Comparative Study on the Phyllanthus Acidus and Phyllanthus Embilca and their Antimicrobial Activity

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ABSTRACT

The present research evaluates the comparative study on the*Phyllanthus acidus* and *Phyllanthus Emblica*and its antimicrobial activity of chloroform, methanol and aqueous extract of *Phyllanthus Emblica* and *Phyllanathus Acidus* against the organisms *staphylococcus aureus* (gram +) and *Escherichia coli* (gram -). The standard drug used is Amikacin.In the phytochemical investigationof*Phyllanthus Emblica* the phytochemical constituents present are carbohydrates, Flavonoids, Tannins, Pectin and Ascorbic acid. In *Phyllanthus Acidus.* The phytochemical constituents present are carbohydrates, Tannins and Ascorbic acid. The estimation of ascorbic acid is more in *Phyllanthus Emblica* than in *Phyllanathus Acidus.* The content of tanninsis more in *Phyllanthus Emblica* than *Phyllanathus Acidus.* The chloroform extract and methanolic extract shows significant antimicrobial activity. The antimicrobial activity is more in *Phyllanathus Acidus* than in*Phyllanthus Emblica*. Also, these plants have bioactive phytochemical compounds with potential medicinal values for the treatment of numerous infections. The present study showed the effectiveness of the plant extract against the tested bacterial strains and indicates the potential use of the extract as antimicrobial agent for the control of infectious diseases.

Key Words: *Phyllanthus acidus, Phyllanthus Emblica, Antimicrobial action, staphylococcus aureus (gram +), Escherichia coli (gram -)*

eIJPPR 2022; 12(1):1-6

HOW TO CITE THIS ARTICLE: Jose EJB, Azariah JS, Radhakrishnan M, Rajagopal K, Sundaramoorthy M, Pillai NP. Comparative Study on the Phyllanthus Acidus and Phyllanthus Embilca and their Antimicrobial Activity. Int J Pharm Phytopharmacol Res. 2022;12(1):1-6. https://doi.org/10.51847/9CSOk0twSN

INTRODUCTION

Phyllanthus acidus, isa plant belongingto the Euphorbiaceae family. It is generallyrecognizedas the Country gooseberry. It is usuallycultivated for ornamentation and itslocalname is Arenelli (Tamil). The fruits of *P. acidus*are collected from different agroclimatic zones of Tamilnadu. The plant is an intermediary between trees and shrubs with edible small yellow berries fruits. Varioussignificantchemical constituents are found in this plant. The plant has medicinal usage, too. The peppered leaves are utilizedto make a poultice to treat sciatica, lumbago and rheumatism (but have been observed to cause low blood pressure when combined with nitrates), At the same time, the seeds are used as a cathartic and the root, if prepared with care, as a purgative. The syrup is used to medicate the stomach, and in India the fruit is usedas a blood-enhancer for the liver. *P. acidus* contains 4-hydroxybenzoic acid, kaempferol,adenosine, caffeic acid, and hypogallic acid. The medicinal activities of Phyllanthusspecies are antipyretic, analgesic, anti-inflammatory, anti-hepatotoxicand antiviral [1-5].

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Received: 10 November 2021; **Revised:** 12 January 2022; **Accepted:** 15 January 2022

Phyllanthus Emblica Linn. (syn. *Emblica Officinalis*), usuallyknown as Indian gooseberry or amla, family Euphorbiaceae, is significant herbal drug used in Unani (Grac- Arab) and ayurvedic systems of medicine. Phyllanthus Emblica is highly nutritious and could be a significant dietary source of minerals, vitamin C, and amino acids. The plant contains emblicol, tannins, phyllembelin, phyllembelic acid, rutin, curcuminoids, and phenolic compounds. All parts of the plant are consumedfor medicinal purposes, especially the fruit, which has been used in Ayurveda as a potent Rasayana and traditional medicine to treat inflammation, jaundice, and diarrhea. Different plant parts have chemopreventive, antiulcerogenic, hypolipidemic, antioxidant, hepatoprotective, antidiabetic, gastroprotective, and antibacterialproperties. *Phyllanthus Emblica*is a maningredient in many Ayurvedic preparationssuch as Chyawanprash and Triphala. The present study intended to investigate the comparative study on estimating ascorbic acid and tannins in *Phyllanthus acidus* and *Phyllanthus Emblica*and their antimicrobial activity [6-12].

MATERIALS AND METHODS

The fruits were collected from thecentral market of Madurai city. All the informations about these two spiceswas collected from Madurai Medical College, Madurai, Al Ameencollege of pharmacy, Bangalore and American college, Madurai.

Materials used

The materialsused were soxhlet apparatus. Chloroform, methanol, and distilled water and shade dried fruits of *Phyllanthus Emblica* and *Phyllanathus Acidus.*

Purification of solvent

The various solvents Chloroform, methanol and distilled water, were purified as follows:

Chloroform

Chloroform was shaken with an equal volume of distilled water twice to remove water-soluble impurities and separated using a separating funnel. It was then dried again with anhydrous potassium carbonate for another 24 hours. It was then dried again with anhydrous potassium carbonate for another 24 hours. It was decanted and distilledthe fraction boiling high at 60°c was collected and stored in a dark brown bottle. Absolute alcohol was added as a preservative.

Methanol

Methanol was time distilled and used.

Water

Distilled water was used for water extraction.

Isolation of ascorbic acidfrom phyllanthus emblica and phyllanathus acidus

The fully ripe fruits (1 kg) were pulped and pressed and the juice (450 ml) was treated with lead acetate. 330 ml and asmall quantity of formic acid. The impurities were present in the precipitate and the ascorbic acid was present in the solution. After separation, the solution was again treated with lead acetate and ammonia was added to make it alkaline as to phenolphthalein. This timethe precipitate was isolated by centrifuging at 5000 rpm for 5 minutes. It was decomposed by adding a sufficient amount of hydrochloric acid (125ml) and the lead chloride was filtered off and washed to recover all ascorbic acid. The filtrate was concentrated rapidly under reduced pressure (150 vapor pressure at 35°c) to a syrupy liquid containing not more than20 % water. This 120 ml was extracted repeatedly with an excess of acetone (50ml). The acetone solution in which most of the ascorbic acid present was evaporated to a sticky syrup liquid 100 ml under reduced pressure (150 vapor pressure at 35°c) and again extracted with dry acetone 15 ml and then treated with butyl alcohol 20 ml and the remaining acetone was removed under reduced pressure (150 vapor pressure at 35°c) for 1 hr. The slowly crystallized from 68ml butyl alcohol solution kept at 0°c for several days. The crystals were separated usingacentrifuge at 5000 rpm for 5 minuteswashed with acetone methanol mixture, and recrystallized from a mixture of methanol and dioxane (4:1). The total amount of ascorbic acid present was found to the 25%.

Estimation of ascorbic acid usingfresh amla fruit juice

About 100mg of the powdered sample of ascorbic acid was dissolved in 100 ml of recently boiled and cooled water. Then add 25 ml of dilute sulphuric acid, and the mixture was titrated against iodine solution taken in the burette using starch mucilage as an indicator. Each ml of N/10 iodine solution was equivalent to 0.008801 gm of ascorbic acid was calculated by the formula

Titre value*equivalent weight factor*strength of iodine *100/weight of the substance*100 (1)

Isolation of tannin from phyllanthus emblica and phyllanathus acidus

The fully ripe fruits were pulped and pressed, and the juice was treated with lead acetate and some formic acid. The precipitate consists of lead tannate. Collect the precipitate by filtration. Then the precipitate was treated with hydrochloric acidlead tannate was converted into tannic acid and lead chloride. Then the lead chloride was separated by filtration. The filtration was collected and concentrated by evaporation. Finally, is left as a residue.

ISSN (Online) 2249-6084 (Print) 2250-1029

Antimicrobial activity

The antimicrobial activity of chloroform, and aqueous extract of *Phyllanthus Emblica* and *Phyllanathus Acidus* against the *staphylococcus aureus* (gram +) *Escherichia coli* (gram -) was carried out by cup plate method. Casein acid hydrolysate, agar, beef extract, starch were added to distilled water and dissolved by heating and filtering. The pH was adjusted to 7.6±0.2. A clear solution was obtainedand it was sterilizedby autoclaving for 175°c for 15 minutes. The media was poured into the Petri plates, and test organisms were inoculated. The temperature of the media has beenmaintained. Four holes were drilled in the media. The two holes served as a extract for both the spices of *Phyllanthus Emblica* than *Phyllanathus Acidus.* Third hole served as a standard drug Amikacin and test used for the control. A uniform concentration of 1000µg/ml of the different extracts were found into the corresponding hole. After 24 hour of incubation of plates at 37 °c the zone of inhibition were measured in nm. The activity was compared with standard drug Amikacin and control. The antimicrobial activity is showed in **(Figures 1-6)**.

RESULTS AND DISCUSSION

Phytochemical investigation

In *Phyllanthus Emblica* the phytochemical constituents present are carbohydrates, Flavonoids, Tannins, Pectin, and Ascorbic acid. In *Phyllanthus Acidus*, the phytochemical constituents present are carbohydrates, Tannins and Ascorbic acid.

Estimation of ascorbic acid from phyllanthus emblica and phyllanathus acidus

Phyllanthus Emblica

S.NO	Weight of Ascorbic Acid	Burette reading Initial	Final	Volume of iodine	Indicator
1	0.098 gm	0ml	7.3ml	7.3ml	Starch mucilage

Percentage purity of ascorbic acid =7.3*0.008801*0.9754*100/0.0986=63.5%.

Phyllanthus Acidus

S.NO	Weight of Ascorbic Acid	Burette reading Initial	Final	Volume of iodine	Indicator
1	0.0975 gm	0ml	6.9ml	6.9ml	Starch mucilage

Percentage purity of ascorbic acid = 6.9*0.008806*0.9754*100/0.0975=60.78%.

Comparative Reportfor the Content of Ascorbic Acid

Variety	Amount of ascorbic acid Present in fresh amla juice	Percentage purity
Phyllanthus Emblica	0.550 gm	63.5%
Phyllanthus Acidus	0.100 gm	60.78%

From the above results the content of ascorbic acid is more in *Phyllanthus Emblica* than in *Phyllanathus.*

Isolation of tannin from phyllanthus emblica and phyllanathus acidus

The amount of tannin obtained from *Phyllanthus Emblica* =4.1 gm

The amount of tannin obtained from *Phyllanathus Acidus*=2.7gm

The content of tannin is more in *Phyllanthus Emblica* than *Phyllanathus Acidus.*

Antimicrobial activity

The species of *Phyllanthus Emblica* and *Phyllanathus Acidus* have antimicrobial activity. The chloroform extract and methanolic extract show significant antimicrobial activity **(Figures 1, 3, 4 and 6)**. The antimicrobial activity is more in *Phyllanathus Acidus* than in *Phyllanthus Emblica* **(Tables 1 and 2)**.

Table 1. Extract of *Phyllanthus Emblica* and *Phyllanthus Acidus* against *staphylococcus aureus* (gram +)

Extract	Cup plate method	Zone of inhibition *P.E* and *P.A*
Chloroform extract	Chloroform	8mm8mm
	Substance	- 8mm
	Positive control	20mm 20mm

Methanolic extract	Methanol Substance Positive control	8mm 8mm 24mm26mm 20mm20mm
Aqueous extract	Distilled water Substance Positive control	- - 8mm 7mm 14mm 14mm
Medium	Muller Hinton Agar	

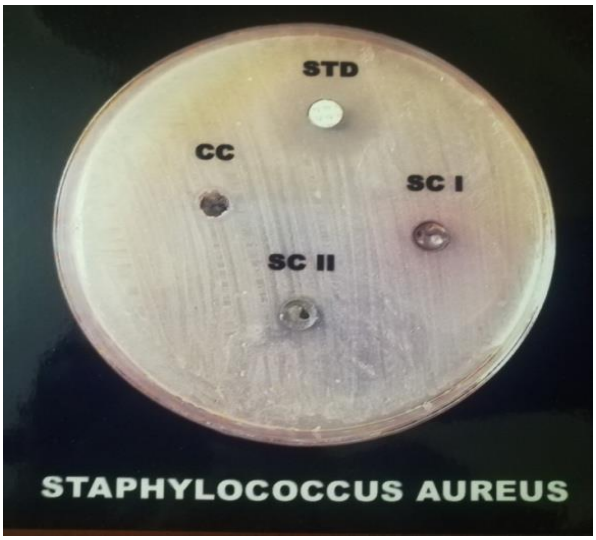

Figure 1. Chloroform extract of *Phyllanthus Emblica* and *Phyllanthus Acidus* against *staphylococcus aureus* (gram +)

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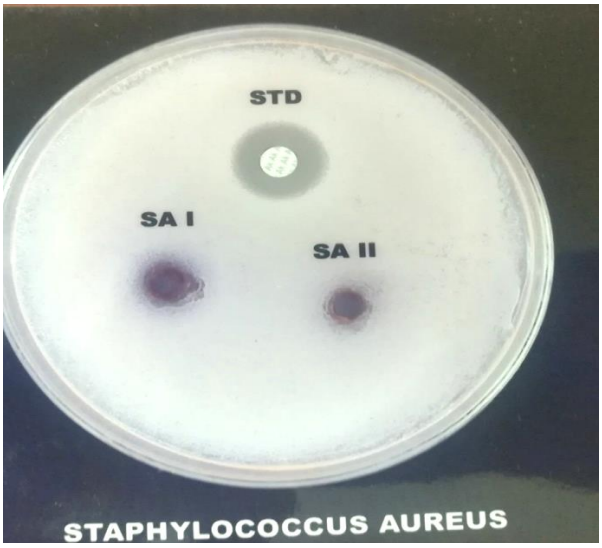

Figure 2. Aqueous extract of *Phyllanthus Emblica* and *Phyllanthus Acidus* against *staphylococcus aureus* (gram +)

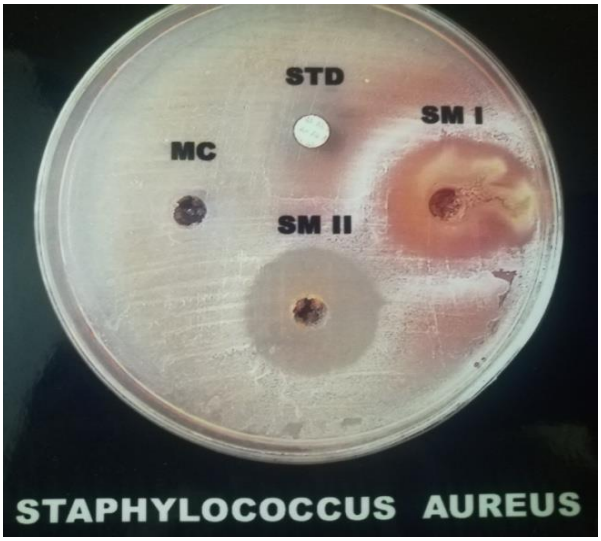

Figure 3. Methanol extract of *Phyllanthus Emblica* and *Phyllanthus Acidus* against *staphylococcus aureus* (gram +)

Table 2. Extract of *Phyllanthus Emblica*and *Phyllanthus Acidus*against *Escherichia coli* (gram -)

Extract	**Cup plate method**	**Zone of inhibition** *P.E* **and** *P.A*
Chloroform extract	Chloroform Substance Positive control	15mm 15mm 14mm 13mm 20mm20mm
Methanolic extract	Methanol Substance Positive control	8mm8mm 18mm 17mm 20mm20mm
Aqueous extract	Distilled water Substance Positive control	- 12mm - 14mm14mm 11mm
Medium	Muller Hinton Agar	

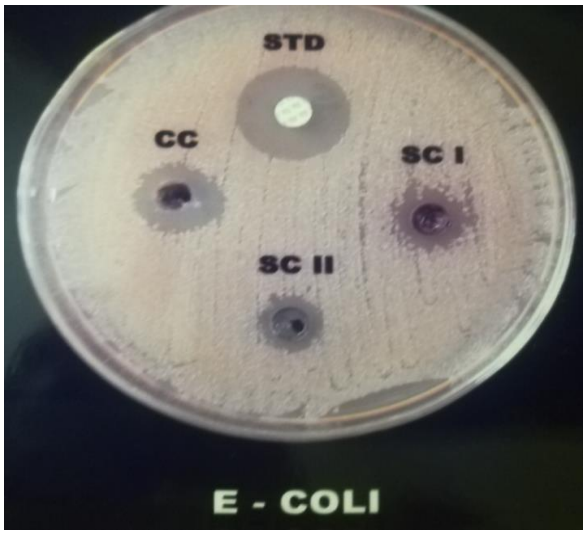

Figure 4. Chloroform extract of *Phyllanthus Emblica* and *Phyllanthus Acidus* against *Escherichia coli* (gram -)

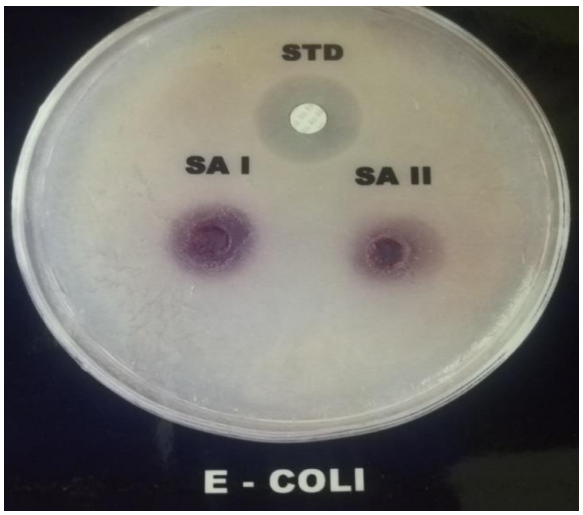

Figure 5. Aqueous extract of *Phyllanthus Emblica* and *Phyllanthus Acidus* against *Escherichia coli* (gram -)

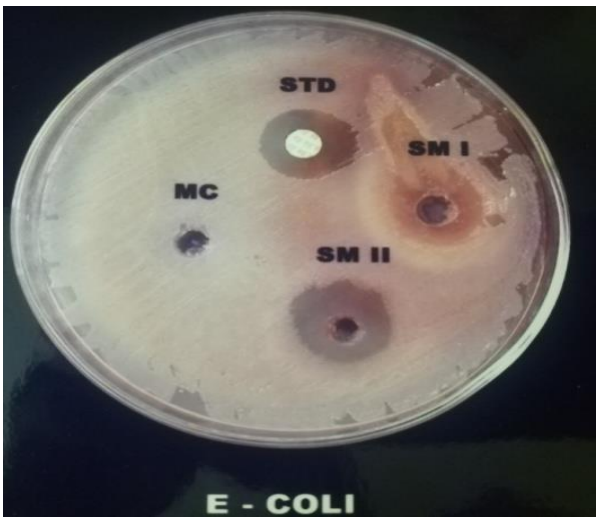

Figure 6. Methanol extract of *Phyllanthus Emblica* and *Phyllanthus Acidus* against *Escherichia coli* (gram -)

CONCLUSION

In the present study, antimicrobial activity of *Phyllanthus Emblica* and *Phyllanathus Acidus* was compared. The chloroform extract and methanolic extract show significant antimicrobial activity. From this result the antimicrobial activity is more in *Phyllanathus Acidus* than in *Phyllanthus Emblica.* Further research on the phytochemicals responsible for this activity can result in formulation of an antibacterial agent. Therefore, this study recommends the use of plantsforantimicrobial and antioxidant resources.

Acknowledgments: We express deep thanks to for providing pathogenic strains from Bose laboratory. Our sincere thanks to the searcher for his practical help.

Conflict of interest: None

Financial support: None

Ethics statement: None

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