Preliminary Phytochemistry and antibacterial activity of *Ipomoea fistulosa*

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Abstract

Phytochemicals are secondary metabolites produced by all plants which have medicinal uses. Ipomoea fistulosa commonly called Pink Morning Glory, belongs to the family Convolvulaceae. The current study was carried out to analyse the Phytochemical Components and Antibacterial Activity of various extracts (Petroleum Ether, Chloroform, and Methanol) of whole plant extracts of Ipomoea fistulosa by using standard procedure. The Antibacterial Activity test conducted against Staphylococcus aureus, Escherichia coli and Salmonella typhi for the solvent extracts of Ipomoea fistulosa by agar well diffusion assay gave varied results. The Ipomoea fistulosa showed a zone of inhibition against these bacteria. The preliminary qualitative phytochemical screening revealed the presence of bioactive constituents such as alkaloids, carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Phlobatannins, Amino acids, proteins, Saponins, Sterols, Tannins, Terpenoids, Quinones, Oxalates, Fats and oils. The findings demonstrate that, in comparison to all other extracts, the methanol and petroleum ether extracts showed greater antibacterial activity.

Key words: Antibacterial Activity, Chloroform, Methanol, Petroleum Ether, Phytochemicals.

1. INTRODUCTION

Ipomoea fistulosa, the pink bush morning glory, is a species of morning glory that grows as a bush. This flowering plant has heart-shaped leaves that are a rich green and 6–9 inches long. It can be easily grown from seeds. Happens in earthbound as well as sea-going environments. Cordate clears out are substitute with long petioles and whole edges, 13-23 cm in length and marginally bushy on both surfaces. Numerous of the plants utilized nowadays were well known to the individuals of

antiquated societies all through the world and they were esteemed for their conservation and restorative powers (Kivanc & Kunduhoğlu, 1997; Peyvast & Khorsandi, 2007). Phytochemicals are confined from the plants, which are valuable and successful for us in this time. Phytochemicals by and large starting from the plant source are nothing but bioactive compounds too known as auxiliary metabolites. Essential metabolites and Auxiliary metabolites. Essential metabolites are critical for the plant's customary digestion system such as development and advancement. Auxiliary metabolites created by plants may have small require for them. These are synthesized in nearly all parts of the plant like bark, clears out, stem, root, blossom, natural products, seeds, etc. Since of these pharmaceutical businesses as well as analysts put a more noteworthy accentuation on phytochemical considers. Too, these phytochemicals display in the distinctive plant parts are utilized by the nearby individuals for the mending of certain clutters(Solomon et al., 2013).

Plants are able with several phytochemical compounds such as Alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, Phlobatannins, amino acids, proteins, saponins, sterols, tannins, terpenoids, quinones, oxalates, fats, oils, and other constituents which are the rich cause of free radical scavengers (Gracelin et al., 2013). Such preliminary phytochemical screening of plants is the need of the hour to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world (Dasgupta et al., 2013; Raphael, 2012).

Ipomoea is one of the imperative plant genera there are awesome therapeutic values and as we all know the moment biggest class of Convolvulaceae family is Ipomoea with around 600 species. *Ipomoea* species are as a rule found in tropic or subtropical districts. Which are solely known for their helpful values such as Anti-inflammatory, alkaloids are known to have appeared potential anticancer properties. Ipomoea fistulosa is a species of blooming plant in the family Convolvulaceae. There are commonly known as 'Blue morning glory'. Several species have been presented in India and numerous species are developed in gardens for decorative reason. The therapeutic properties of plants are ascribed to the auxiliary metabolites synthesized in the plants and expanding consideration has been coordinated towards the utilize of these for the treatment of numerous irresistible infections(Okunade, 2002).

There has been an expanding rate of different resistances in human pathogenic microorganisms in later a long time, generally due to aimless utilize of commercial antimicrobial drugs commonly utilized in the treatment of irresistible illnesses. This has constrained researcher to look for modern antimicrobial substances from different sources like the restorative plants. The show examination was bargains with the recognizable proof of bioactive compounds and to screen the antibacterial test of *Ipomoea fistulosa* plant.

2. MATERIALS AND METHODS

The selected plant species namely *Ipomoea fistulosa* plant were collected from Gangasamudra lake in Chitradurga District, Karnataka, India. The collected samples were carefully stored in sterile polythene bags. Plant was identified Flora of the presidency of madras by J. S Gamble book from Department of Botany, Kuvempu University, Shankaraghatta, Karnataka, India.

2.1.Phytochemical Analysis and Antibacterial Activity:

In the Soxhlet apparatus, 50 grams of coarsely powdered and air-dried plant material were extracted thoroughly using petroleum ether for approximately 40 cycles, or around 18 hours. Under a fan, the petroleum ether extract was separated and allowed to evaporate. Following air drying, the plant material was repacked in a Soxhlet apparatus and thoroughly extracted using chloroform for approximately 40 cycles (≈20 hours). The methanol extraction process takes around 24 hours to complete 40 cycles. The extracted materials were gathered individually, labelled, and stored in dry, clean screw-capped bottles. The extract yield was noted following each further solvent extraction. Thus, the initial phytochemical analysis and antimicrobial assay were performed using the obtained solvent extracts.

2.2.Preliminary Phytochemical Group Test and Antibacterial Activity:

The preliminary phytochemical group tests and the assessment of antibacterial activity of the plant solvent extracts were carried out using standard procedures as described previously (Dasgupta et al., 2013; Harborne, 1998; Valgas et al., 2007).

2.3.Preliminary phytochemical screening Test for Alkaloids (Wagner's reagent):

Three to five drops of Wagner's reagent (prepared by dissolving 1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) were added to a portion of the extract. The mixture was then observed for the formation of a reddish-brown precipitate or coloration, indicating a positive result.

2.4.Test for Carbohydrates (Molisch's test)

A small amount of Molisch's reagent was added to the 2 ml fraction containing the various extracts. Subsequently, 2 ml of concentrated sulfuric acid (H_2SO_4) was carefully added down the side of the test tube. The mixture was then allowed to stand for two to three minutes. A positive result was indicated by the appearance of a reddish-violet or dull violet color at the interface between the two layers.

2.5.Test for Cardiac glycosides (Keller Kelliani's test)

In a test tube, 5 millilitres of each extract were treated with 2 millilitres of glacial acetic acid, followed by the addition of one drop of ferric chloride solution. This mixture was carefully underlaid with 1 millilitre of concentrated sulfuric acid. The presence of deoxy sugars, indicative of cardenolides, was confirmed by the formation of a brown ring at the interface. Additionally, a violet ring may appear below the brown ring, and a greenish ring may develop within the acetic acid layer.

2.6.Test for Flavonoids (Alkaline reagent test)

Two millilitres of each extract were treated dropwise with a 20% sodium hydroxide solution. The development of a bright yellow colour indicated the presence of flavonoids. This yellow coloration disappeared upon the addition of dilute hydrochloric acid, confirming the result.

2.7.Test for Phenols (Ferric chloride test)

A portion of the extracts was treated with 5% aqueous ferric chloride solution, and the mixture was observed for the development of a deep blue or black coloration, indicating the presence of phenolic compounds or tannins.

2.8.Test for Phlobatannins (Precipitate Test):

Two millilitres of the extract were heated with 1 millilitre of 1% aqueous hydrochloric acid. The formation of a red precipitate indicated the presence of phlobatannins.

2.9.Test for Amino Acids and Proteins (Ninhydrin Test):

Two to five drops of 1% ninhydrin solution (prepared in acetone) were added to 2 millilitres of the filtrate. The mixture was then boiled for 1 to 2 minutes. The appearance of a purple colour confirmed the presence of amino acids or proteins.

2.10. Test for Saponins (Foam Test):

Two millilitres of the extract were mixed with 6 millilitres of water in a test tube and shaken vigorously. The formation of a stable, persistent froth indicated the presence of saponins.

2.11. Test for Sterols (Liebermann-Burchard Test):

To 1 millilitre of the extract, chloroform, acetic anhydride, and concentrated sulfuric acid were added. The development of a dark pink or red coloration indicated the presence of sterols.

2.12. Test for Tannins (Braymer's Test):

Two millilitres of the extract were treated with 10% alcoholic ferric chloride solution. The formation of a blue or greenish-coloured solution suggested the presence of tannins.

2.13. Test for Terpenoids (Salkowski's Test):

Two millilitres of the extract were mixed with 1 millilitre of chloroform, followed by the addition of a few drops of concentrated sulfuric acid. The appearance of a reddish-brown precipitate indicated the presence of terpenoids

2.14. **Test for Quinones:**

A small amount of the extract was treated with concentrated hydrochloric acid.

The formation of a yellow precipitate or coloration indicated the presence of quinones.

2.15. **Test for Oxalates:**

A few drops of glacial acetic acid were added to a 3-millilitre fraction of the extract. The appearance of a greenish-black colour indicated the presence of oxalates.

2.16. **Test for Fats and Oils:**

Five drops of the sample were mixed with 1 millilitre of 1% copper sulfate solution and a few drops of 10% sodium hydroxide. The development of a clear blue solution confirmed the presence of fats and oils.

2.17. **In Vitro Antibacterial Assay:**

Extracts of *Ipomoea fistulosa* obtained using petroleum ether, chloroform, and methanol were tested for antibacterial activity against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*.

2.18. Culture Preparation

Luria Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) 30mL was prepared in 3 respective flasks by adding Tryptone 0.3g, Sodium chloride 0.3g, Yeast extract 0.18g, Distilled water 30mL and autoclaved at 121°C for 15 minutes. Later, *E. coli* (MTCC 433), *S. typhi* (MTCC 735), and *S. aureus* (MTCC 96) strains were inoculated respectively in 30mL of sterilized LB broth flasks and incubated for 24h at 37˚ C. 24h Cultured organisms (*E. coli*, *S. typhi*, and *S. aureus*) were centrifuged at 6000rpm for 10 minutes respectively, the supernatant was discarded and the pellets were dissolved in 1% (w/v) Sodium chloride and adjusted to absorbance 1.000 at 600nm under UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer).

2.19. Sample Preparation

Sample (*Ipomoea fistulosa*) 10mg were dissolved in 1mL of Dimethyl sulfoxide (DMSO) respectively. Various sample concentrations were created by the sample were prepared by diluting samples to obtain 1mg, 0.1, and 0.01mg, and the final volume was made up to 50µL by adding DMSO.

2.20. Standard Preparation

Tetracycline 10mg was dissolved in 1mL of DMSO 100µg was prepared by pipetting 10µL and the final volume was made up to 50µL by adding DMSO.

2.21. Antibacterial assay of plant extract by agar well diffusion method

Approximately 25mL of the media was poured into the sterilized Petri plates and allowed to solidify. 200µL prepared inoculum (*E. coli, S. typhi*, and *S.aureus*) was poured respectively on agar plates and spread thoroughly using a plate spreader. 5 wells measuring 0.6cm were made in each plate using the borer and 50µL of prepared sample and Standard was loaded into the respective plate wells, the plates were incubated at 37°C for 24h. Later, the diameter of the zone of inhibition was recorded in mm (Millimetre).

3. RESULT AND DISCUSSION

3.1. Phytochemical analysis

As shown in the table 1, The phytochemical investigation of bulk quantities of *Ipomoea fistulosa.* Phytochemical screening carried out with fresh *Ipomoea fistulosa* has revealed the presence of many secondary metabolites which in turn contributes to its antibacterial activity.

Tests	*Ipomoea fistulosa*		
	Petroleum Ether	Chloroform	Methanol
Alkaloids	-	+	+
Carbohydrates	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	-
Phenols	-	+	+
Phlobatannins	-	-	-
Amino acids and proteins	-	-	-
Saponins	-	-	-
Sterols	-	-	-
Tannins	+	+	+
Terpenoids	-	-	-
Quinones	+	-	+
Oxalates	-	+	-
Fats and oils	-	-	-

Table 1: Preliminary Phytochemical analysis of *Ipomoea fistulosa* crude extracts

While secondary metabolites like alkaloids, phenols, phlobatannins, amino acids and proteins, saponins, sterols, terpenoids, oxalates, fats, and oils were found to be absent, the preliminary qualitative phytochemical screening of the whole plant extracts of Ipomoea fistulosa in the petroleum ether extract revealed the presence of carbohydrates, cardiac glycosides, flavonoids, tannins, and quinones. Alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, and oxalates were also present in the whole plant extract of Ipomoea fistulosa that was obtained in chloroform extract. In contrast, it was discovered that secondary metabolites such as phlobatannins, proteins, amino acids, terpenoids, saponins, quinones, fats and oils were missing. Similarly, metabolites of whole plant extracts obtained in the methanolic extract revealed the absence of flavonoids, phlobatannins, amino acids and proteins, saponins, sterols, terpenoids, oxalates, fats, and oils and the presence of alkaloids, carbohydrates, cardiac glycosides, phenols, tannins, and quinones.Among the three solvent extracts of whole plant extracts the major secondary metabolites were observed in chloroform extraction then the methanolic extraction and petroleum ether.

Solvent extract in whole plant extracts, alkaloids, and phenols, were found in chloroform and methanolic extract except petroleum ether extract. while carbohydrates, cardiac glycosides, and tannins, were discovered to be prevalent in all three solvent extracts. Flavonoids are found in petroleum ether extract and chloroform extract except methanol extract. Phlobatannins and amino acids, proteins, saponins, sterols, terpenoids, fats, and oils were found to be completely absent in all three solvents. Quinones are found in petroleum ether and methanol extract except chloroform extract. Whereas oxalates were present in chloroform extract, except petroleum ether and methanolic extract.

It is imperative that plants undergo preliminary phytochemical screening in order to find and create novel medicinal compounds with increased efficacy. These kinds of investigations have also been reported globally by other study organizations (Dasgupta et al., 2013; Raphael, 2012).

3.2.Extract the phytochemicals with solvents by Soxhlet apparatus.

Our current investigation showed that three solvents (petroleum ether, chloroform, and methanol) to extract the phytochemicals from the experimental plant (*Ipomoea fistulosa*). The yield of the raw materials in different solvents has been tabulated (Table. 2).

Plant materials (whole plant extract-50gm in 200ml of solvent)		
Sl. No.	**Solvent**	**Yield (gm)**
		Ipomoea fistulosa
1	Petroleum ether	2.76
2	Chloroform	1.32
3	Methanol	3.17

Table 2: Yield of raw plants materials in different solvents

The antibacterial potential of *Ipomoea fistulosa* crude extract against bacteria.

The findings of the antibacterial activity test for the solvent extracts of *Ipomoea fistulosa* against *Salmonella typhi, Escherichia coli,* and *Staphylococcus aureus* were inconsistent. Tables 3, 4, and 5 provide information regarding the plant

extracts' antibacterial capacity. Tetracycline is the standard drug used in this test; it serves as the positive control and 10% DMSO serves as the negative control.

Extract	Zone of Inhibition (in mm) Test Organism- *Staphylococcus aureus*		
	1mg	0.1mg	0.01mg
Petroleum ether	-	-	-
Chloroform	-	-	-
Methanol	-	-	-

Table 3: Antibacterial activity of *Ipomoea fistulosa* against *Staphylococcus aureus* (gram +ve)

Figure 1. Antibacterial activity of *Ipomoea fistulosa* extracts against *Staphylococcus aureus* (Gram-positive). Petroleum ether, chloroform, and methanol extracts were tested using the in vitro agar well diffusion method. Zones of inhibition were observed to evaluate the antibacterial potential of each extract.

There is no inhibition zone seen in the current study of *Ipomoea fistulosa* antibacterial activity against *Staphylococcus aureus*.

Extract	Zone of Inhibition (in mm) Test Organism- *Salmonella typhi*		
	1mg	0.1mg	0.01mg
Petroleum ether	14	10	-
Chloroform	11	-	-
Methanol	12	-	-

Table 4: Antibacterial activity of *Ipomoea fistulosa* against *Salmonella typhi* (Gram -ve)

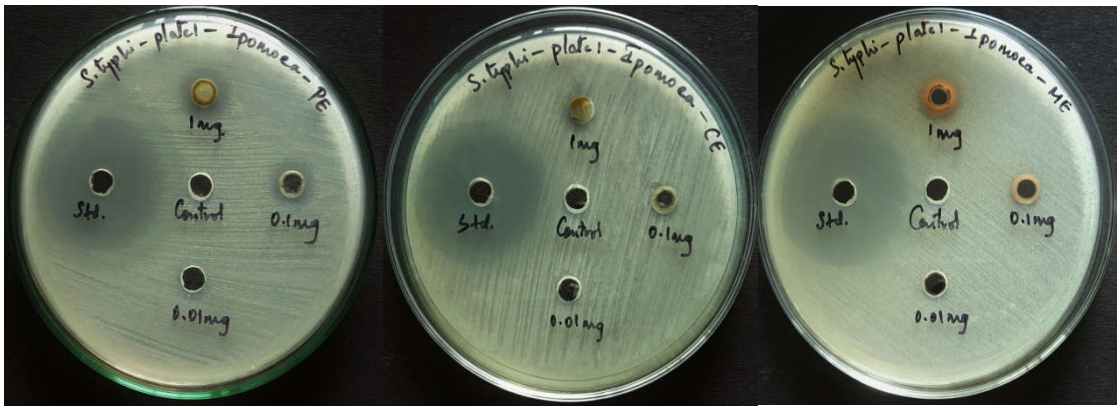

Figure 2. Antibacterial activity of *Ipomoea fistulosa* extracts against *Salmonella typhi* (Gram-negative). Petroleum ether, chloroform, and methanol extracts were tested using the in vitro agar well diffusion method. The formation of zones of inhibition indicated the antibacterial effect of each extract.

The present investigation of antibacterial activity of *Ipomoea fistulosa* against *Salmonella typhi* whereas the present research work revealed that, petroleum ether extract showed at 14mm of 1 mg concentration, 10 mm of 0.1 mg concentration, methanol extract showed at 12 mm of 1 mg concentration and chloroform extract showed at 11 mm of 1 mg concentration against *Salmonella typhi*. among the three solvent extracts, petroleum ether extracts reported significant antibacterial activity against *Salmonella typhi*, then the methanol and chloroform extract. However, methanolic extract showed the moderate activity, whereas, least activity was observed in chloroform extract.

Extract	**Zone of Inhibition (in mm) Test Organism- *Escherichia coli***		
	1mg	**0.1mg**	**0.01mg**
Petroleum ether	-	-	-
Chloroform	-	-	-
Methanol	-	-	-

Table 5: Antibacterial activity of *Ipomoea fistulosa* against *Escherichia coli* (Gram -ve)

Figure 3. Antibacterial activity of *Ipomoea fistulosa* extracts against *Escherichia coli* (Gram-negative). Petroleum ether, chloroform, and methanol extracts were evaluated using the in vitro agar well diffusion method. Zones of inhibition were measured to determine the antibacterial effectiveness of each extract.

There is no inhibitory zone visible in the current study on *Ipomoea fistulosa* antibacterial efficacy against *Escherichia coli*. *Ipomoea aquatica* has antimicrobial and anti-inflammatory effects which provide pharmacological evidence for folk uses of *I. aquatica.* Humans use *Ipomoea* for their content of medical and psychoactive compounds, mainly alkaloids.The genus contains food crops; two economically significant food items are the water spinach leaves and the sweet potato tubers. It was found that flavonoids were responsible for the antimicrobial activity of *I. aquatica.*

Conclusion

Phytochemical screening is essential for detecting the presence of bioactive compounds in plant extracts. In the present study, preliminary qualitative analysis of whole plant extracts of *Ipomoea fistulosa* revealed a diverse range of phytoconstituents. The petroleum ether extract indicated the presence of carbohydrates, cardiac glycosides, flavonoids, tannins, and quinones. The chloroform extract tested positive for alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, and oxalates. Similarly, the methanol extract contained alkaloids, carbohydrates, cardiac glycosides, phenols, tannins, and quinones, highlighting the rich phytochemical profile of the plant.

The antibacterial activity of the petroleum ether, chloroform, and methanol extracts of *Ipomoea fistulosa* was evaluated using the agar well diffusion method against three bacterial strains: *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. The results showed that the zones of inhibition varied across the bacterial species and types of extracts. Among the tested extracts, the methanol and petroleum ether extracts demonstrated comparatively higher antibacterial activity, suggesting that these solvents were more effective in extracting antimicrobial compounds from the plant.

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CONFLICT OF INTEREST
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