Original article

Phytochemical and antibacterial screening of different fractions of root part of *Ipomea Turpethum*

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Abstract

Objective: In folk medicines, different herbs and plants have been used for many thousands of years .Now it is important to investigate these plants and herbs scientifically which have been used in traditional medicines. The aim of this study was to investigate the antibacterial activity and preliminary phytochemical screening of root of *Ipomea turpethum* extracted in methanol and its fractions. ***Methods:*** The methanol extract was further extracted with three solvents ethyl acetate, chloroform and hexane and analyzed for their antibacterial activity using by agar well diffusion method. They were tested against six bacteria; *Echrichia coli, Staphylococcus aureus, Staphylococcus epidermidus, Proteus vulgaris, Pseudomonas auroginosa* and *Salmonella typhi*. The susceptibility of microorganisms to all three fractions was compared with each other and with standard antibotic (Ampicillin). The fractions of *Ipomea turpethum* was also qualitatively analyzed for the presence of chemical components, i.e. saponins, alkaloids, flavonoids, tannins and glycosides. ***Result:*** Among all fractions methanol exhibited highest antibacterial activity (average zone of inhibition 23.53mm ± 1.3) while ethyl acetate exhibited least antibacterial activity (average zone of inhibition 18.50mm ± 3.5). Minimum inhibitory concentration of methanol, ethyl acetate, chloroform and hexane fractions was found in the range of 650ug/ml to 2500ugl/ml against microorganisms. ***Conclusion:*** Results obtained from this preliminary in-vitro experiment indicate that, all three fractions of *Ipomea turpethum* has good antibacterial activity against all microorganisms used. By phytochemical analysis of extract, it has been found to contain some nutrient and chemical components which support its ethenomedicinal use but further work is required for development of new antibiotic compounds.

Keywords: *Ipomea turpethu;,* phytochemical screening; antibacterial activity.

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Introduction

Due to indiscriminate use of antimicrobial drugs against many infectious diseases, organisms have developed resistance to various antibiotics[1]. Rapidly increase in resistance of microorganisms to currently used antibiotics and their side effects; in addition to high cost of production of synthetic compounds; there is an urgent need to search for the alternatives[2]. In view of this, the searches for new antimicrobial agents from medicinal plants are even more vital in developing countries like Pakistan, India and Bangladesh. Considering the high costs of synthetic drugs and there various side effects, the search for alternative products from plants used in folklore medicine is further justified. *Ipomea turpethum* (family:convolulaceae) is a large perennial and climbing herb found in Southern, South east and the Barendra region of Bangladesh, India, Pakistan Nepal , Srilanka and other tropical region of the world. Commonly known as Dudh kalmi in Bangladesh, in

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Sanskrit known as kalammeshi Rechani, Kutarana Bhandi and in English Turpeth root. Root of Ipomea is used to treat obesity, ascites, piles, snake bites, fever cough, asthma, dyspepsia, flatulence, paralysis, gout, rheumatism, melancholia[3,4]. It is used to relieve constipation, flatulence, colic and in treatment of obesity. It is also used to treat gout, rheumatism and other inflammations[5]. The root extract of *Ipomea turpethum* has been used as an effective hepatoprotective agent[6].

Plants are excellent source of secondary metabolites such as sterols, alkaloids, glycosides, saponins, flavonides, tannins and carbohydrates. These metabolites have antimicrobial properties[7]. Previous work about the phytochemical studies revealed that Ipomea is a rich source of some triterpenoids; B sterols, betulin and lupeol[8] ; glycosidic resin and volatile oil[9].

Due to the reported medicinal properties of this plant, a study is designed to explore and compare the antibacterial activity in different fractions of root part of the plant against wide range of bacteria and phytochemical screening of root extract and fractions to provide scientific evidence for its use as a traditional folk remedy.

Experimental

Plant material

The roots of *Ipomea turpethum* was purchased from local market and identified by botanist. After cutting into small pieces, dried in shade, dried root part of *Ipomea turpethum* was washed and allow to air dry at room temperature. The dried roots were grinded to fine powder and stored in an air tight jar.

Extraction

The powdered plant material (200g) was soaked in 70% methanol (1.5 L) for 3 days at room temperature. Mixture was stirred every 24 h using a sterile glass rod. The solvent- containing extract was then decanted and filtered. The extraction was further repeated with methanol .The filtrate from each extraction was combined and excess solvent was evaporated under reduced pressure using a rotary evaporator to give crude methanol extract. The ethanol extract was further extracted with different solvents such as chloroform, ethyl acetate and hexane. The crude extract and fractions were stored at 4ºC for determination of antibacterial activity and phytochemical analysis[10].

Phytochemical Screening

The freshly prepared extract and fractions of *Ipomea turpethum* was qualitatively analyzed for the presence of chemical components, i.e. saponins, alkaloids, flavonoids, tannins and glycosides. Different chemicals and reagents were used, for example alkaloids were tested by, dragendroffs, reagent, flavonoids by Mg and HCl, tannins with ferric chloride and potassium dichromate solutions. Steroids and reducing sugars were identified by Libermann_Burchard reagent and Benedict's reagent respectively[11].

Test microorganism

The antibacterial activity was carried out against six microorganisms *Echrichia coli, Staphylococcus aureus, Staphylococcus epidermidus, Proteus vulgaris, Pseudomonas auroginosa* and *Salmonella typhi.* All microorganisms used in the present study were clinically isolated. All the organisms were maintained on tryptic soya agar slants at 4ºC prior to testing.

Preparation of Solution

Extract was dissolved in 6% dimethylsulfoxide (DMSO) to give strength of 100mg/ml. Ampicillin was used as reference standard (positive control) while 6% dimethylsulfoxide (DMSO) used as negative control.

Inoculums Preparation

A 10ml of sterile distilled water was poured in test tubes. General colonies of each test organism were taken directly from the plate emulsified in distilled water tubes and the suspension was adjusted to match the 0.5 McFarland's standard ($1x10^8$ CFU/ml)[12].

Antibacterial assay

The agar well diffusion method was used to determine antibacterial activity by plant extract and its fractions[13]. According to this method; 100 ul of diluted inoculums (10^6 CFU/ ml) of test culture was thoroughly mixed with 20 ml of molten sterile tryptic soya agar and poured in to pre-sterilized petri dishes under sterile condition. All plates were left to set at 4ºC for 30-40 minutes. Holes of 6 mm diameter were made in the center of each seeded plates. Holes were then filled aseptically with 0.1 ml of test solution (crude methanol and fractioned extracts.). Standard disc of antibiotic ampicilin (10ug) served as positive antibacterial control. DMSO is used as negative control. All plates were then incubated at 37ºC ±1ºC for 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition around the well. The diameter of inhibition zone was measured in millimeters by Vernier caliper. All tests were performed in triplicate to minimize test error.

Minimum Inhibitory Concentration

The minimum inhibitory concentration of the extract was calculated by broth dilution method[14]. Freshly

prepared nutrient broth was used as diluents. Two fold serial dilutions of all fractions were made. Each inoculum was prepared in nutrient broth and density was adjusted to 0.5 Mcfarland standards ($1x10^8$ CFU/mL).50ul of each inoculum was added to each test tube except negative control tube. All tubes were incubated at 37ºC and MIC was recorded after incubation period. The MIC is the lowest concentration of extract at which the microorganisms tested do not have visible growth.

Results

Qualitative phytochemical analysis showed the presence of alkaloids, steroilds, triterpenoids, coumarins, flavonoids and phenolics (Table-1).

Table-1: Phytochemical analysis of different fractions of *Ipomea turpethum* root.

Chemical Name	Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	+	+	-	-
Steroids	+	+	+	+
Triterpenoids	-	-	+	-
Coumarins	-	+	-	-
Flavonoids	+	-	-	-
Phenolics	-	+	-	-
Saponins	-	-	-	+

Crude methanol extract of *Ipomea turpethum* and its fractions (ethyle acetate, hexane and chloroform) exhibited a varied degree of antibacterial activity against wide range of bacteria including *Echrichia coli, Staphylococcus aureus, Staphylococcus epidermidus, Proteus vulgaris, Pseudomonas auroginosa* and *Salmonella typhi*. The results of antimicrobial activity are presented in Table 2.

DMSO is taken as negative control, which exhibited antimicrobial activity against microorganism up to 8mm zone of inhibition. Hence a zone of inhibition of 8mm and less is considered as no activity.

The study not only gives a preliminary account of the antibacterial components in the root part of *Ipomea turpethum* but also compares the activity of extract in different solvents. Among all fractions, methanol fraction exhibited highest antibacterial activity with average zone of inhibition 23.53mm ± 1.3 while ethyl acetate fraction showed least antibacterial activity with average zone of inhibition 18.50mm ± 3.3 as shown in table 2. In methanol fraction maximum activity was observed against *E. coli* (26.5mm ± 0.5) and least activity was recorded in *Proteus vulgaris* measured 17.6 mm ± 1.21. The ethyl acetate fraction was also found effective against tested organisms with 20.95mm ± 4.5 average zone of inhibition. Maximum antibacterial activity was observed against salmonella typhi (26.33mm ± 1.52) at 100mg/ml concentration and least activity was obtained against *Proteus vulgaris* (17.16 mm ± 0.76). In Chloroform fraction, *Salmonella typhi* and *Proteus vulgaris* showed remarked sensitivity with inhibition zone of 26.16mm ± 1.04and 25.16mm ± 1.04 respectively, while all other organisms exhibited moderate activity with average zone of inhibition 18.50mm ± 3.3. Hexane fraction also exhibited good antibacterial activity with average zone of inhibition 20.69 ± 1.78 and maximum activity was observed against *Salmonella typhi* i.e 23.5mm ± 0.5.

Minimum inhibitory concentration (MIC) of methanol, ethyl acetate, hexane and chloroform extracts against microorganisms are presented in table 3. Among all fractions, the least MIC value (650ug/mL) was shown by methanol fraction against *Staphylococcus aureus*.

Discussion

In folk medicines, different herbs and plants have been used for many thousands of years .Now it is

Table-2: Antibacterial activity exhibited by different fractions of *Ipomea turpethum* root.

Sr. #	Name of Organisms.	Zone of Inhibition; dia. (mm)					
		Concentration 100mg/ml					
		Methanol	Hexane	Ethyl Acetate	Chloroform	Standard	DMSO
	E. coli	26.5±0.5	20.16±0.76	19.16±0.76	18.76±0.25	30.5±0.5	7.5±0.5
	S. aureus	26.16±0.76	19.83±.28	22.16±1.04	17.33±1.25	29.33+0.763	7.3±.76
	Ps. auroginosa	20.5±0.5	18.16±1.25	19.5±0.5	21.16±1.04	29.5+0.5	8±0.5
	S. typhi	25.5±0.5	23.5±0.5	26.33±1.52	26.16±1.04	28.33±.763	7.3±.32
	S. epidermides	22.83±0.76	21.33±0.76	21.43±0.60	19.8±0.76	29.83±1.25	7.6±.36
	P. vulgaris	17.6±1.21	21.16±1.04	17.16±0.76	25.16±1.04	31.16±.86	7.6+.28
	Average	**23.53±.3.57**	**20.69±1.78**	**20.65±3.17**	**18.50±3.5**	**29.77±.98**	**7.55±.25**

Table-3: Minimum inhibitory concentration of different fractions of *Ipomea turpethum* root

Sr. #	Organisms	Methanol	Hexane	E. Acetate	Chloroform
	E. coli	1250 ug	2500 ug	5000 ug	5000 ug
	S. aureus	650 ug	5000ug	2500 ug	5000 ug
	Ps. auroginosa	2500 ug	5000 ug	2500 ug	2500 ug
	S. typhi	2500 ug	2500 ug	2500 ug	1250 ug
	S. epidermides	5000 ug	5000 ug	5000 ug	2500 ug
	P. vulgaris	2500 ug	2500 ug	5000 ug	2500 ug

important to investigate these plants and herbs scientifically which have been used in traditional medicines. Plant extracts are potential source of antibacterial compounds. This study shows that plant extracts inhibit the bacterial growth but their effectiveness varied. The antibacterial activity has been attributed to the presence of different natural compounds[15]. An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to rupture the lipid layer of the bacterial cell membrane and mitochondria, disturbing the cell structures and expose them for permeability. Heavy leakage from bacterial cells and excretion of vital molecules and ions will lead to death[16]. Presence of secondary metabolites in *Ipomea turpethum* is in line with earlier studies[17,18]. The plant is reported to contain four new Dammarane-type saponins[19]. Many biological activities such as bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory have been attributed to the presence of saponins[20]. In support of antibacterial activity of saponins against microorganisms many reports are available[21]. Photochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds of plant provide the protection against many microorganisms. The antibacterial activity of flavonoids may be due to their ability to make a complex with cell walls of bacteria and with extra cellular and soluble proteins. Alkaloids are also known to have antimicrobial activity[22].

In present study the different fractions of crude methanol extract of root of *Ipomea turpethum* showed the antibacterial activity against *Echrichia coli, Staphylococcus aureus, Staphylococcus epidermidus, Proteus vulgaris, Pseudomonas auroginosa and Salmonella typhi*. In methanol fraction maximum activity was observed against *E. coli*. These results also validate the traditional medicinal use of *Ipomea turpethum* root as principal ingredient in the Ayurvedic formulation for the treatment of gastric ulcer and related gastrointestinal disturbances[23].

The ethyl acetate fraction was also found effective against tested micro-organisms and maximum antibacterial activity was observed against *Salmonella typhi*. In chloroform fraction, *Salmonella typhi* and *Proteus vulgaris* showed remarked sensitivity while all other organisms exhibited moderate activity. These results are in alignment with antibacterial activity of root and leaf parts in chloroform extract, crude petroleum ether and ethyl acetate extracts against pathogenic bacteria[24,25]. Hexane fraction also exhibited good antibacterial activity and maximum activity was observed against *Salmonella typhi*.

Minimum inhibitory concentration (MIC) of methanol, ethyl acetate, hexane and chloroform extracts against microorganisms are presented in table 3. Among all fractions, the least MIC value (650ug/mL) was shown by methanol fraction against *Staphylococcus aureus.* The preliminary results of this study indicate that root part of *Ipomea turpethum* has high potential of antibacterial activity and provide a rationale for the use in folk medicine.

Conclusion:

The preliminary results of phytochemical analysis and antibacterial study indicate that root part of *Ipomea turpethum* has high potential of nutrients and antibacterial compounds that provide a rationale for the use in folk medicine. Further work is required for development of new antibiotic compounds.

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