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Shreyasi PradhanDepartment of Zoology, Khejuri
College, Khejuri, West Bengal,
India**Subhabrata Goswami**Department of Microbiology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India**Satyabrata Manna**Department of Microbiology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India**Tapati Adak**Department of Microbiology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India**Ajit Pradhan**Department of Microbiology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India**Sumana Dolui**Department of Microbiology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India**Gora Chand Ghosh**Department of Microbiology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India**Somnath De**Department of Biotechnology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India**Corresponding Author:****Somnath De**Department of Biotechnology,
Panskura Banamali College
(Autonomous), Panskura, West
Bengal, India

Phytochemical Analysis of *Citrullus colocynthis* (L.) Schrad and study of its antibacterial activity *in vitro*

Shreyasi Pradhan, Subhabrata Goswami, Satyabrata Manna, Tapati Adak, Ajit Pradhan, Sumana Dolui, Gora Chand Ghosh and Somnath De

Abstract

Citrullus colocynthis (L.) an important herbal to the tribal not founded as vivid day up till now. Except few fragmentary findings no investigation in respect to phytochemical screening. And antibacterial activity of the present day has not been done keeping this point in mind phytochemical screening and anti-bacterial activity recently done against four bacteria (*S. typhi*, *P. aeruginosa*, *K. pneumoniae*, *B. subtilis*) using Acetone, Ethanol, and Aqueous as solvent extracts. Flavonoids as marker of phytochemicals identified for best results where Acetone extracts for *B. subtilis* and Ethanol extract for *P. aeruginosa* showed excellent results rather than extract in aqueous solution.

Keywords: *Citrullus colocynthis* (L.), phytochemical analysis, antibacterial activity

Introduction

Worldwide, the use of fruit and their extracts for disease prevention and treatment has a long history and is becoming more prevalent [1-4]. Medicinal fruits have therapeutic qualities because of secondary metabolites which are a collection of complex chemical compounds with varied compositions [5, 6]. All plant parts, including the bark, leaves, flowers, roots, fruits, and seeds, can be used to make plant-based medicines [6, 7]. Alkaloids, glycosides, flavonoids, saponins, tannins, sugars, and essential oils are only a few of the secondary metabolites that are categorized. These organic substances give rise to distinct physiological effects on the human body. *Citrullus colocynthis* is a desert plant that contains a variety of bioactive substances, including fatty acids, glycosides, flavonoids, alkaloids, and glycoside glycosides. Plant medicines strengthen the immune system. The dried fruit pulp of *C. colocynthis* has been used to cure intestinal parasites as well as gastrointestinal diseases like indigestion and gastroenteritis. Excellent pharmacological qualities of *C. colocynthis* include laxative and purgative effects, as well as anti-diabetic, anti-inflammatory, anthelmintic, and anti-cancerous effects. The fruit's antibacterial, antioxidant, and anti-inflammatory properties have been the subject of much research [8]. Antioxidant, anti-inflammatory, anti-diabetic, and antibacterial properties are some of its therapeutic traits [9]. Its pharmacological characteristics include antioxidant, hypoglycemic, antibacterial, anti-cancerous, anti-inflammatory, analgesic, antidiabetic, profibrinolytic, antiallergic, antimicrobial, pesticidal, and immune-stimulating actions. It also has an impact on fertility and the reproductive system [10]. The natural insecticide

C. colocynthis is gaining popularity and has been tested for effectiveness against numerous economically significant pest species. It has been recommended as an effective insecticide to safeguard the ecology and enhance general well-being. Numerous researches has demonstrated the effectiveness of *C. colocynthis* and other plants as potent insecticides, but only a small number have demonstrated the nematocidal qualities of *C. colocynthis* and urged that their total efficacy be assessed in order to protect the environment [11]. By employing a variety of solvents, the current phytochemical study on *Citrullus colocynthis* was conducted to identify the presence of diverse compositions of components such alkaloids, glycosides, flavonoids, phenols, steroids, and tannins in leaf, stem, tendril, root, fruit peel, fruit pulp, and seeds. We want to expand the applications of *C. colocynthis* and raise scientists' and veterinarians' understanding of the advantages of this plant for human and poultry health.

Taxonomic account of *Citrullus colocynthis* (Linnaeus) Schrader

- Kingdom - Plantae.
- Sub kingdom - Tracheobionta.
- Super division - Spermatophyta.
- Division - Magnoliophyta.
- Class - Magnoliopsida.
- Sub- class - Dilleniidae.
- Order - Cucurbitales.
- Family - Cucurbitaceae.
- Genus - *Citrullus*.
- Species epithet - *Colocynthis* (L.) Schrad.



Fig 1: Unripe and ripped fruit of *C. colocynthis* in field

Materials and Methods

Selection of fruit material

Most of the villagers and tribal people used the *Citrullus colocynthis* (L.) Schrad for their common diseases regularly. On that context we have chosen the material as experimental tool. It is collected in the month of September, 2022 from Panskura, Purba Medinipur district (Latitude- N 22° 23'44.26728", Longitude- E 87° 44'23.4078", Altitude- 7 meters from mean sea level), West Bengal, India and it is not available in any season of year.

Fruit material extraction process

After surface sterilization of whole fruit materials of *Citrullus colocynthis* (L.) Schrad cut into small pieces, kept it few days for drying under shade. Then the air-dried fruit material grinded into powder. The powdered material was extracted with acetone, ethanol and aqueous using Soxhlet apparatus. About 10 grams of powder was loaded in Soxhlet extraction unit and exhaustively extracted using 100 ml of solvents such as acetone, ethanol and aqueous respectively at 60 °C for 12 hours. Thereafter, it was filtered with the help of Whatman No.1 filter paper and use for various phytochemical analysis.

Bacterial strain and culture condition

Three Gram Negative and one Gram positive indicator bacteria used for antibacterial assay respectively *Klebsiella pneumonia* (MTCC 109), *Salmonella typhi* (MTCC 890), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 3541) were provided by National Institute of Cholera and Enteric Diseases, Department of Health Research, Ministry of Health and Family Welfare, Govt. of India.

Phytochemical Screening

Phytochemical analysis of the test sample was carried out according to standard methods [12, 13].

Test for Alkaloids

The alkaloids test was performed by the help of Wagner's reagent (100 ml of water contains 2 gm of potassium iodide and 1.27 gm of iodine). The different solvent extracts of fruit were added to this reagent and observed for the formation of reddish-brown precipitate.

Test for Cardiac glycosides

In a test tube 5ml of each fruit extract was taken and glacial acetic acid (2ml) used to treat them and addition of few drops of ferric chloride solution. Then carefully sulphuric acid (1 ml) was added to the tested solution. At the junction of the two solutions a brown ring is appeared which indicates the presence of deoxy-ribose sugar characteristic of cardenolides. After that greenish ring may form to indicate the presence of Cardiac glycosides.

Test for Flavonoids

A portion of different crude extract of fruit was added of 5ml of dilute ammonium solution, followed by addition of concentrated H₂SO₄. After that a yellow coloration may formed in each of the plant extract indicates the presence of flavonoids.

Test for Phenols

1ml of various solvent extracts of fruit kept in to the different test tubes, 2ml of distilled water and few drops of 10% ferric chloride solution was added. Then blue or green colour was formed which indicate the presence of phenols.

Test for Tannins

In a test tube 1ml of various fruit extract was taken and then addition of 1ml of 0.008 M potassium ferric cyanide. After that, 1ml of 0.02 M ferric chloride containing 0.1 N HCL was added and observed for blue -black coloration.

Test for Steroids

Salkowski test: 1ml of fruit extract was treated with few drops of conc. H₂SO₄; formation of red color indicated the presence of steroids whereas yellow color showed presence of triterpenoids.

Antibacterial activity

The antimicrobial activity was determined in the *Citrullus colocynthis* (L.) Schrad extract using agar well diffusion method. The antibacterial activities of *Citrullus colocynthis* (L.) Schrad extract was tested against *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhi* (MTCC 890), and *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 3541). Zone of inhibition of *Citrullus colocynthis* (L.) Schrad extract was compared with standards like ampicillin for antibacterial activity. The results showed that the remarkable inhibition of the bacterial growth was against the tested organisms [14].

Results and Discussion

Preliminary phytochemical screening

Different tribal communities totally depend upon medicinal fruits for their healthcare because it has great significance. The fruit extracts of the experimental tool phytochemical analysed and different biochemical constituents have been proved their existence. After careful analysis of the *Citrullus colocynthis* (L.) Schrad fruit extracts revealed the presence of phytochemicals, such as alkaloid, cardiac glycosides, flavonoids, phenols, tannins and steroids. During my present study, preliminary phytochemical analysis revealed a large number of flavonoids and alkaloids present in different whole fruit extract of *Citrullus colocynthis* (L.) Schrad. The phytochemical analysis of whole fruit of *Citrullus colocynthis* (L.) Schrad, represents interesting results in phytochemical screening shown in Table 1. The positive phytochemicals of this experiment lead to investigate further their pharmacological activities. In future, detail research on this plant can reveal a new era of phytochemistry and provide more active phytoconstituents for pharmacological signi-

ficance. It should be finding new generations of drug that may be treat the very serious problems of diseases.

Table 1: Preliminary phytochemical screening of *Citrullus colocynthis* (L.) Schrad

Phytochemicals Constituents	Acetone Extract (AE)	Chloroform Extract (CE)	Ethanol Extract (EE)	Methanol Extract (ME)
Alkaloids	+	+	+	+
Glycosides	+	+	-	+
Flavonoids	+	+	+	+
Phenols	+	-	+	+
Tannins	+	-	+	+
Steroids	+	-	+	+

N.B + = Positive, - = Negative. A.E. = Acetone extract, C.E. = Chloroform Extract, E.E. = Ethanol Extract, M.E. = Methanol extract
Antibacterial activity

Table 2: Antibacterial activity of different solvent of *Citrullus colocynthis* (L.) Schrad against human enteric pathogen

Different Solvent	<i>K. pneumoniae</i> (MTCC 109)	<i>P. aeruginosa</i> (MTCC 354)	<i>B. subtilis</i> (MTCC 441)	<i>S. typhi</i> (MTCC 890)
Ampicillin	16.3	17	13.3	24.3
Acetone (A.E.)	5	7.3	7.6	5.6
Ethanol (E.E)	6.6	8.6	3.3	5.6
Aqueous	0	3	0	0

A.E = Acetone extract, M.E = Methanol extract, P.E = Petroleum ether extract, Values are mean of three replicates, ± values represent SD.

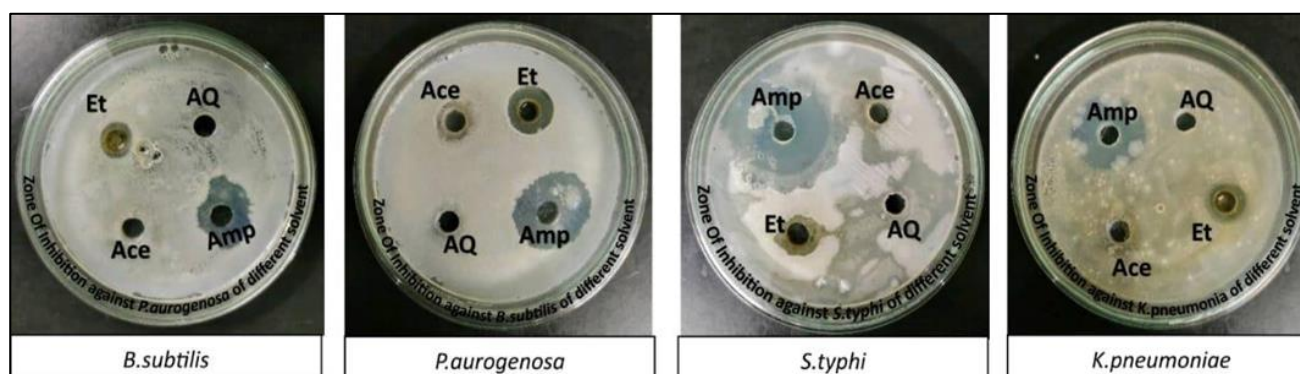


Fig 2: Agar well diffusion method of different solvent whole plant extracts of *Citrullus colocynthis* (L.) Schrad against human enteric pathogens

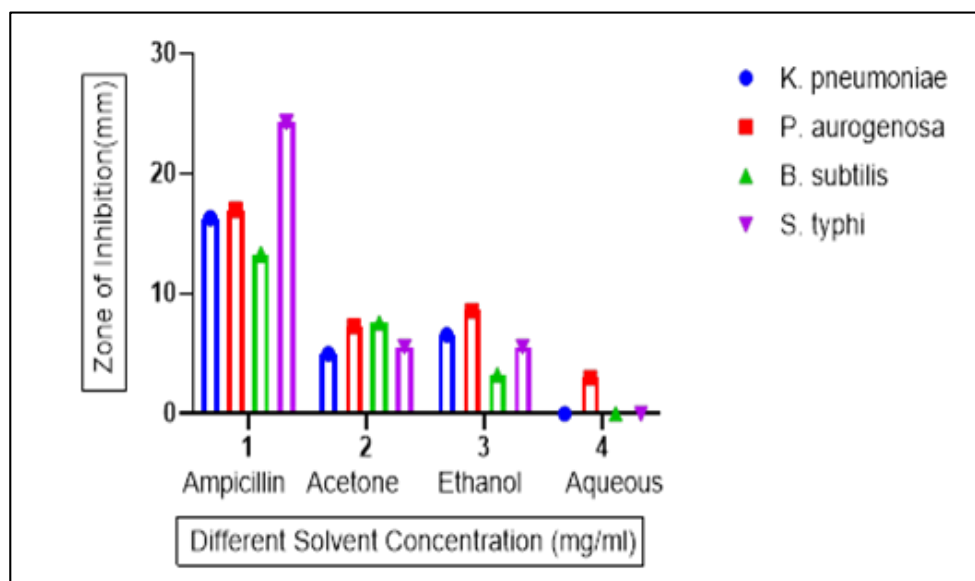


Fig 3: Antibacterial activity of different solvent whole plant extracts *Citrullus colocynthis* (L.) Schrad against human enteric pathogens

Conclusion

Phytochemicals found present in whole fruit extracts of *Citrullus colocynthis* (L.) Schrad indicates their potential as a source of principles that may supply novel medicines. Further

Experimental findings reveal *Citrullus colocynthis* (L.) Schrad is the best herbal remedy to control specially *K. pneumoniae*, *S. typhi*, *B. subtilis* and *P. aeruginosa*. The phytochemical constituents which are responsible for many pharmacological activities, may be useful for the evolution of pharmaceutical and for the therapy of ailments. This is the first ever experimental findings of antibacterial activity as well as demonstration of any biological properties in the globe but deserve further investigation to develop new medicine that may help in combating several diseases in tropical countries to some extent. Further detail and accurate studies on this fruit will explore a dynamic field of bioactive compound which is help to develop new antibacterial medication shown in Table 2, Figure 3 and Figure 4.

studies are therefore suggested to ascertain their anti-microbial, antispasmodic and anti-helminthic activities. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies.

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