some researchers like (Naziri et al., 2012; Vasconcelous et al., 2003) stated that Punicagranatum peel extracts in different concentrations were effective against Streptococcistrains (Streptococci mutans; Streptococci aureus; Streptococci salivarius; Streptococci sanguinis and Streptococci epidermidis). It was confirmed that this antibacterial activity may be connected to the presence of polyphenolics and hydrolysable tannins in the pomegranate extract specifically gallagic acid and punicalagin (Reddy et al., 2007).

Hence, the antibacterial activity of Atalantia monophylla Linn may be related to alkaloids, flavonoids, Cardiac glycosides and Phenolic compounds, interact with proteins and disturb coaggregation of microorganisms. This is in agreement with the earlier findings of several authors (Naziriet al ., 2012; Al-zoreky, 2009) reported that the antibacterial activity of peel extracts of pomegranate against both Gram positive and negative bacteria strains and mention MIC values ranging from 0.25 to 4.0 mg/ml against the tested bacteria. The results obtained showed that Ethanol, Chloroform and Ethyl acetate extracts of Atalantia monophylla Linn, had inhibitory effect on the growth of isolates.Gram negative bacteria Escherichia coli and the Gram positive bacteria Staphylococcus aureus showed the highest zone of inhibition in all the three ethanol, chloroform and Ethyl acetate extracts.The results obtained were shown the positive results for the presence of flavonoid, alkaloid, and phenol. In conclusion, it is suggested that this Atalantia monophylla Linn plants may be recommended as useful sources to prepare natural bioactive products from which we can develop new antimicrobial drugs. In the search for new pharmaceuticals, screening of such various natural organic compounds and identification of active agents must be considered as a fruitful approach for now a day.

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