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Phytochemical Evaluation and Antibacterial Activity of *Limonia Acidissima* Fruit Shell Extract

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Abstract

In ancient historical time onwards the medicinal plants play a vital role in maintaining human health in good condition. Phytochemicals are most important biologically active compounds found in various fruits, vegetables, grains, beans and numerous plant materials which can be used in drug development. The traditional medicinal plant *Limonia acidissima* has several pharmacological activities and various parts of these plants were used for the ailment of various disease. The main objective of the present study is to evaluate the phytochemicals present in the aqueous extract of the *Limonia acidissima* fruit shell and study their antibacterial activity. Aqueous extract of *Limonia acidissima* fruit shell showed the presence of phytoconstituents - Carbohydrates, Flavonoids and Anthraquinones. Antibacterial activity was assessed by agar well diffusion method against both Gram Positive and Gram Negative pathogenic bacterial strains. The antibacterial activity results showed the highest inhibitory effect against gram negative bacteria - *Klebsiella pneumoniae* and *Salmonella typhi*. The lowest zone of inhibition was observed against gram positive bacteria - *Staphylococcus aureus*. These findings suggest that aqueous extract of *Limonia acidissima* fruit shell has complimentary potential to treat various bacterial infectious diseases. Therefore this plant could be an important source of new antimicrobial compounds to treat the diseases.

Keywords: *Limonia acidissima*, Fruit Shell, Aqueous extract, Phytochemicals, Antibacterial activity.

1 Introduction

The World Health Organization (WHO) estimates that 80% of the world's population depends on medicinal plants for their primary health care [1]. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs [2]. *Limonia Acidissima L.* is globally distributed and in India, it is cultivated for its fruits throughout the plains of India [3]. The present study was aimed to screen preliminary phytochemicals present in the aqueous extract of the *Limonia acidissima* fruit shell and to study their Antibacterial activity.

2 Experimental

2.1 Collection of Limonia Acidissima Fruit Shell

The Limonia acidissima fruit was brought from local Tirupattur market and its shell was used as sample for phytochemical analysis and antibacterial activity.

2.2 Preparation of Aqueous Extract

Limonia acidissima fruit shell extract was washed thoroughly with double distilled water and dried under sunshade for one week. Then it would be grained as a fine powder.10 g of Limonia acidissima fruit shell powder was boiled with 50 ml of double distilled water for 60 minutes at 100 º C and it was cooled and filtrated. Then the filtrate would be stored at 4ºC for about 1 week and could be utilized for further studies.

2.3 Phytochemical Evaluation

2.3.1 Test for Alkaloids

2.3.1.1 Mayer's Test- To a few ml of extract and added few drops of Mayer's reagent. Appearance of white creamy precipitate expresses the presence of alkaloids.

2.3.1.2 Wagner's Test- To few drops of Wagner's reagent and added few drops of extract. A reddish brown precipitate expresses the presence of alkaloids.

2.3.1.3 Hager's Test- Few ml of extract is allowed to react with few drops of Hager's reagent. An orange precipitate expresses the presence of alkaloids.

2.3.2 Test for Amino Acids

2.3.2.1 Ninhydrin Test- To a few ml of extract added an equal quantity of Ninhydrin reagent and heated in a boiling water bath. The violet color expresses the presence of amino acids.

2.3.3 Test for Carbohydrates

2.3.3.1 Molish's Test- To two ml of extract and added two ml Molish's reagent. Then slowly added a sulphuric acid on one side and appearance of violet ring shows the presence

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of carbohydrate.

2.3.3.2 Benedict's Test- To 1ml of extract added 1 ml of Benedict's reagent and then heated for two minutes. The orange red precipitate indicates the presence of carbohydrate.

2.3.3.3 Fehling's Test- To 2ml of extract added few drops of Fehling's A and B reagent and heated for two minutes. A red color precipitate indicates the presence of carbohydrate.

2.3.4 Test for Fixed Oil and Fats

2.3.4.1 Saponification Test- Few ml of 0.5N alcoholic potassium hydroxide was added to few ml of extract along the tube and added a drop of phenolphthalein. Then heated in boiling water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

2.3.5 Test for Phenols and Tannins

2.3.5.1 Ferric Chloride Test - To 5 ml of extract added few drops of 5% ferric chloride solution and formation of red color indicates the presence of phenols.

2.3.5.2 Lead Acetate Test - To 3ml of extract added 1ml of lead acetate and formation of white precipitate indicates the presence of phenol.

2.3.5.3 Gelatin Test - To 5ml of extract added 2ml of 5% gelatin solution containing 10% NaCl. Formation of white precipitate indicates the presence of phenol.

2.3.5.4 Braemer's Test - To 2ml of extract added 10% alcoholi ferric chloride. Formation of dark blue or greenish grey color indicates the presence of phenol.

2.3.6 Test for Proteins

2.3.6.1 Millons Test - To 3ml of extract added a few drops of Millon's reagent and formation of red color indicates the presence of peptide bonds.

2.3.6.2 Biuret Test- To 2ml of extract added 2 drops of 2% copper sulphate and potassium hydroxide pellets. Formation of violet color indicates the presence of Proteins.

2.3.7 Test for Saponins

2.3.7.1 Foam Test- 1ml of extract was diluted with 20 ml of distilled water and shaken for 15 minutes. 1cm layer of foam formation indicates the presence of Saponins.

2.3.8 Test for Gum and Muicilages

To 10 ml of extract added 2 ml of alcohol with constant stirring. A white or cloudy precipitate indicates the presence of gums and Mucilages.

2.3.9 Test for Flavonoids

2.3.9.1 Alkaline Reagent Test - To 2ml of extract added few drops of NaOH. Yellow color formation indicates the presence of Flavonoids.

2.3.10 Test for Anthraquinones

2.3.10.1 Borntrager's Test- 5 ml of extract was heated with 10% ferric chloride solution and added 1 ml Conc. HCl. Extract. It was cooled and filtrated. The filtrate was shaken with diethyl ether. Formation of pink or red color indicates the presence of Anthraquinones.

2.4 Antibacterial Activity

2.4.1 Sample Preparation

The aqueous fruit shell extract of Limonia acidissima is prepared and filtered. Then the filtrate was kept under hot air oven at 100 º C for 1 hour and the fruit shell powder was dissolved in DMSO for antibacterial study.

2.4.2 Culture of Pathogens

Three clinical bacterial pathogens were collected which includes Salmonella typhi, Klebsiella pneumoniae and Staphylococcus aureus.

2.4.3 Determination of Antibacterial Activity of Aqueous Extract

Antibacterial activity of fruit shell extract was assessed by agar well diffusion method. Muller Hinton agar medium was prepared and sterilized. After sterilization the media would be poured into plates. After solidification of agar plates, bacterial strains were swabbed on the surface of the agar medium. Three different bacterial strains were cultured in four separate culture plate. Totally five wells have been made in each culture plate, four wells were loaded with fruit shell extract at various concentrations such as 50µl, 100µl, 150µl, 200µl and fifth well was loaded with 100µl of DMSO. After 24 hours incubation period plates were observed for antibacterial activity by calculating the zone of inhibition around the wells as diameter in mm.

3 Results and Discussion

3.1 Phytochemical Evaluation

Phytochemicals are plant secondary metabolites which give protection to plant from environmental stress. Phytochemical possess various important pharmacological properties. The presence of Anthraquinones was confirmed by Borntrager's test. Carbohydrates presence was confirmed by Molish test, Benedict's test and Fehling's test. Limonia acidissima fruit shell extract gives positive result for Alkaline Reagent test and thereby confirms the presence of flavonoids.

Alkaloids, Amino acids, Fixed oil and Fats, Proteins, Saponins, Phenols, Tannins, Gum and Mucilage phytochemicals are found to be absent in the Limonia acidissima fruit shell extract. The presence of various phytoingredients may provide the several pharmacological activity of the Limonia acidissima fruit shell extract.

The Carcia papaya peel extract of different solvents Aqueous, Acetone, Petroleum ether, hexane, ethanol showed the presence of compounds alkaloids, flavonoids, Quinones, Saponins, glycoside, Coumarins, Terpenes [4].

Phytochemical screening of aqueous extract of Garlic (*Allium sativum*) bulbs showed the presence of phytochemicals alkaloids, flavonoids, Saponins, tannins and cardiac glycosides [5].

Table: 1 Phytochemical evaluation of aqueous extract of *Limonia acidissima* fruit shell

S.NO	Phytochemicals	Tests	Result
1.	Alkaloids	Mayer's Wagner's Hager's	_ _ _
2.	Aminoacids	Ninhydrin	_
3.	Anthraquinones	Borntrag-er's	+
4.	Carbohydrate	Molish's Benedict's Fehling's	+ + +
5.	Fixed Oils and Fat	Saponifica-tion	_
6.	Flavonoids	Alkaline re-agent	+
7.	Phenols and Tan-nins	Ferric chlo-ride Gelatin Lead ace-tate Braemer's	_ _ _ _
8.	Protein	Millon's Biuret	_ _
9.	Saponins	Foam	_
10.	Gum and Muci-lage	Gum and muci-lage	_

(+) indicates Presence, (-) indicates Absence

Similar study was proved by Eneh *et al*., [6] on the bark of S*accharum officinerum* aqueous and ethanolic extract and the study shows the presence of phytochemicals fla-vonoids, carbohydrate, anthracene glycosides, tannins and Saponins.

Anumol *et al*., [7] analysed the phytochemical powder isolated from dorsal side of leaves of *Cocos nucifera* (Arecaceae) and showed the presence of flavonoids and tannins.

3.2 Antibacterial Activity

Many diseases are caused by pathogens. The activity of the pathogens could be decreased by using active components that are present in the plants. The antibacterial activity of *Limonia acidissima* fruit shell extract obtained was given the Figure 1.

The *Limonia acidissima* fruit shell extract was studied against three clinical pathogens - *Staphylococcus aureus,* a gram positive bacteria and *Klebsiella pneumonia and Salmonella typhi*, gram negative bacteria. The *Limonia acidissima* fruit shell extract shows zone of inhibition at the concentration level of 100µl, 150µl and 200µl against all the selected microorganisms. The antimicrobial activity of *Limonia acidissima* fruit shell may be due to the presence of bioactive compounds and it can be used to treat the clinical pathogens or microorganisms. The data obtained from this study shows that the fruit shell extract shows potent antibacterial activity at dose dependent manner. DMSO serves as negative control and did not show any zone of inhibition on the bacterial culture.

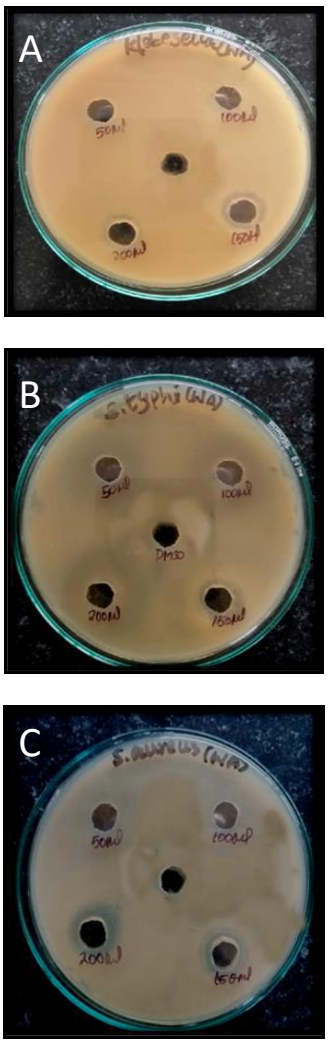

Fig.1. Antibacterial activity of aqueous extract of *Limonia acidissima* fruit shell against A. *Klebsiella pneumonia,* B. *Salmonella typhi* and C. *Staphylococcus aureus.*

The 150 µl of *Limonia acidissima* fruit shell extract shows highest zone of inhibition and lowest zone was formed at 100 µl concentrations against *Klebsiella pneumonia* and *Salmonella typhi Staphylococcus aureus* shows maximum zone of inhibition at 200 µl and lowest zone of inhibition at 100 µl. At 50 µl concentration of *Limonia acidissima* fruit shell extract shows no activity against all the three microorganisms.

Nada *et al.,* [8] in their studied showed the antimicrobial activity of aqueous lemon peel extract. The aqueous extract of lemon peel showed the activity against *Klebsiella pneumoniae, E. aerogens, Enterococcus faecalis* and *M. catarrhalis*.

Table 2. Antibacterial activity of *Limonia acidissima* fruit shell

S. No	ORGANISMS	DMSO	CONCENTRATION OF EXTRACT			
			50µl	100µl	150µl	200µl
			Zone of inhibition (diameter in mm)			
1	*Klebsiella pneumonia*	NIL	NIL	9	12	10
2	*Salmonella typhi*	NIL	NIL	8	13	9
3	*Staphylococcus aureus*	NIL	NIL	8	18	20

Antimicrobial activity of ginger extract against food-borne pathogenic bacteria was studied by Kamrul *et al.,* [9]. Soyabean oil extract of ginger showed activity against *Salmonella* species. Lowest zone of inhibition was showed against *Escherichia coli.*

4 Conclusions

The aqueous *Limonia acidissima* fruit shell extract possess various biologically important plant secondary metabolites. Phytochemicals serve as nutritional supplements and other pharmacological industries for preparing new drugs and health care products. These secondary metabolites may responsible for the antibacterial activity of the fruit shell extract. In the phytochemical study, Anthraquinones, Carbohydrate and Flavonoids were found to be present in aqueous extract of *Limonia Acidissima* Fruit shell. These findings confirm that aqueous extract of *Limonia acidissima* fruit shell has potential to treat bacterial infectious diseases like Urinary tract infection, Meningitis and Respiratory disease. The gram bacterial infection lead to High Fever, Diarrheoa, vomiting etc., The gram positive bacteria generally causes a skin infection such as Pimples, Folliculitis, Scalded skin, Osteomyelitis, Sepsis etc., The *Limonia acidissima* fruit shell have the capability to treat both gram positive and gram negative bacteria causing disease. We hope that important phytochemicals are identified in aqueous extract of *Limonia acidissima* fruit shell and these phytochemicals it contains a pharmacological properties and helpful for treating diseases

Conflict of Interest:

All authors have no conflict of interest to report.

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