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An In-Vitro Assessment of Antimicrobial, Thrombolytic and Cytotoxic Activity on Ipomoea Pes-Tigridis

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ABSTRACT

To explore activities antimicrobial, thrombolytic, cytotoxic of the ethyl acetate extract and n-hexane extract of Ipomoea pes-tigridis leaves. Antimicrobial study was measured by noticing zone of inhibition. In vitro thrombolytic activity by clot lysis method and cytotoxic activity was considered by using brine shrimp lethality bioassay.Anti-microbiological screening ethyl acetate extract of leaves showed mild antimicrobial activity with zone of inhibition ranging from 11 to 14 mm and n hexane also showed mild antimicrobial activity with zone of inhibition ranging from 4 to 9 mm as compared to standard ciprofloxacin (zone of inhibition of 50 mm). In vitro thrombolytic model was applied to find out the clot lysis effect of ethyl acetate and n-hexane extracts of Ipomoea pes-tigridis where streptokinase and water were employed as a positive and negative controls, respectively. The extracts showed thrombolytic activity 14.90% and 8.18%. In Brine Shrimp Lethality Bioassay, ethyl acetate extract of Ipomoea pes-tigridis exhibited LC50 at 14.125µg/ml and n hexane extract of Ipomoea pes-tigridis exhibited LC50 at 33.113µg/ml and which showed that the extract is pharmacologically dynamic. Investigation disclosed that the plant is pharmacologically active and it possesses mild antimicrobial activity, thrombolytic activity as well as remarkable cytotoxic effect. Thus , this plant can be a good source of some useful drugs of therapeutic.

Keyword: Antimicrobial, Thrombolytic, Cytotoxic, Ipomoea pes-tigridis.

INTRODUCTION

The importance of studying the medicinal values of plants in order to see the sights new resources against the threat of new and recent diseases is rising constantly all over the world especially in Asia and Africa. Countless features of plant i.e., low toxicity, fewer side effects and potent pharmacological activity and economic feasibility contribute to their growing popularity[1]. Nevertheless, several lead compounds were revealed from plants till now. Furthermore, more than 30% of global pharmaceutical preparations depend on plant resources[2]. Drug resistance occurring from careless and irregular administration of commercial antimicrobial drugs, and adverse effects such as, allergic reaction, immune suppression, abdominal pain, anorexia, hypersensitivity etc. motivate the scientists to discover new and effective antimicrobial agents that could be a better substitute of the current treatments[3-5]. Nevertheless, the role of plant secondary metabolites in protecting the plant against microorganisms made plant a possible source of active compounds against microbe [6]. Blood Clotting can block the normal blood flow and thereby can cause necrosis and infarction. Conversely, more or less all the currently available thrombolytic treatments have few vital limitations including limited fibrin specificity, significant bleeding tendency and large dose prerequisite[6-9]. Highly Bioactive compounds are always toxic to the living body at some higher doses and it justifies the report "Pharmacology is simply toxicology at higher doses and toxicology is simply pharmacology at lower doses". Medicinal plants would be the best source to obtain various drugs and consequently such plants should be studied to understand better about their belongings, efficacy and safety [10-14]. Hence, at present researchers shift their concentration on natural resources to find more effective alternative treatment which can counter the problem.

Ipomoea pes-tigridis is a flowering plant of the Convolvulaceae family. This family comprises plants whose characteristics are of high industrial, pharmaceutical, scientific, and cultural significance. Its geographical distribution comprises the Sahel zone from Senegal to Niger and North Nigeria, and distributed across tropical Africa and into Asia, Mascarene Island, Malaysia and Australasia, [15]. Typically, it is used as folk medication for the treatment of hemorrhoids, diabetes, bronchitis and arthritis, [16-18].

Literature review exhibited that diverse species *Ipomoea pes-tigridis* of have a variety of biological activities. *Ipomoea pes-tigridis* is a herb conventionally used by the tribes of kerala as a single drug to treat painful conditions like headache etc, swellings, poisonous wounds and snake bites [19]. Roots are

laxative; used for the cure of carbuncles, bolis and dog-bites. EtOH(50%) extract of plant is spasmolytic[18]. Leaves are used to treat poulticing sores and pimples, haemorrhoids,arthritis, rheumatism, dropsy, swellings, oedema, gout, venereal diseases, in boils, carbuncles and dog bites.

Petiole is used as diuretics, laxatives and pain killer. Leaf sap is used as antidotes for venomous stings, snake bites, etc. Seeds are used to treat stomach troubles. Stem is used in the treatment of tumors and cancers. Entire creeper is crushed and the juice extracted and taken orally for treatment of or prevention of rabies if bitten by a rabid dog. The plant is used for healing wound and Leaf powder is smoked to get relief from bronchial spasm [16-19]. In contrast , chemical investigations of *Ipomoea pes-tigridis* species have revealed the existence Alkaloids,saponin glycosides ,Cardenolides and Bufadionolides ,Flavonoids, Tannins and Polyphenolic compounds, Anthraquinones, Cyanogenic glycosides, Carbohydrates, Fixed oils, Fats, and Volatile oils[20]. Due to its copious and widespread disposal, the objective of the study was to explore the prospective of the *Ipomoea pes-tigridis* leaf extract as antimicrobial, thrombolytic and cytotoxic agent. Moreover, the report of preceding studies [21-24] on analgesic activities conducting using aqueous leaf extract of Ipomoea pes-tigridis influenced us to explore similar type of activities from the crude extract of leaves [25-26]. Prior to the assessment of cytotoxic, antimicrobial and thrombolytic activity, primary phytochemical screenings of the extract were assessed.

MATERIALS AND METHOD

Chemicals and drugs:

Ethyl acetate and n-hexane used as solvent for the extraction, DMSO used in cytotoxic investigation. The drug Ciprofloxacin, streptokinase used as standard which were purchased from Merck, Germany. Normal saline were collected from Square Pharmaceuticals Ltd. Bangladesh.

Plant material:

The leaves of *Ipomoea pes-tigridis* were collected from Chittagong University's area in (Date: 14/03/2013 to 22/03/2013) and its identification was verified by Bangladesh Forest Research Institute (BFRI), Chittagong. The leaves of the plant was cut in to small pieces and ground into fine powder with the help of grinder. Then the powder of the plant stored in air tight container and placed in a cool, dry dark place.

Preparation of the Extract:

200 grams of dried powder was cold macerated in 700 ml ethyl acetate as well as in n hexane for 15 days with occasional shaking and stirring. The whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporation and dried in oven. For ethyl acetate extract percent of yield was 2.4 gm.

Antimicrobial Assay:

Seven bacterial strains (gram positive and gram negative) were collected from Akin scientific store, Chittagong. Solutions of known concentration (3µg/ml) of the test samples are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature (4 ^{0}C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials dissolve and diffuse out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel .As a result there is a gradual change of test materials concentration in the media surrounding the discs.[27, 28]

The plates are then incubated at 37 ^{0}C for 24 hours to allow maximum growth of the organisms. If the test materials have any antimicrobial activity, it will inhibit the growth of the microorganisms and a clear, distinct zone of inhibition will be visualized surrounding the medium. The antimicrobial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out more than once and the mean of the readings is required. [29]

Thrombolytic Activity:

In vitro thrombolytic potential of each extract of *Ipomoea pes-tigridis* was assessed by the method developed by Daginawala using streptokinase as the standard material [30]. 5 mL venous blood drawn from 2 healthy volunteers (1 male and 1 female) without a history of oral contraceptive or anticoagulant therapy was moved to 10 pre-weighed sterilized alpine tubes and incubated for 45 minutes at 37^{o}C. Subsequently clot formation, serum was totally aspirated out without unsettling the clot formed and the weight of clot in each tube was weighed. To each alpine tube containing pre-weighed clot, 100µl aqueous solution of different extracts of concentration of 10 mg/ml was taken distinctly. Thereafter, 100µl of streptokinase (SK) and 100µl of distilled water were separately taken to the control tube as positive and negative controls in some respects. All the tubes were then incubated at 37°C for 90 minutes and noticed for clot lysis. After doing incubation, the released fluid was withdrawn and tubes were again weighed to observe the difference in weight after clot lysis. Difference obtained in weight taken before and after clot disruption was expressed as percentage of clot lysis as shown below: % of clot lysis = (wt of released clot /clot wt) × 100.

Cytotoxic activity:

Biologically active compounds are toxic to the living body at upper doses and it justifies the statement "Pharmacology is simply toxicology at higher doses and toxicology is simply pharmacology at lower doses". [31]. The Brine shrimp lethality bioassay is a quick and comprehensive bioassay for the bioactive compound. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. In this method, in vivo lethality in Brine shrimp nauplii is used as a favorable monitor for screening and fractionation in the discovery of new bioactive products. It indicates cytotoxically as well as a wide range of pharmacological activities such as antimicrobial, antiviral, pesticidal and anti-tumor etc. of the compounds. Generally the LD_{50} values for cytotoxicities are one tenth of LC_{50} values in the Brine shrimp Lethality Test.

RESULT AND DISCUSSION

Antimicrobial Assay:

The extracts of the sample were tested for antimicrobial activity against a number of both gram positive and gram-negative bacteria. Gram positive bacteria are *Bacillus subtilis,Staphylococcus aureus* and gram negative bacteria are *Escherichia coli*, *Pseudomonus aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi*, and *Vibrio cholera*. Standard antibiotic disk of Ciprofloxacin was used for comparison purposes. Ethyl acetate extracts & n hexane extracts of the plant show moderately

antimicrobial activity against some of the test organisms. The results of the antimicrobial activity, measured in terms of diameter of zone of inhibition in mm are showed in table 1 and comparison between both extract and standard are showed in figure A.

Table 1: Results of anti-microbial investigation.

Microorganisms	Zone of inhibition (diameter)		
	Ethyl acetate extract	N hexane extract	Standard
Escherichia coli	No zone of Inhibition	No zone of inhibition	40 mm
Pseudomonus Aeruginosa	No zone of Inhibition	No zone of inhibition	50 mm
Salmonella paratyphi	No zone of Inhibition	No zone of inhibition	50 mm
Staphylococcus aureus	14 mm	9 mm	40 mm
Salmonella typhi	No zone of Inhibition	No zone of inhibition	39 mm
Bacillus subtilis	13 mm	7 mm	35 mm
Vibrio cholerae	11 mm	5 mm	35 mm

Graphical representation:

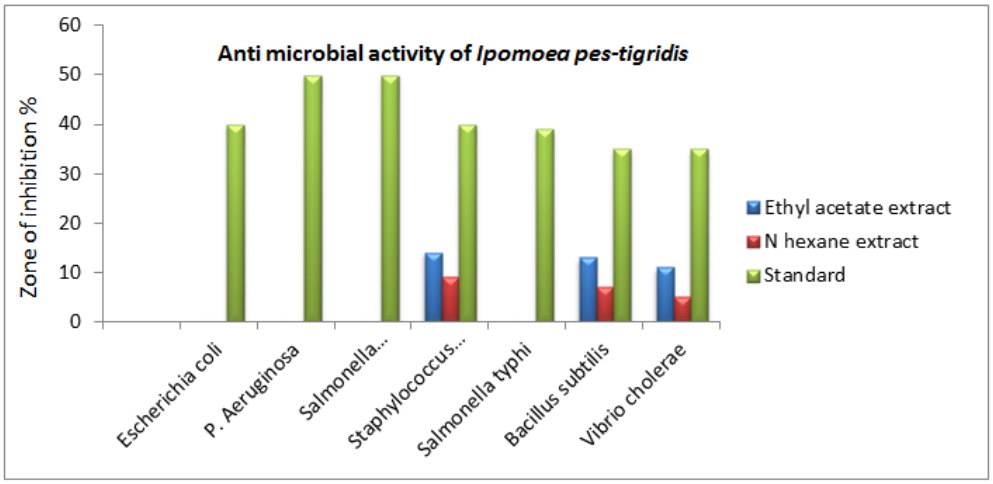

Fig A : comparison of Z one of inhibition of ethyl acetate and n hexane extract of ipomoea pes-tigridis with ciprofloxacin (standard).

The ethyl acetate extract and n hexane extract of *Ipomoea pes-tigridis* showed antimicrobial activity against three test organisms' namely-*Staphylococcus aureus, Bacillus subtilis* and *vibrio cholera.* But had no effect on *Escherichia coli, pseudommonas aeruginosa, salmonella paratyphi,* and *salmonella typhi.*

On the basis of the experiments done on the extract of *Ipomoea pes-tigridis*, we can conclude that it exhibit antibacterial action against very certain bacteria that means it is not much more effective against bacteria and bacterial infection.

Thrombolytic Activity:

Addition of 100-µl Streptokinase, a positive control to the clots along with 90 minutes of incubation at 37°C, showed 86.3% clotlysis and mean % of clot lysis for water was found 5.69%. Clots when treated with 100µl of ethyl acetate and n-hexane extract of *Ipomoea pes-tigridis* showed average clot lysis 14.9% and 8.18% respectively. Among these clotlysis *Ipomoea pes-tigridis* showed minor significant clotlysis activity.

Table 2: Determination of thrombolytic effect of ipomoea pes-tigridis. (Ethyl acetate extract)

No of Alpine Tube	Weight of blank alpine tube	Weight of tube with clot	Weight of tube with clot after Lysis	Weight of clot	Weight of clot after Lysis	% of lysis	Average
1	0.791	1.31	1.21	0.519	0.1	19.27	
2	0.791	1.27	1.17	0.479	0.1	20.88	
3	0.824	1.34	1.28	0.516	0.06	11.63	
4	0.827	1.34	1.26	0.513	0.08	15.59	
5	0.801	1.32	1.19	0.519	0.13	25.05	14.90
6	0.808	1.32	1.26	0.512	0.06	11.72	
7	0.800	1.32	1.25	0.520	0.07	13.46	
8	0.853	1.38	1.35	0.527	0.03	5.69	
9	0.792	1.25	1.21	0.458	0.04	8.73	
10	0.783	1.25	1.17	0.467	0.08	17.13	

Table 3: Determination of thrombolytic effect of Ipomoea pes-tigridis. (n- hexane)

No of alpine Tube	Weight of blank alpine tube	Weight of tube with clot	Weight of tube with clot after Lysis	Weight of clot	Weight of clot after lysis	% of lysis	average
1	0.884	1.42	1.36	0.536	0.06	11.19	
2	0.808	1.25	1.24	0.442	0.01	2.26	
3	0.825	1.27	1.21	0.445	0.06	13.48	
4	0.818	1.21	1.20	0.392	0.01	2.55	
5	0.791	1.27	1.21	0.479	0.06	12.53	

6	0.797	1.19	1.16	0.393	0.03	7.63	8.18
7	0.855	1.26	1.23	0.405	0.03	7.41	
8	0.810	1.27	1.25	0.460	0.02	4.35	
9	0.820	1.33	1.27	0.510	0.06	11.76	
10	0.809	1.27	1.23	0.461	0.04	8.68	

Graphical representation:

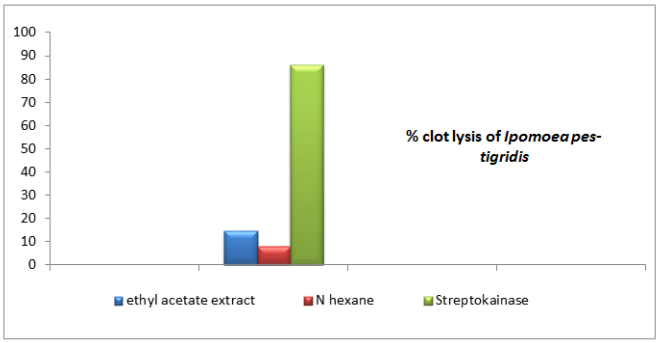

Figure B: Comparative % clot lysis in vitro study of ethyl acetate extract and n hexane extract of Ipomea pes-tigridis with standard drug (streptokinase)

So finally we can conclude that, ethyl acetate and n hexane extract of *Ipomoea pes-tigridis* showed minor significant clot lytic properties in different blood samples. The percent clot lytic activity was compared with water (negative control) and standard enzyme streptokinase (positive control).

Cytotoxic Activity:

The ethyl acetate and n hexane extracts of ipomoea pes-tigridis were tested for Brine shrimp lethality bioassay using brine shrimp nauplii and DMSO as a solvent. The 3 extracts showed positive result on brine shrimp lethality bioassay with high concentration. The LC50 for the extracts were obtained from the graph 3 and graph 4. Control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality. For the extract, the number of nauplii died and percent mortality was counted. The result is shown in the following table 4 and 5.

Table 4: Brine Shrimp Lethality Bioassay for the ipomea pes-tigridis (ethyl acetate extract)

No of test Tube	Sample Concentration, C (µg/ml)	Log C	Total Shrimp number	No of alive shrimp	No of death shrimp	% Mortality	LC_{50} (µg/ml)
1	1000	3.00	10	0	10	100	
2	500	2.70	10	0	10	100	
3	250	2.40	10	0	10	100	
4	125	2.10	10	0	10	100	
5	62.50	1.80	10	0	10	100	14.125
6	31.25	1.50	10	4	6	60	
7	15.62	1.20	10	4	6	60	
8	7.80	0.89	10	5	5	50	
9	3.91	0.59	10	10	0	0	

(Control)							

Graphical representation:

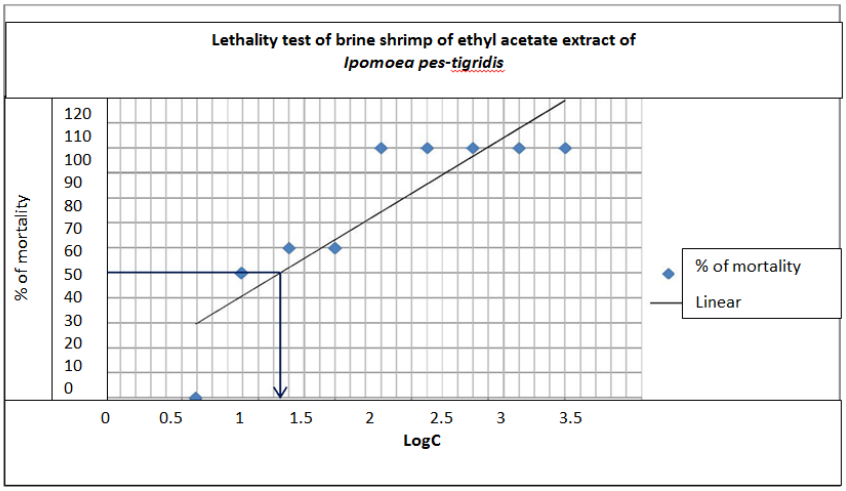

Fig C: Determination of LC50 of ethyl acetate extraxt of Ipomoea pes-tigridis.

Table 5: Brine Shrimp Lethality Bioassay for the Ipomea pes-tigridis (n-hexane extract)

No of test Tube	Sample Concentration, C (µg/ml)	Log C	Total Shrimp Number	No of alive shrimp	No of death shrimp	% mortality	LC_{50} (µg/ml)
1	1000	3.00	10	0	10	100	
2	500	2.70	10	0	10	100	
3	250	2.40	10	3	7	70	
4	125	2.10	10	3	7	70	
5	62.50	1.80	10	4	6	60	33.113
6	31.25	1.50	10	5	5	50	
7	15.62	1.20	10	5	5	50	
8	7.80	0.89	10	6	4	40	
9 (Control)	3.91	0.59	10	10	0	0	

Graphical representation:

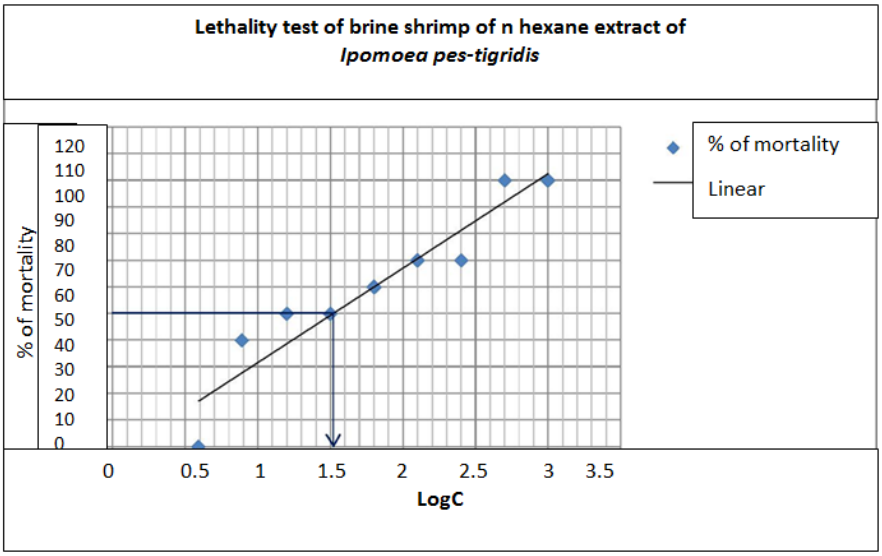

Fig D: Determination of LC50 of n hexane extract of Ipomoea pes-tigridis.

In Brine Shrimp Lethality Bioassay, ethyl acetate extract and n hexane extract of *Ipomoea pes-tigridis* showed LC_{50} at 14.125µg/ml, 33.113µg/ml respectively which revealed that the extract is pharmacologically active. The LC_{50} value of the ethyl acetate and n hexane extracts showed significant lethality against brine shrimp and it can be considered for compound isolation in order to detect future anti-tumor compounds. Moreover, this significant lethality of the ethyl acetate and ne hexane extract of *Ipomoea pes-tigridis* to brine shrimp are indicative of the presence of potent cytotoxic and probably insecticidal compounds which deserves further investigation.

CONCLUSION:

The study is designed to check the antimicrobial activity of two different solvent extracts of *Ipomoea pes-tigridis* against seven pathogenic bacteria. The ethyl acetate extract and n hexane extract of *Ipomea pes-tigridis* showed antimicrobial activity against three test organisms namely- *Staphylococcus aureus*, *Bacillus subtilis and vibrio cholera*. Ethyl acetate and n-hexane extract of *Ipomoea pes-tigridis* showed clot lytic properties in blood samples. The mean % of clot lysis for water (negative control) and streptokinase(standard) was found 5.69% and 86.30% respectively, whereas the mean percent clot lytic activity of ethyl acetate and n hexane extract of *Ipomoea pes-tigridis* was found 14.9% and 8.18% respectively compare with the positive and negative control. The ethyl acetate extract and n hexane extract of *Ipomoea pes-tigridis* showed LC_{50} at 14.125µg/ml, 33.113µg/ml which revealed that the extract is pharmacologically active. It is very useful by providing a preliminary screen that can be supported by a more specific bioassay, once the active compound has been isolated. Thus, some useful drugs of therapeutic importance may develop out of the research work.

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Conflict of interest statement: All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work. Besides, we have no conflicts of interest in this work.

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