

IN VITRO ANTIMICROBIAL AND PHYTOCHEMICAL SCREENING OF THREE REPUTED BANGLADESHI MEDICINAL PLANTS

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ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand in the present world for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. The antimicrobial activity of extracts of different plants has been recognized for many years for screening purpose. In the present study, crude ethanol extracts of leaves of three medicinal plants of Bangladesh namely, *Annona squamosa* Linn. (Fam: Annonaceae), *Calotropis procera* R. Br. (Fam: Asclepiadaceae), and *Piper betel* Linn (Fam: Piperaceae) were prepared and investigated for their *in vitro* antimicrobial properties and for determining their phytochemical constituents. All the crude extracts were tested against four different gram positive and gram negative bacteria by the disc diffusion technique, where kanamycin (30 µg/disc) disc was used as a standard. Among all, the ethanol extracts of *Piper betel* Linn. showed the maximum antibacterial activity against *Escherichia coli* with the maximum zone of inhibition, whereas the *Annona squamosa* Linn. and *Calotropis procera* R. Br. showed significant zone of inhibition against the bacteria used in the study. In phytochemical screening, various tests were performed for the determination of the presence or absence of alkaloids, phenols, steroids, tannins, flavonoids, saponins, glycoside and volatile oils. From the tested results, it was evident that steroids, tannins, saponins, glycosides and volatile oils were present in the *Annona squamosa* Linn. leaf extract whereas all the constituents for which the tests were carried out were present in the leaf extract of *Calotropis procera* R. Br. *Piper betel* Linn. leaf extract was found to contain alkaloids, phenols, steroids, tannins and volatile oils.

Keywords: Antimicrobial activity, Disc diffusion, *Annona squamosa*, *Calotropis procera*, *Piper betel*, Phytochemical constituents.

INTRODUCTION

Annona squamosa Linn. (Fam: Annonaceae) is a small tree, about 3 to 6 meters high, with oblong to lanceolate leaves, greenish flowers and warty skinned segmented sweet fruits, planted as a fruit plant in different areas of Bangladesh. Leaves and tender stems of it contain the alkaloids, anonaine, roemerine, norcorydine, corydine, isocorydine, nor-isocorydine, norlaureline, glaucine, xylopin and lanuginosine¹. Its seed oil is rich in the unsaturated fatty acids- oleic and linoleic acids². Leaves and fruits of *Annona squamosa* Linn. are used for treating tumors and their extracts possess spasmogenic, spasmolytic and oxytocic properties. They also show significant anti-cancer activities. Leaves, bark and unripe fruits of the plant are used to treat diarrhea and dysentery³. Ripe fruit can be used as tonic, laxative and anthelmintic. Root is considered as a drastic purgative. Seeds are abortifacient. Leaves, fruits and seeds are also used as insecticides. The acetogenin and squamotacin possesses antitumor property. The aporphines and oxoaporphines isolated from the plant possess strong antiplatelet and vasorelaxing actions⁴. *Calotropis procera* R. Br. (Fam: Asclepiadaceae) is a plant with broad obovate fleshy leaves but it is a white flowered and small in size. It is perennial shrub abounding in milky latex and it grows commonly in wastelands and graveyards in all over Bangladesh. Root bark of the plant is useful in treating chronic cases of dyspepsia, flatulence, constipation, loss of appetite, indigestion and mucus in stool.

Extracts of its leaves and roots stimulate respiration and blood pressure in dog, and are used in cardiac arrhythmia, rheumatism and cancer. Flowers of the plant are useful in asthma. Calotropin has digitalis-like actions⁵. *Piper betel* Linn. (Fam: Piperaceae) is a stout twining climber with broadly ovate oblong or ovate cordate leaves, tiny yellow-green flowers and small spherical fruits. It is extensively cultivated as a cash crop throughout Bangladesh. Leaves of the plant are popularly used as carminative, astringent, stimulant and antiseptic drug. They are also used in headache and cough of children. Leaf stalk of the said plant is used as a suppository for rectal evacuation in children, while leaf, mixed with honey, is a remedy for coughs. Juice of leaf is used as an eye drop in painful infections and night blindness, and also to relieve cerebral

congestion. Roots of the plant induce permanent sterility in women and extract of its leaves exerts anti-tumor activity in carcinogenesis, and suppresses mutagenic and carcinogenic actions of tobacco specific nitrosamines⁶.

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand in the present world for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. We, therefore, report here in the results of preliminary antimicrobial and phytochemical screening of the ethanolic crude extracts of the leaves of the above mentioned three Bangladeshi medicinal plants, which have much folklore reputation.

MATERIALS AND METHODS

Collection and Preparation of the Plant Materials

Annona squamosa Linn. And *Calotropis procera* R. Br. leaves were collected from Jahangirnagar University campus, Dhaka, Bangladesh while *Piper betel* Linn. was collected from the local market of Dhaka, Bangladesh in February 2011, of which voucher specimens (nos. 39316, 37537 and 37536, respectively) have been deposited in the Bangladesh National Herbarium for future reference. Leaves of the above mentioned plants were shed dried for several days after washing. The plant materials were then ground to a coarse powder. Each of the powdered materials (300 gm) was then soaked in ethanol (1.5 liter) separately and kept for 14 days at room temperature with occasional shaking. The crude extracts were then filtered through cotton plug followed by Whatman no. 1 filter paper (Bibby RE200, Sterilin Ltd., UK) individually and the extracts were finally concentrated one by one with a vacuum rotary evaporator (Buchi, Switzerland).

Test organisms

Both Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*), bacterial strains used for the experiment were collected from the Microbiology Laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) located at Mohakhali, Dhaka-1212, Bangladesh.

Experimental Procedures

Antibacterial screening : The antimicrobial study was carried out by the disc diffusion technique for bacteria⁷. Standard kanamycin disc (30 µg/disc) and discs containing the test materials (500 µg/disc) and the respective solvents were used as the positive control, test sample and the negative control, respectively. According to this method, the antimicrobial potencies of the test samples were measured by determining the diameter of the zones of inhibition in millimeter⁸.

Phytochemical screening

The phytochemical screening of the crude ethanol extracts of three plants were carried out by using standard chromogenic reagents-

lead acetate, potassium dichromate, ferric chloride, hydrochloric acid, sulphuric acid, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Hager's reagent, Molisch reagent, Benedict's reagent and Fehling's solutions were used to detect steroids, alkaloids, gums, flavonoids, saponins, tannins, and reducing sugars using standard protocol⁹.

The extracts of three plants were subjected for this screening using for its different chemical groups as alkaloids, phenols, steroids, tannins, flavonoids, saponins, glycosides and volatile oils as the different chemical groups are reported for specific biologic activities¹⁰. The colour intensity or the precipitate formation was used as analytical responses to these tests¹¹. In each test 10% (w/v) solution of the extract in ethanol was taken.

Table 1: In vitro anti-microbial activity of ethanolic extract of leaves of *piper betel* linn, *calotropis procera* r. br and *anonna squamosa* linn

Bacterial strains	Diameter of zone of inhibition in mm					
	<i>Piper betel</i> Linn.		<i>Calotropis procera</i> R. Br.		<i>Anonna squamosa</i> Linn.	
	Kanamycin (50µg/disc)	Ethanolic extract (500µg/disc)	Kanamycin (50µg/disc)	Ethanolic extract (500µg/disc)	Kanamycin (50µg/disc)	Ethanolic extract (500µg/disc)
Gram +ve <i>B.subtilis</i>	25	13	25	9	25	5
<i>S.aureus</i>	20	12	20	7	20	5
Gram -ve <i>P.aeriginosa</i>	20	12	20	9	20	6
<i>E.coli</i>	15	10	15	10	15	9

Table 2: Status of phytoconstituents in the ethanolic extract of leaves of *anonna squamosa* linn, *calotropis procera* r. br. and *piper betel* linn

Phytoconstituents	Leaves extract of Plants		
	<i>Anonna squamosa</i> Linn.	<i>Calotropis procera</i> R. Br.	<i>Piper betel</i> Linn.
Alkaloids	-	+	+
Phenols	-	+	+
Steroids	+	+	+
Tannins	+	+	+
Flavonoids	-	+	-
Saponins	+	+	-
Glycosides	+	+	-
Volatile oils	+	+	+

+ = present; - = absent

RESULTS AND DISCUSSION

The results of the *in vitro* antimicrobial screening assay of the ethanolic crude extracts of the leaves of *Anonna squamosa* Linn., *Calotropis procera* R. Br. and *Piper betel* Linn. against the tested Gram positive and Gram negative strains are shown in the TABLE 1. Among all, the ethanolic extract of leaves of *Piper betel* Linn. at a concentration of 500 µg/disc showed the maximum antimicrobial activity against *Bacillus subtilis* with zone of inhibition 13 mm (TABLE 1) followed by *Staphylococcus aureus* (12 mm) and *Pseudomonas aeruginosa* (12 mm) and *Escherichia coli* (10 mm).

The ethanolic extract of the leaves of *Calotropis procera* R. Br. at the same concentration showed the maximum antimicrobial activity against *E coli* with zone of inhibition 10 mm (TABLE 1) followed by *Pseudomonas aeruginosa* (9 mm), *Bacillus subtilis* (9 mm) and *Staphylococcus aureus* (7 mm). Similarly, the ethanolic extract of *Anonna squamosa* Linn. leaves showed the maximum antimicrobial activity against *Escherichia coli* having zone of inhibition 9 mm (TABLE 1). It was followed by *Pseudomonas aeruginosa* (6 mm), *Bacillus subtilis* (5 mm) and *Staphylococcus aureus* (5 mm). Finally, as a whole, the maximum zone of inhibition was found for *Piper betel* Linn. followed by *Calotropis procera* R. Br. and *Anonna squamosa* Linn. irrespective of the microorganisms used (TABLE 1). On the other hand, the zones of inhibition in case of the standard kanamycin with *B subtilis*, *S aureus*, *P aeruginosa* and *E coli* were 25, 20, 20 and 15 mm, respectively (TABLE 1). From the above stated data for both of the standard and test samples, it is clear that the zone of inhibition showed by the extracts of the above mentioned plants leaves are significantly comparable with that of the kanamycin standard. So the present investigation provide evidence for the antimicrobial nature of the ethanolic extracts of the

leaves of *Piper betel* Linn., *Calotropis procera* R. Br. and *Anonna squamosa* Linn. and the probable presence of antimicrobial phytoconstituents in their ethanolic extracts. Overall antimicrobial activity of the ethanolic extract of the studied plants is *Piper betel* Linn. > *Calotropis procera* R. Br. > *Anonna squamosa* Linn. Bioassay guided separation of the extracts may led to the isolation of compounds responsible for antimicrobial activity¹².

In phytochemical screening (TABLE 2), various tests were performed for the determination of the presence or absence of alkaloids, phenols, steroids, tannins, flavonoids, saponins, glycoside and volatile oils in the prepared extracts. Steroids, tannins, saponins, glycosides and volatile oils are found to be present in the leaf extract of the *A. squamosa* Linn. (TABLE 2) but alkaloids, phenols and flavonoids are found to be absent in the same extract (TABLE 2). On the other hand, all the constituents for which the tests were carried out are found to be present in the *Calotropis procera* R. Br. leaves extract (TABLE 2).

The *Piper betel* Linn leaves extract is found to contain alkaloids, phenols, steroids, tannins and volatile oils, whereas the tests for flavonoids, saponins and glycoside are negative, indicating their absence in the extract of the *Piper betel* Linn. leaves (TABLE 2). Traditional practitioners use widely and effectively the plants, that are investigated in our present study, for the treatment of various infectious diseases. The phytoconstituents that are found to be present in the investigated plants from the present study and also that are reported in the literatures¹⁻², could be responsible for the remedies results in from the use of the plants and hence for the folklore reputation. However, these plants need to be subjected for further research to explore their extensive pharmacological and other relevant activities.

CONCLUSION

Annona squamosa Linn, *Calotropis procera* R. Br. and *Piper betel* Linn. etc. are medicinally important plants and are used in the treatment of various diseases in traditional systems of medicine. This report provides valuable antimicrobial and phytochemical information about the plants. Such information may serve as a base for new phytochemical, pharmacological, toxicological and clinical research. The future prospects of the present research work include isolation and purification of antimicrobials from the active extracts and their further pharmacological evaluation and clinical trials.

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