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Prabhu Thangavel
PG and Research, Department of Botany, Jamal Mohamed College Autonomous, Tiruchirappalli, Tamil Nadu, India

Ravi Kumar Ramasamy
PG and Research, Department of Botany, Jamal Mohamed College Autonomous, Tiruchirappalli, Tamil Nadu, India

Correspondence
Prabhu Thangavel
PG and Research, Department of Botany, Jamal Mohamed College Autonomous, Tiruchirappalli, Tamil Nadu, India

Phytochemical screening and antibacterial and antifungal activity of the stem, leaf and fruit extracts using different solvent of *Citrullus colocynthis* (L.) Schrad

Prabhu Thangavel and Ravi Kumar Ramasamy

Abstract
The present investigate, the preliminary phytochemical analysis revealed the presence of different phytoconstituents such as Tannins, Saponins, Flavonoids, Steroids, Terpenoids, Cardiac glycosides and Alkaloids observed for *Citrullus colocynthis* (L.) Schrad. stem, leaf and fruit extract using different solvent methanol, chloroform and acetone. The antimicrobial effect of *C. colocynthis* stem, leaf and fruit extract tested against Gram-positive and Gram-negative pathogenic bacteria and antifungal were investigated. The *C. colocynthis* extract have been used in the treatment of pneumonia, bloodstream infection, kidney failure; wound infection and urinary tract infections.

Keywords: Antibacterial, *Citrullus colocynthis*

1. Introduction
Infectious Diseases are caused various living organism such as bacteria, fungi, viruses and parasites. There may be still a major threat to public health departments, despite the tremendous progress in human medicine. This impacts predominantly large in developing countries due to relative unavailability of medicine and emergence of widespread drug resistant microorganisms [1]. Therefore, search for new antimicrobial substance must be continued and all possible strategies must be explored [2]. Increasingly adverse drug reactions to the synthetic antibiotics and the increasing resistance of some pathogens to synthetic antibiotics [3, 4, 5].
The medicinal plants have been widely studied and is demanded by consumers, especially for protection against cardiovascular disorder, cancer and other diseases, as well as for general health benefits. *C. colocynthis* has been widely used in traditional medicine for centuries [6]. In premodern medicine, it was an ingredient in the electuary called *confectio hamech*, or diacatholicon, and other laxative pills [7]. In Arabia the colocynth had numerous uses in traditional medicine, such as a laxative, diuretic, or for insect bites [8]. The powder of colocynthis was sometimes used externally with aloes, unguents, or bandages [7]. Troches made of colocynthis were called "troches of alhandal" used as an emetic [7].
In order to explore new strategies to identify and develop the next generation of drugs or agents to control bacterial infections, the antimicrobial properties of the *C. colocynthis* extract are examined with the phytochemical studies.

2. Experimental and Methods
2.1 Collection of plant materials
The Plant of *C. colocynthis* was collected from its natural habitats, in Bharathidasan University and was identified by Tiruchirappalli, Tamil Nadu, India.

2.2 Screening of phytochemical components Tannins
One ml of water and 1-2 drops of ferric chloride solution were added in 0.5 ml of extracted solution. Blue colour was observed for Gallic tannins and green black for catecholic tannins.

2.3 Saponins (Foam test)
Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicated the presence of saponins.

2.4 Flavonoids (Alkaline Reagent test)
Extractions were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which because colourless on addition of dilute acid, indicates the presence of flavonoids.

2.4 Steroids
Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The colour changed from violet or blue or green in some samples indicating the presence of steroids.

2.6 Terpenoids (Salkowski test)
Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish-brown colouration of the interface was formed to show the presence of terpenoids.

2.7 Cardiac glycosides (Keller-Killani test)
Five ml of each extract was treated with 2 ml of glacial acetic acid containing drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of inter face indicates a deoxy suger characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.8 Alkaloids
Alkaloids are baric nitrogenous compounds with definite physiological and pharmacological activity. Alkaloids solution produces white yellowish precipitated when a few drops of Mayer's reagents are added.

2.9 Anthraquinones
Frager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red, or violet colouration in the ammomia phase indicated the presences of anthraquinone.

2.10 Antibacterial assay
The antibacterial activity of the *Citrullus colocynthis* stem, fruit and leaf extract using different solvent acetone, chloroform and methanol were studied against gram positive G+ (Staphylococcus aureus, Streptococcus pneumoniae and Bacillus subtilis) and gram-negative G- (Klebsiella pneumoniae, Shigella dysenteriae and Escherichia coli) bacterial strains using the well diffusion method. Petri plates were prepared with 25 ml of sterile Muller Hinton agar (MHA, Himedia) and each bacterial pathogen was individually swabbed on MHA in separate plates. The antibacterial activity was tested at a concentration of 40 µl/ml, 50 µl/ml and 60 µl/ml with the required quantity of the *C. colocynthis* stem, leaf and fruit extract using different solvent acetone, chloroform and methanol. The zone of inhibition levels (mm) was measured after 24 h, it was incubated overnight at 37 °C. The standard antibiotic Amoxicillin was used as the positive control.

2.11 Antifungal assay
Antifungal activity was determined by an agar disc diffusion method against the test fungi *C. albicans* using potato dextrose agar. The test strain was transferred into potato dextrose broth (PDB) and incubated at 30 °C until it achieved the turbidity of 0.5 McFarland standard. The media plates were inoculated with the test strain by streaking for 2-3 times by rotating the plate at a 60° angle for each streak to ensure uniform distribution of inoculum. Subsequently, well (6mm) loaded with 40 µl/ml, 50 µl/ml and 60 µl/ml of test *Citrullus colocynthis* stem, leaf and fruit extract using different solvent acetone, chloroform and methanol was placed onto the inoculated plates using sterile forceps and incubated at 30 °C for 24 h under visible light. The zone of inhibition formed around the disc was measured and recorded.

3. Results and discussion

3.1 Phytochemical analysis
The preliminary phytochemical test revealed that the *Citrullus colocynthis* stem, leaf and fruit extract using different solvent methanol, chloroform and acetone, contain the phytochemicals *viz*., Tannins, Saponins, Flavonoids, Steroids, Terpenoids, Cardiac glycosides and Alkaloids as shown in the Table -1. From the observation stem, leaf and fruit extract using different solvent methanol, chloroform and acetone extract presence only Cardiac glycosides, Terpenoids and absence of Saponins. However, other phytochemical constituent's Tannins, Flavonoids, Steroids, and Alkaloids randomly presence and absence are observed.

Table 1: Preliminary phytochemical analysis of Methanol, chloroform, acetone extract of Stem (S), leaf (L), and fruit (F) of *C.* colocynthis

Test	Methanol			Chloroform			Acetone		
	S	L	F	S	L	F	S	L	F
Tannins	-	+	-	-	+	-	+	+	-
Saponins	-	-	-	-	-	-	-	-	-
Flavonoids	+	-	+	+	-	+	+	+	+
Steroids	+	-	-	-	+	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+
Alkaloids	+	-	-	+	+	-	+	-	-

3.2 Antibacteria and antifungal Activity
Citrullus colocynthis (L.) Schrad. stem, leaf and fruit extract using different solvent methanol, chloroform and acetone were screened for their antimicrobial activity against a three Gram positive (*Staphylococcus aureus*, *Streptococcus pneumoniae* and *Bacillus subtilis)* and three-gram negative bacterial (*Klebsiella pneumoniae*, *Shigella dysenteriae* and *Escherichia coli*) strain and fungal strain like *candida albicans*. Antibacterial potential of stem, leaf and fruit extract using different solvent methanol, chloroform and acetone were assessed in terms of their inhibition zone of bacterial and fungal growth using well diffusion method. The results of the antimicrobial activity showed various degrees of inhibition against the 6 bacteria and 1 fungal tested are shown in Table: 2-4. We have observed that the *C. colocynthis* (L.) part and the type of solvent can have a great impact on the antibacterial and antifungal activity. Among the tested extracts, the leaf with methanol extract showed strong inhibition compared to other fruit and stem extracts on all tested bacterial and fungal strains. The observed differences in the inhibition zones could be probably due to cell membrane permeability or other genetic factors.

Table 2: Antibacterial and antifungal activity of *in-vivo* Stem extracts of *Citrullus colocynthis* by agar disc diffusion method

Solvent	Concentration	Zone of inhibition (mm)						
		K. p	S. a	S. d	E. c	S. p	Bac	C. a
Acetone	40 µl	9 mm	11 mm	11.5 mm	12 mm	10.5 mm	11.5 mm	11.5 mm
	50 µl	9.5 mm	13 mm	13 mm	11.5 mm	11 mm	13.5 mm	12.5 mm
	60 µl	9.5 mm	13 mm	12 mm	12 mm	12 mm	13.5 mm	12 mm
Chloroform	40 µl	10 mm	10 mm	11 mm	10 mm	10 mm	8.5 mm	10.5 mm
	50 µl	13 mm	7 mm	8.5 mm	7 mm	9 mm	8 mm	9.5 mm
	60 µl	14 mm	10 mm	9 mm	7.5 mm	9 mm	8.5 mm	8.5 mm
Methanol	40 µl	11 mm	13 mm	12.5 mm	10.5 mm	12 mm	10.5 mm	11 mm
	50 µl	13.5 mm	13 mm	11.5 mm	12.5 mm	12 mm	10.5 mm	12 mm
	60 µl	11.5 mm	13.5 mm	13 mm	11 mm	14 mm	11 mm	12 mm

(K.p - *Klebsiella pneumoniae,* S.a- *Staphylococcus aureus*, S.d-*Shigella dysenteriae* E.c-*Escherichia coli,* S.p–*Streptococcus pneumonia,* Bac - *Bacillus subtilis* strain and fungal strain like C.a - *Candida albicans*)

Table 3: Antibacterial and antifungal activity of *in-vivo* Leaf extracts of *Citrullus colocynthis* by agar disc diffusion method

Solvent	Concentration	Zone of inhibition (mm)						
		K.p	S.a	S.d	E.c	S.P	Bac	C.a
Acetone	40 µl	13.5 mm	13 mm	12.5 mm	12 mm	10.5 mm	10.5 mm	11.5 mm
	50 µl	14 mm	13.5 mm	13 mm	12.5 mm	11 mm	11 mm	12 mm
	60 µl	13.5 mm	15 mm	14 mm	12.5 mm	11.5 mm	12.5 mm	12 mm
Chloroform	40 µl	11.5 mm	11.5 mm	11.5 mm	10 mm	8 mm	10 mm	10 mm
	50 µl	12.5 mm	12 mm	11.5 mm	10.5 mm	9.5 mm	11 mm	11 mm
	60 µl	13 mm	12.5 mm	11.5 mm	10.5 mm	10 mm	10 mm	11 mm
Methanol	40 µl	11 mm	12 mm	12.5 mm	10.5 mm	12.5 mm	12 mm	16.5 mm
	50 µl	13.5 mm	15 mm	13.5 mm	12.5 mm	13 mm	13 mm	14 mm
	60 µl	13 mm	16 mm	13.5 mm	13.5 mm	13 mm	13 mm	13.5 mm

(K.p-Klebsiella pneumoniae, S.a-Staphylococcus aureus, S.d-Shigella dysenteriae E.c-Escherichia coli, S.p- Streptococcus pneumonia, Bac - Bacillus subtilis strain and fungal strain like C.a - Candida albicans)

Table 4: Antibacterial and antifungal activity of *in-vivo* Fruit extracts of *Citrullus colocynthis* by agar disc diffusion method

Solvent	Concentration	Zone of inhibition (mm)						
		K.p	S.a	S.d	E.c	S.p	Bac	C.a
Acetone	40 µl	14 mm	14 mm	11.5 mm	11.5 mm	13 mm	12 mm	11.5 mm
	50 µl	11 mm	16 mm	12.5 mm	15 mm	16 mm	13.5 mm	12 mm
	60 µl	11 mm	16.5 mm	15 mm	12 mm	17 mm	15 mm	16 mm
Chloroform	40 µl	10.5 mm	10 mm	12 mm	10 mm	9 mm	9 mm	9 mm
	50 µl	11 mm	11 mm	13 mm	12 mm	10 mm	10 mm	10 mm
	60 µl	12 mm	11 mm	13.5 mm	12.5 mm	12 mm	12.5 mm	12 mm
Methanol	40 µl	10.5 mm	12 mm	12 mm	10.5 mm	12.5 mm	11.5 mm	11.5 mm
	50 µl	11 mm	12.5 mm	13 mm	11.5 mm	11.5 mm	11 mm	13 mm
	60 µl	11 mm	12 mm	14 mm	11.5 mm	12.5 mm	12 mm	12 mm

(K.p - Klebsiella pneumoniae, S.a- Staphylococcus aureus, S.d - Shigella dysenteriae E.c - Escherichia coli, S.p – Streptococcus pneumonia, Bac - Bacillus subtilis strain and fungal strain like C.a - Candida albicans)

4. Conclusion

The phytochemical screening of *C. colocynthi*s stem, leaf and fruit extract using different solvent acetone, chloroform and methanol revealed the presence in large amount of Terpenoids and Cardiac glycosides in the stem, leaf and fruit for all the solvent extracts. Furthermore, the results obtained in this study clearly demonstrate that *C. colocynthi*s leaf menthol extract exhibited potential antibacterial and antifungal activity against Gram-positive, Gram-negative bacteria and fungal strains.

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