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Antibacterial activity of Senna alata leaf extracts against coliform bacteria isolated from Bhogdoi river water of Jorhat District of Assam, India

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Abstract: Infectious diseases are increasing day by day due various factors such as climate change, population explosion, antibiotic resistance etc. To treat these diseases synthetic drugs have been used. But use of these drugs gives adverse side effects on human body. So, an alternative way of treating these diseases is a much-needed call of this era. Natural or plant-based medicines could be used in place of synthetic drugs as they are cheaper in price and less or no toxic side effects. North-east India specially Assam is blessed with biological diversity, hence numerous medicinally important plants and herbs are available here. But only a small section of it have been explored scientifically for their activities. In this study, Senna alata (Fabaceae) had been selected to evaluate its antibacterial activity against Coliform bacteria (E. coli) isolated from Bhogdoi river water from Jorhat district of Assam. This plant is traditionally used by the local people of Assam against skin infections like ringworm and also used as a laxative. Phytochemical analysis of the leaf extract showed the presence of phytochemicals like phenol, flavonoid, tannin, steroid, alkaloid etc. Methanolic and aqueous leaf extract of Senna alata were tested against Coliforms (E. coli) and results showed that methanolic leaf extract possessed higher antibacterial activity than the aqueous extract. These findings confirmed that due to presence of phytochemicals present in the leaf extracts of Senna alata showed antibacterial activity Coliform (E. coli) bacteria. However further study would be required to isolate its bioactive components and its pharmaceutical potential.

Keywords: Senna alata; antibacterial; methanolic; aqueous; Coliforms; phytochemicals

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1. Introduction

Infectious diseases impacting public health systems and economies worldwide as they create a significant global burden (1). They are responsible for 17 million deaths per year worldwide, roughly 30% of the global mortality rate. Microorganisms like bacteria, fungus, protozoa are responsible for these infectious diseases. In India specially Assam due its hot and humid climate bacterial and fungal diseases are very much prevalent as the climate favours their growth. To control microbial diseases antibiotic drugs have been used, but inappropriate use of these synthetic drugs triggers antibiotic resistance in microbes (2). The increase in multidrug resistance in microbes has encouraged scientists and

researchers to concentrate on the search for new antimicrobial substances as an alternative to synthetic drugs. Medicinal plants have been used traditionally to treat infectious diseases. Different parts of plants such as leaves, seeds, roots, stems possess bioactive compound which are effective against microbes (3). These compounds are generally the secondary metabolites like phenol, flavonoid, glycosides, alkaloids and due to which plants show activities such as antimicrobial, antidiabetic, anthelminthic etc (4). Assam is blessed with biological diversity, hence numerous medicinally important plants and herbs are available here. In this study Senna alata plant was selected to evaluate its antibacterial activity against coliforms (E.coli) isolate from Bhogdoi River water. This plant is traditionally used in skin infections like ringworm and other fungal infection (5).

2. Materials and Method

2.1 Collection of samples: Leaves of Senna alata was collected from Meleg village of Jorhat, Assam and identified with help of local people. Senna alata is locally known as Khorgos and comes under the family Fabaceaae.

Collected leaves were washed properly with clean water and dried under shade for seven days. Then these were ground into powder by using a mechanical grinder. These powdered samples were kept in airtight container for further use.

Fig1: ***Senna alata***

2.2 Collection of water sample: Water sample collected from Bhogdoi river and was taken to the Biotech Hub of Bahona College.

2.3 Coliform examinations: For antimicrobial activity analysis, Coliform bacteria were isolate from Bhogdoi river water. Bhogdoi river is tributary of the Brahmaputra in India. It flows through the Jorhat city and then merges with other rivers and got the name Gelabil. By using specific media (Mckonky media) Coliform bacteria were isolate from the river water. Bacteriological analysis was carried out for indicator organisms; total and fecal coliform (*E. coli*) using MPN technique (APHA, 2004). This was carried out according to the three-stage process of presumptive test, confirmed test and completed test. These isolated bacteria were used as test organisms (2).

Fig 2: Colonies of Coliform bacteria on Mckonky Agar

2.4 Preparation of Extracts

Aqueous and methanol leaf extracts of *Senna alata* were prepared by using **cold extraction** method (6). 10gm of air-dried seed powder of *Senna alata* was mixed in 100ml of solvent in a conical flask, plugged with cotton wool and kept on the shaker incubator at a speed of 350rpm for 72hours. After 72 hours, extracts were filtered using Whatman N0.1 filter paper. The filtrates were concentrated on water bath at 60^{o}C temperature to get the residues. The residues were weighted and dissolved in Dimethyl sulphoxide -DMSO (1mg/ml). These extracts were stored at 4^{0}C in airtight bottles and were used for detection of phytochemicals

2.4 Qualitative Phytochemical Analysis

2.4.1 Test for protein:

a) Biuret test: To 3ml of test solution (seed extract), 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 were added and observed for the formation of pinkish or purple violet color.

b) Millon's test: 1ml of test solution acidified with sulphuric acid was added to Millon's reagent and boiled this solution and observed for the formation of a white precipitate. After warming, the precipitate turned brick red or the precipitate dissolved giving a red colored solution.

2.4.2 Test for amino acids

1ml of the extract was mixed with few drops of Ninhydrin reagent. The appearance of purple color shows the presence of amino acid.

2.4.3 Test for carbohydrate

A small quantity of extract was dissolved in 5ml of distilled water and filtered. The filtrates were then subjected to following tests to detect the presence of carbohydrate.

(a) Molisch's test: To 3ml of the filtrate, few drops of α-naphthol (20% in ethyl alcohol) were added. The mixture was shaken well. Then 1ml of concentrated sulphuric acid was added along the side of the test tube. The Reddish violet colored ring at the junction of two layers appeared in the presence of carbohydrates.

(b) Fehling's reagent test: 1ml of the Fehling's solution A mixed with 1ml of Fehling's Solution B. This mixture was then boiled for 5minutes. An equal volume of filtrate was then added to the mixture and allows it to heat for 5-10 minutes in water bath at 60^0C. Appearance of red precipitate indicates the presence of sugars.

(c) Benedict's test: Equal volume of Benedict's reagent was added to the extract and the mixtures were boiled for 5minutes on a water bath. Formation of green or yellow color indicates the presence of reducing sugar.

(d) Iodine test: 2ml of the filtrate was mixed with few drops of iodine solution. The appearance of blue color, which disappears on boiling and reappears on cooling, indicates the presence of nonreducing sugars.

2.4.4 Test for flavonoids

(a) Shinoda test: A small quantity of the extract was taken in 5ml of alcohol (95% ethanol), was treated with few drops of concentrated hydrochloric acid (HCl) and 0.5g of magnesium turnings was added and observed for the formation of pink color.

(b) Alkaline reagent test: 1ml of the extract was mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on the addition of few drops of diluted acid which indicated the presence of flavonoids.

2.4.5 Test for glycosides

(i) For cardiac glycosides

(a) Liebermann's test: 1ml of the extract was mixed with each of 2ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice and then concentrated H_2SO_4 was added carefully. A color change from violet to blue to green indicates the presence of glycoside.

(b) Salkowski's test: 1ml of the extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish-brown color indicated the presence of glycoside.

(c) Keller-kilani test: 1ml of extracts was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

(ii) For anthraquinone glycosides

(a) Borntrangers test: 2ml of the extract was mixed with dilute H_2SO_4 and the solutions were boiled and filtered and then cooled. To this cold filtrate, an equal volume of chloroform was added and shakes well. After sometime organic solvent was separate and followed by addition of ammonia. Ammonical layer turns into pink red indicate the presence of anthraquinone glycosides.

2.4.6 Test for saponins

Foam test: 1ml of the extract was mixed with equal volume of distilled water and shaken vigorously and observed the formation of persistent foam which indicates the presence of saponins in the extract.

2.4.7 Test for steroids

1ml of the extract was dissolved in 10 ml of chloroform and an equal volume of concentrated H_2SO_4 was added by sides of the test tube. The upper layer showed yellow with green fluorescence. This indicates the presence of steroids.

2.4.8 Test for phenols and tannins

1ml of the extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue -green or black coloration indicated the presence of phenols and tannins.

2.4.9 Test for Alkaloid

5 gm of powdered sample was treated with petroleum ether and filtered. The residue was taken and treated with chloroform and filtered, the obtained residue was treated with methanol and filtered. Now the filtrate was evaporated and pH is maintained at 2 and the volume was made up to 50 ml with distilled water. Then the filtrate was taken to test with Mayer's, Wagner`sand Dragendorff' alkaloid reagent.

(a) Mayer's reagent test: To 3ml of filtrate few drops of Mayer's reagent (potassium mercuric iodide solution), was added and observed for the formation of white or cream-colored precipitate which indicates the presence of alkaloid in the extract.

(b) Wagner's reagent test: To 3ml of the filtrate, few drops of Wagner's reagent (iodine in potassium iodide), was added and observed for the formation of a red-brown precipitate.

(c) Dragendorff reagent test: To 3ml of the filtrate, few drops of Dragendorff's reagent (potassium bismuth iodide solution), was added and observed for the formation of an orange-brown precipitate.

2.5 Evaluation of antibacterial activity

Antibacterial activity of extracts in different solvents was determined by Agar well diffusion method on Muller Hinton Agar medium (8).

15ml of Muller-Hinton agar was poured on sterilized glass plates and allowed to solidify. After solidification of agar, plates were inoculated with 100 µl of overnight grown bacterial culture which were adjusted to 0.5 McFarland turbidity standards, on Muller-Hinton agar plates. After inoculation, wells were made using sterile cork borer and 100 µl of extracts which prepared in different solvents were dispersed into wells. Plates were incubated for 24 hours at 35oC in BOD incubator under static condition and zones of inhibition were measured. DMSO was taken as negative control whereas Chloramphenicol (30µg/ml) was taken as positive control.

3. RESULTS

3.1 Phytochemical Analysis

Qualitative phytochemical analysis of the leaf of the plant in water and methanol showed the presence of most of the phytochemicals. Carbohydrate was detected in both solvent extract of the plant. Protein as well as amino acids were detected in both aqueous and methanolic extracts. Reducing sugar was also detected in both solvent extracts. Phenolics were detected in aqueous as well as methanolic extracts of the plant. Flavonoid was detected in the methanolic extracts but not detected in aqueous leaf extract of Senna alata. Tannin and anthraquinone was detected in both aqueous and methanolic leaf extracts of the plant. Alkaloid and steroid were detected in the methanolic leaf extract but absent in aqueous extract. Whereas saponin was detected in both of the extracts.

Table 1: Phytochemicals present in the seed extracts of *Senna alata* prepared in different solvents

solvents / Phytochemicals	water	methanol
Carbohydrate	+	+
Protein	+	+
Amino acid	+	+
Reducing sugar	+	+
Phenolics	+	+
Flavonoid	-	+
Tannin	+	+
Glycosides	+	+
Anthraquinone	+	+
Alkaloid	-	+
Steroid	-	+
Saponins	+	+

(+ means present, - means absent)

3.2 antimicrobial activity analysis

Solvents → / Microbes ↓	Water	Methanol	Negative	Standard Antibiotic (Chloramhenicol)
	Zone of inhibition in mm			
Coliform *(E. Coli)*	10.67 ±0.33	36.33 ±0.67	------	40.33 ±0.33

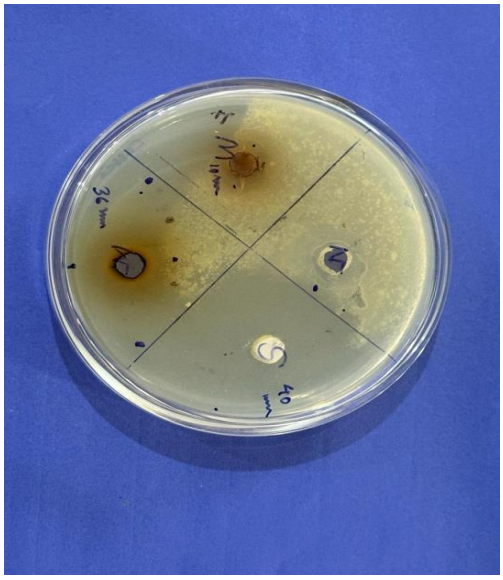

Fig 3: Antibacterial activity of aqueous and methanolic leaf extract of *Senna alata* against Coliform (*E. coli*) bacteria isolated from Bhogdoi river water

4. DISCUSSION:

North-east India specially Assam is blessed with rich biodiversity and hence numerous medicinally important plants and herbs are available here (9,10). To combat the adverse effect of synthetic drugs plant-based medicine could be used. Senna alata L is traditionally used in skin infections by the local people of Assam. From this study it was found that seed extract of Senna alata. contains active phytochemicals like phenol, flavonoid, tannin, saponin, steroids etc. Among the water and methanol solvent extract of Senna alata L., methanolic leaf extract showed highest antimicrobial activity against tested bacterial strain.

From the above findings it is confirmed that the extracts from the methanolic leaf extract of Senna alata contain bioactive compound which is effective against coliform bacteria which is responsible for many stomachs related diseases and faecal pollution.

5. CONCLUSION

This study stated that leaf extracts of Senna alata. contain bioactive compound which needed to be isolate, characterized and tested for its pharmaceutical potential.

Conflict of Interest

We have no conflict of interest.

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