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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *CITRULLUS COLOCYNTHIS*.(LINN.) SCHARED

Sandhya V. Rodge and *S.D. Biradar
**Department of Botany, Dnyanopasak College Parbhani 431401(M.S.)*
**Author for Correspondence*

ABSTRACT
C*itrullus colocynthis* (Linn). Schred is an important medicinal plant of family Cucurbitaceae. The crude fruit extract were examined for antimicrobial potentialities against Gram positive, Gram negative bacteria and fungi. Fruit extract was evaluated on bacterial strains like *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella shigella* and *Candida albicans* .The solvent used for the extraction of plants were water, methanol, petroleum ether, ethanol and acetone. The acetone, methanol, ethanol and distilled water fruit extracts exhibit significant activity against all test organisms. The petroleum ether showed least activity against test bacterial strains which were investigated. The *in vitro* antimicrobial activity of the active extract was compared with standard antibiotic Cephlexin. The MIC values of the extracts were also determined. The plant contains tannins, saponins, flavanoids, terepenoids, alkaloids, steroids and cardiac glycoside.

Key Words: *Phytochemical Screening, Citrullus Colocynthis*

INTRODUCTION
Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to relative unavailability of medicine and emergence of widespread drug resistant microorganisms (Okeke *et al*., 2005). Therefore search for new antimicrobial substance must be continued and all possible strategies should be exploring (Clardy and Walsh, 2004). Rational drug design does not always yield effective antimicrobials. In past, potent enzyme inhibitors have been successfully designed and synthesized but they had only moderate antimicrobial activity (Silver, 1990). Current research on nature of molecules and products primarily focused on plant since they can be sourced more easily and be selected on the basis of their ethanomedicinal uses (Verpoorte *et a*l., 2005). The use of ethanopharmacological knowledge is one of the attractive ways to enhance the probability of success in new drug finding efforts (Patwardhan, 2005 and Cordell and Colvard, 2005).
Citrullus colocynthis is an annual herbaceous plant. Stem is angular and rough, leaves rough, 3 to 7 lobed, 5 to 10cm long, flowers are monoecious, solitary, peduncled, axillary, corolla 5-lobed, ovary villous, fruit is pepo, nearly globular, 4 to 10cm in diameter with somewhat elliptical fissures, small orange, green and becoming yellow when ripe. Seeds numerous, smooth and dark brown.

Medicinal Uses
The Citrullus *colocynthis* of family Cucurbitacece is useful against fever, intestinal parasities, hepatic and abdominal diseases, visceral and cerebral congestions. Fruit juice with sugar is a house hold remedy in dropsy (Anonymous, 1970). Root extract is used against jaundice, urinary diseases, rheumatism etc. (Dastur, 1962). Seeds are diuretic (Vohora and Khan, 1981). Fruits are used against tumors of gastrointestinal tract. It is more pronouncedly used in anticancerous drug. It is effective in leukemia and joint pains.

MATERIALS AND METHODS

Plant Material

The root, stem and leaves of *Citrullus colocynthis* were collected during November 2010 from various places of Parbhani district and identified using standard floras such as Naik (1979); Naik, *et al*., (1998) and Singh and Karthikeyan (2001). The collected plant material was shade dried, powdered and stored in airtight container.

Preparation of Extracts

Powder obtained was subjected to successive soxhlet extraction with increasing order of polarity i.e. Acetone (56 to 60^0c), Ethanol (60^0c), Petroleum ether (60-80c) Methanol (60.5-65.5c) and Water (Daniel, 1991).

Test Microorganisms

Escherichia coli, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella shigella*, *Candida albicans* were obtained from stock cultures of Department of life Sciences, SRTMU Nanded and maintained on Muller Hinton agar and potato dextrose agar slant for bacteria and fungi respectively and stored at 6^0c until used. The slants were incubated at 37^oC for 24 hours and inoculums were prepared by Mac Farland turbidity standards.

Antimicrobial Assay

Antimicrobial activity was determined by agar well diffusion method using Nutrient agar for bacteria and Potato dextrose for fungi. Plant extracts were dissolved in DMSO (Dimethyl Sulphoxside) at concentration of 2mg /ml and Cephalexin 20μg/ml was used as standard. Each plate was inoculated with 20mg/ml microbial suspension having a concentration of 10^8 cells/ml 0.1ml extract was added to each well. The bacterial plates were incubated at 37^0C for 24 hours and those containing fungi were incubated at 25^0C for seven days. The antimicrobial activity was observed as inhibition zone which was compared with standard. MIC was also determined by broth dilution method. The cultures were diluted in nutrient agar broth at a density adjusted to turbidity of 0.5 Mac Farland standards. Equal volume of each extracts and nutrient broth was mixed in test tubes; 0.1ml standard inoculums were added to each tube. The lowest concentration of the extract that effects visible bacterial growth and compared with standard regarded as MIC.

RESULTS

Antimicrobial assay was performed by agar well diffusion method against four bacterial species and one fungal species. Table 1 summarizes the zone of inhibition for microbial growth of both aqueous and different solvent extracts of *C. colocynthis*. The ethanol, acetone, distilled water and methanol extract exhibit significant antimicrobial activity. Whereas, petroleum ether exhibit less antimicrobial activity as compared to other extracts.

The maximum antimicrobial activity was exhibited by acetone, ethanol, methanol and distilled water extract against Escherichia *coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella shigella* and *Candida albicans.* Whereas petroleum ether extract is less effective against test strains. The antimicrobial activity of plant extracts was compared to the standard antibiotic Cephalexin 20μg/ml (figure 1).

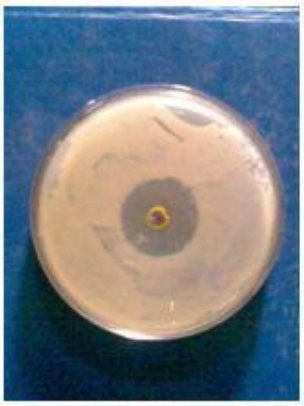
Table 1: Antimicrobial activities of *Citrullus colocynthis*

Sr.No	Organisms	Zone of inhibition(mm)					
		Plant extract					Control
		ETF	DTF	ATF	MTF	PTF	
1	*Escherichia coli*	8	2	15	--	---	10.1
2	*Salmonella typhi*	8	7	16	7	----	5.1
3	*Staphylococcus aureus*	4	1	6	11	2	5.0
4	*Shigella shigella*	9	5	2	7	----	8.1
5	*Candida albicans*	8	1	13	8	1	10.2

ETF: Ethanol fruit extract, DTF: Distilled water fruit extract, ATF: Acetone fruit extract, MTF: methanol fruit extract, PTF: Petroleum ether fruit extract.

***Eschreschia coli* in acetone extract**

***Salmonella tyhpi* in acetone extract**



***Staphylococcus aureus* in Methanol extract**

***Candida albicans* in ethanol extract**

Figure 1: Photos Showing Antimicrobial Assay

Table 2: Minimum inhibitory concentration (mg/ml)

Sr.No	Organisms	Minimal inhibitory zone				
		ETL	DTL	ATL	MTL	PTL
1	*Escherichia coli*	20	50	10	---	----
2	*Salmonella tyhpi*	20	20	10	20	---
3	*Staphylococcus auerus*	40	50	30	50	50
4	*Shigella shigella*	20	50	50	40	----
5	*Candida albicans*	20	50	10	40	50

Table 3: Phytochemical screening of fruit extracts of *Citrullus colocynthis*

solvent	Tannin	Flavanoid	Steroid	terpenoid	Cardice glycoiside	Saponin	Alkaloid
Ethanol	+	+	--	+	+	+	+
Methanol	--	+	-	+	+	+	+
Acetone	-	--	+	+	+	+	+
Petroleum ether	+	+	--	--	+	+	--
Distilled water	+	+	-	+	+	+	+

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Phytochemical Analysis

Phytochemical analysis of plant extract revealed that the presence of tannins, saponins, flavanoids, terpenoids, alkaloids, steroids and cardic glycoloids (Table 3).

DISCUSSION

The antimicrobial study shows that the fruit extracts of *C. colocynthis* inhibits the growth of gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli, Shigella shigella* and *Salmonella tyhpi.* Similar results observed by Memon *et al*., (2003) that the ethanolic extract of *Citrullus colocynthis* which is active against gram positive bacteria i.e. *Bacillus pumilus and Staphylococcus aureus* whereas it is inactive against gram negative bacteria *Eschreschia coli and Pseudomonas aeruginosa.* But present study reveals that methanolic, ethanolic, acetone and distilled water extract shows potent activity againt *Escherichia coli.* Phate *et al*., (2011) supports the results of present study.

Tannins has been reported to prevent the development of microorganisms such as fungi, bacteria, yeast, viruses by precipitating microbial protein and converting it into unavailable form (Sadipo *et al.*,1991,Chung *et al*,1998 and Phate *et al.*, 2011).

Phytochemical screening of fruit extract of *Citrullus colocynthis* reveal that most of the phytochemicals, tannin, terpenoids, steroids, cardicglycosides, alkaloids are soluble in ethanol, methanol, acetone and distilled water. In petroleum ether the steroids, terpenoids and alkaloids were insoluble. Ambi *et al*. (2007) screened the phytochemicals of *Citrullus colocynthis* and observed that the presence of alkaloid, seteroids, glycoside and flavanoids but in this observed study it is noted that that the presence of tannins, saponins, cardic glycosides, alkaloids, terpenoids, flavanoids .The earlier studies of Najafi *et al*., (2010) also exhibited the presence of similar phytochemicals in fruit extract of *Citrullus colocynthis.*

Conclusion

The present study concludes that the crude fruit extract of *Citrullus colocynthis* show active response against the Escherichia *coli*, *Staphylococcus aureus, Salmonella typhi, Shigella shigella* and *Candida albicans.* It is may be due to secondary metabolites which are identified in *Citrullus colocynthis.* The present investigation proves that antimicrobial activity of fruit extracts was higher than that of antibiotic Cephalexin against studied test organisms.

The study indicates that fruit extract possess potent antimicrobial activity and proves that use of *Citrullus colocynthis* for the treatment of diseases caused by the test organisms. There is still an urgent need to identify novel substances that are active towards pathogens with high resistance.

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