

Phytochemical and antibacterial evaluation of various extracts of *Amoora ruhituka* bark

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ABSTRACT. Preliminary phytochemical screening of various extracts of *Amoora ruhituka* bark were investigated which reveals the presence of several secondary metabolites in each extracts. The antibacterial activity of all the extracts was tested against four gram negative bacterial strains. The results indicated the zone of inhibition which ranges from 11.30 ± 5.77 to 18.7 ± 0.577 for different extracts in which Methanol extract has shown highest zone of inhibition for *Salmonella typhimurium* followed by *Enterobacter aerogenes*, *E coli* and *P aeruginosa* whereas benzene extract has showed the least zone of inhibition and the minimum inhibitory concentration (MIC) of the different extracts ranging from 0.78 mg/ml to 6.25mg/ml. The complete results of this study provides a essential data for the use of *Amoora ruhituka* for the treatment of infection associated diseases

1. INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the world. Medicinal plants are being used since centuries as remedies for human diseases because of their therapeutic value [1]. The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. In India thousands of plant species are known to have medicinal value and various parts of several medicinal plants used to cure specific ailments and continued as has been in trend since ancient times. The traditional medicine still plays an important role in the primary health care in India. Antibiotic resistance has become a global concern [2]. In recent years most of the synthetic antibiotics which are commercially available have major setback due to the multiple resistance developed by pathogenic microorganisms. In addition to this problem, antibiotics are sometimes associated with causing adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Thus, there is a need for search of new and more potent, safe and cheap antimicrobial compounds of natural origin to combat the activities of these pathogens. Hence the studies of medicinal plants based on the traditional medicine have fascinated the attentions of numerous researchers in finding solutions for the bacterial infections.

Aphanamixis polystachya (Wall) Parker (Meliaceae) is also known as *Amoora ruhituka* is a traditional plant used as medicine extensively in Asian countries[3,4]. It has been shown the high potential biological activity and elevated number of secondary compounds and their medicinal properties [5]. It is widely distributed in higher altitudes in Western Ghats and Eastern Ghats of South India, rarely found in Sub Himalayan tracks from upper area going eastwards to Bengal, Sikkim and Assam[4,6,7]. The plant extract possess Anti-oxidant, Thrombolytic activity [8], Anti-cancer [9-10], Insecticidal, Antifeedant [11], Laxative [7] and Anti-microbial [12]. The present study is aimed to screen for phytochemicals and to evaluate antibacterial activity of crude extracts of *Amoora ruhituka* bark and to find out minimum inhibitory concentration (MIC) of different extracts against Gram negative bacteria.

2. MATERIALS AND METHOD

2.1 Collection of plant materials and extraction

Stem bark of *Amoora ruhituka* was collected from in and around Thiruvananthapuram, Kerala, INDIA in the month of march 2013 and the plant was duly identified and authenticated by Dr.Kotresha, Associate professor, Taxonomy and Floristic Laboratory, Department of Botany, Karnataka Science College, Dharwad, Karnataka, INDIA.

The stem bark was allowed to dry in shade for two to four weeks. Precaution was taken to avoid direct sun light exposure otherwise it will destroy the active compounds of bark. After drying, the bark was grinded finely and stored in airtight container. The air dried bark powder (150 g) was successively extracted by soxhlet extraction with solvents of increasing polarity i.e., petroleum ether (60-80 C), benzene, chloroform and methanol. The extracts were dried and stored in a sterile container for further use.

2.2. Phytochemical screening of crude extracts.

The phytochemical components of the *A. ruhituka* bark was screened for using the standard method described by Harbone[13]. The components found in the extracts are alkaloids, carbohydrates, steroids, proteins, saponins, flavonoids, phenol, tannin, terpinoids, oils and fats.

2.3. Source of microorganisms

The microorganisms used for testing are *Enterobacter aerogenes* (MTCC111), *Salmonella typhimurium* (MTCC 98), *Pseudomonas aeruginosa*(MTCC 424), *Escherichia coli* (Clinical strain). The above organisms were obtained from the department of Microbiology and Biotechnology, Gulbarga University, Gulbarga, Karnataka, India.

2.4. Standardization of microorganisms

200 µl of overnight cultures of each microorganisms are dispensed into 20 ml of sterilized nutrient broth and incubated at 37° C for 4-6 h to standardize the culture to 10⁶ CFU/ml. A loopful of the standard cultures are used for the antimicrobial assay [14].

2.5. Screening for antibacterial activity (agar well diffusion assay)

In vitro antibacterial activities of all different extracts of *Amoora ruhituka* was determined by standard agar well diffusion assay [15]. MullereHinton Agar (MHA) plates are seeded with 18 h old culture of the standard strains. All the extracts are dissolved in 5% Tween 80 in deionized water and made the final concentration of 50 mg/ml, from this 50 µl of different extracts were added into the sterile 6 mm diameter well. 5% Tween 80 and sterilized distilled water was used as negative controls while chloramphenicol antibiotic disc (30 mcg, Hi-Media) was used as positive control. A loopful each of the standardized culture of test organisms are streaked on the solidified medium and incubated for 24 h at 37°C. The experiment was performed in triplicate under strict aseptic conditions and the antibacterial activity of each extract was expressed in terms of the mean diameter of zone of inhibition (in mm) produced by the respective plant extract.

2.6 Minimum inhibitory concentration

The antibacterial activity of the extracts was examined by determining the MIC in accordance with Clinical and Laboratory Standard Institute (CLSI) methodology [16]. Minimum inhibitory concentration (MIC) was defined as the lowest concentration where no visible turbidity was observed in the test tubes. The MIC was determined for the microorganisms that showed maximum sensitivity to the test extracts. All tests are performed in Mueller–Hinton broth supplemented with DMSO at a final concentration of 10% (v/v) which enhance their solubility. In this method the broth dilution technique is adopted and different extracts are prepared to the highest concentration of 25 mg/ml (stock concentration). Test strains are suspended in MHB to give a final density of 5X10⁵ cfu/ml and this was confirmed by viable counts. A dilution ranging from 0.098 to 25 mg/ml of the

extracts is prepared in tubes, including one growth control and one positive control which contain 10% DMSO (v/v). The lowest concentration of the tube that did not show any visible growth can be considered as the minimum inhibitory concentration.

2.7 Statistical analysis

Results are expressed as Mean \pm SEM. The statistical analysis was carried out using one way ANOVA analysis. The p-value of 0.05 or less was considered significant for all experiment.

3. RESULTS

Phytochemical screening of crude extracts of the bark of the *Amoora ruhituka* reveals the presence of steroids, phenol, saponins, terpinoids, oils and fats in both petroleum ether and benzene extract. Methanol extract contains alkaloids, carbohydrates, proteins, flavonoids, phenol and tannins, whereas chloroform extract contains all the secondary metabolites except oils and fats which is shown in Table 1

Table 1. Phytochemical Screening of different extracts of *Amoora ruhituka* bark

Sl. No.	Test	Petroleum Ether Extract	Benzene Extract	Chloroform Extract	Methanol Extract
1	Alkaloids	-	-	+	+
2	Carbohydrates	-	-	+	+
3	Steroids	+	+	+	-
4	Proteins	-	-	+	+
5	Saponins	+	+	+	-
6	Flavonoids	-	-	+	+
7	Phenols	+	+	+	+
8	Tannins	-	-	+	+
9	Oils and fats	+	+	-	-
10	Terpinoids	+	+	+	-

+ Presence of the Compound; - Absence of the Compound

Table 2. Antibacterial activity of *Amoora ruhituka*

Sl No	Test organisms	MTCC code	P E	B E	CE	ME	-ve Control	+ve Control
1	<i>E.aerogenes</i>	MTCC 111	14.7 \pm 0.577	15.7 \pm 0.577	18.7 \pm 0.577	17.3 \pm 2.08	---	22.3 \pm 0.577
2	<i>S.typhimurium</i>	MTCC 98	13.3 \pm 1.15	11.3 \pm 0.577	15.3 \pm 0.577	18.3 \pm 0.577	---	21.3 \pm 0.577
3	<i>P.aeruginosa</i>	MTCC 424	14.7 \pm 0.577	12.3 \pm 0.577	12.7 \pm 0.577	14 \pm 1	---	22.3 \pm 0.577
4	<i>E.coli</i>	Clinical isolate	14.3 \pm 0.577	12 \pm 0.577	12.7 \pm 0.577	17.3 \pm 0.577	---	24 \pm 0.577

The antimicrobial activity of various extracts of *Amoora ruhituka* bark against different organisms have shown with varying zone of inhibition which ranges from 11.30 \pm 577 to 18.7 \pm 0.577 as shown in Table 2. Methanol extract has shown highest zone of inhibition for *Salmonella typhimurium* followed by *Enterobacter aerogenes*, *E coli* and *P aeruginosa*. Whereas benzene extract has shown least zone of inhibition, however petroleum ether and chloroform extract have shown moderate zone of inhibition. The results of MIC assay as shown in the table 3 which reveals that the methanol extract of *Amoora ruhituka* exhibits the significant antibacterial efficacy for *S. typhimurium* and *E aerogenes* at 0.78mg/ml and petroleum ether extract and it is highest for *P aeruginosa* whereas the other extracts effects are ranges from 1.563mg/ml to 6.25mg/ml.

Table 3. Minimum inhibitory concentration (Mic in mg/ml) of bark extracts on different bacterial strains

Organisms	Concentration (mg/ml)									
	EXT	0.098	0.195	0.39	0.78	1.563	3.125	6.25	12.50	25.00
<i>Enterobacter aerogenes</i>	PE	+	+	+	+	***	-	-	-	-
	BE	+	+	+	+	+	***	-	-	-
	CE	+	+	+	+	+	***	-	-	-
	ME	+	+	+	***	-	-	-	-	-
<i>Salmonella typhimurium</i>	PE	+	+	+	+	+	***	-	-	-
	BE	+	+	+	+	+	+	***	-	-
	CE	+	+	+	+	+	***	-	-	-
	ME	+	+	+	***	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	PE	+	+	+	***	-	-	-	-	-
	BE	+	+	+	+	+	+	***	-	-
	CE	+	+	+	+	+	***	-	-	-
	ME	+	+	+	+	***	-	-	-	-
<i>E coli</i>	PE	+	+	+	+	+	***	-	-	-
	BE	+	+	+	+	+	+	***	-	-
	CE	+	+	+	+	+	***	-	-	-
	ME	+	+	+	+	***	-	-	-	-

PE= Petroleum ether extract, BE= Benzene Extract CE= Chloroform Extract, ME= Methanol Extract, + =Turbidity observed; - = No turbidity observed; * = MIC value.**

4. DISSCUSSION

Approximately 60-80% of the world's population is still relies on traditional medicines for the treatment of common illnesses specifically in developing countries. Currently most of the attention has been made towards the plant origin active compounds isolated from the plant species for new broad spectrum antimicrobial drugs. Even today the use of medicinal plants plays a significant role in developing countries for primary health needs which offers a new source of antimicrobial agent with a potent activity against infective microorganisms [17]. Phytochemical screening of different extracts of the *Amoora ruhituka* indicated the presence of various secondary metabolites

The chloroform extract of *Amoora ruhituka* consists of maximum number of phytoconstituents when compared with other extracts. This was the preliminary report concerning the chemical constituents usually provided by the qualitative phytochemical screening of the plant extracts. In the present investigation the preliminary phytochemical studies serves as groundwork to isolate the pharmacologically active principles present in the plant. Previous researchers have reported the antibacterial activities of the leaves [18-19], Fruit [8], Bark[20-21]of the plant. The current result also correlates with the previous studies. Presence of varieties of chemical compounds in the plant is directly or indirectly responsible for the significant amount of biological activities.

5. CONCLUSION

Based on the experimental results of the present study it can be conclude that methanol and chloroform extracts of *Amoora ruhituka* bark has exhibited significant antibacterial activity against gram negative organisms this is due to presence of different secondary metabolites in these extracts. Methanolic extract of the bark also exhibited a maximum zone of inhibition for the tested organisms with minimum MIC values. Hence, this work justifies the use of *A.ruhituka* in ethnomedicine and further research on this plant can be exploited for new potent antimicrobial agent

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