

Phytochemical and Antifungal Activity of *Citrullus colocynthis* Seeds Solvent Extracts

Prasad. M. P

Department of Microbiology/Biotechnology, Sangenomics Research Lab, Domlur Layout, Bangalore 560071, India

* Corresponding Author: E Mail- drprasadmp@gmail.com; Ph.: 9844357929

Abstract: Plant extracts and their constituents have a long history as an important source of secondary metabolites and antifungal agents, but their use as fungicides, has been rarely reported. The aim of this study was to assess the phytochemical constituents and in vitro antifungal activity of different solvent extracts from *Citrullus colocynthis* seeds. Alkaloids, Glycosides, Terpenoids, Tannins, Anthraquinone and Carbohydrates were seen present in all the 3 extracts. Saponins and phenols were absent in all the solvent extracts. Antifungal activity was checked for five different solvent extracts against 3 fungal isolates. The maximum zone of inhibition was seen against *Rhizopus* spp., by methanolic extract of the seed sample. Chloroform and diethylether extracts did not show much activity against the fungal isolates. The minimum inhibitory concentration was seen at 0.625mg/ml concentration for all the fungal isolates with the ethanolic extract. *Rhizopus* and *Aspergillus niger* showed minimum inhibitory concentration at 0.625mg/ml concentration of the methanolic extract with a zone of 6mm and 7mm respectively. *Gliocladium* showed MIC value of 2.5mg/ml with the methanolic extract of the seed sample. The hexane extract showed less antifungal activity and the MIC value was 0.625mg/ml, 2.5mg/ml and 5mg/ml for *Rhizopus*, *Aspergillus niger* and *Gliocladium* respectively.

Keywords: *Citrullus colocynthis*, Phytochemical compounds, Antifungal assay, MIC

1. Introduction

India is one the diversified country in plants, animals and microorganisms due to the diverse environmental conditions being suitable for millions of species. The plants have been studied for curing specific ailments. Different parts of plants have been used from ancient times. The indigenous system of medicine, namely, Ayurvedic, Siddha, and Unani, which were followed in India during ancestral period have been used these days as a cure for modern diseases and the medicines have been commercialised in the market as well (Kumar S et al., 2010). People of different ethnic groups inhabiting various terrains, possess their own distinct culture, religious rites, food habit and a rich knowledge of traditional medicine which can be used various ailments (Parinitha M et al., 2005).

Citrullus colocynthis have proved to have several active chemical constituents like colocynthin, colocynthetin, cucurbitacins, á-elaterin (Adam, et al., 2001), cucurbitacines (Sturm, et al., 2009), cucurbitacin glycosides (Hatam, et al., 1989, Seger, et al., 2005 and Abbas, et al., 2006), flavonoids and flavone glycosides (Maatooq, et al., 1997 and Abbas, et al., 2006).

Plants have high immune response mechanisms by production of secondary metabolites, these secondary metabolites help the plants from different bacteria and fungi which mainly effect the growth of the plant as these microorganisms causes various kinds of diseases in the plants. The role of antimicrobial agents is mainly shift the balance in favour of the host plant.

Plant extracts and their constituents have a long history as antifungal agents, but their use in biotechnology as preservatives is less reported, due to the increasing resistance of fungi to fungicides.

The aim of this study was to assess in vitro antifungal activity of hexane extract, ethanol extract, methanol extract, chloroform extract and diethyl ether extract of *Citrullus colocynthis* seeds.

2. Materials and Methodology

Plant Material: The fruits of *C.colocynthis* were collected at maturity between September and November. In the laboratory, seeds were salvaged, dried, sheltered from whilst light, and eventually stored up to the time of further use at ambient temperature.

Extraction Protocol: The extractions were performed on the seeds of *C.colocynthis*. Plant materials were washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite. Afterwards, the seeds are ready for extraction. Solvent used in the study where ethanol methanol and hexane.

Solvent extraction: Ten grams of seeds were ground with a mixer and added to 100 ml of methanol, ethanol, chloroform, diethyl ether and hexane are stored in a glass bottle and kept for 48 hrs undisturbed. After 48 hrs the mixture was then filtered using filter paper (What man No 1). The filtrate is used for further assay.

Phytochemical Screening: The Phytochemical Screening tests were carried out in the extracts using standard procedure to identify the constituents as described by Trease and Evans et al., (1989).

3. Test for Alkaloids

1ml of extract was added with 2-3 drops of Mayer's reagent (dissolve 1.36g of mercuric chloride in 60ml of H₂O and pour into the solution of 5g of potassium iodide in 100ml of

H₂O). The appearance of cream colour precipitate or pale yellow colour precipitate indicates the presence of alkaloids.

Test for Terpenoids (salkowski test):

1ml of the extract was mixed with 2ml of chloroform and concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Test for Flavonoids:

1ml of extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

Test for Tannins:

1ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicates of tannins.

Test for Steroids:

1ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for Saponins:

About 1ml of solvent extract was introduced into a tube containing 1 ml of distilled water, the mixture was vigorously shaken for 2 min, and formation of froth indicated the presence of saponins.

Test for Phenols:

2ml of ferric chloride solution was added in 2ml of solvent extract. Formation of deep bluish green solution indicated the presence of phenols.

Test for Anthraquinones:

0.5g of crude powder was added in 10ml of benzene and filtered. Then 0.5ml of ammonia solution was added in the filtrate and shaken well. Violet color in the layer phase indicated the presence of anthraquinones.

Test for Cardiac glycoside:

0.5g of extracts was dissolved in 2ml of glacial acetic acid containing 1 drop of ferric chloride. Then 2ml of conc. sulphuric acid was added under layered. Brown ring was formed at interphase indicated the presence of deoxysugar which is the characteristic of cardiac glycoside.

Reducing sugar:

To 1ml of extract few drops of fehllings A and few drops of fehilings B solution are added the tubes are heated in boiling water bath for few minutes .presence of red precipitate.

Carbohydrates:

Few drops of molisch reagent were added to 2ml of extract followed by the addition of 2ml of con sulphuric acid along the sides of the tube and allowed to stand for 2to 3 minutes.

Formation of red or dull violet colour at the interphase of the 2 layers.

Antifungal Activity

The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. One hundred micro liter of the fungal spore suspension was evenly spread on the SDA using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer and the wells filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 h and later Observed for zone of inhibition. The effect of the extract on fungal isolates was compared with Amphotericin B and Miconazole at a concentration of 1 mg/ml.

Minimum inhibitory concentration (MIC) was determined for the samples which showed greater zone of inhibition against the fungal isolates.

4. Results

Phytochemical screening: Alkaloids, Glycosides, Terpenoids, Tannins, Anthraquinone and Carbohydrates were seen present in all the 3 extracts. Saponins and phenols were absent in all the solvent extracts. Flavoniods was mainly seen in ethanol extract whereas steroids were present in methanol extract. Reducing sugar was seen in hexane and ethanolic extract (Table 1).

Table 1: Qualitative phytochemical analysis

Tests	Hexane	Ethanol	Methanol
Alkaloids	+	+	+
Flavonoids	-	+	-
Steroids	-	-	+
Glycosides	+	+	+
Terpenoids	+	+	+
Tannins	+	+	+
Saponins	-	-	-
Reducing sugar	+	+	-
Phenol	-	-	-
Anthraquinone	+	+	+
Carbohydrates	+	+	+

5. Antifungal Activity

Five different solvent extracts were checked for its antifungal activity against 3 fungal isolates. The maximum zone of inhibition was seen against *Rhizopus spp.*, by methanolic extract of the seed sample. Chloroform and diethylether extracts did not show much activity against the fungal isolates.

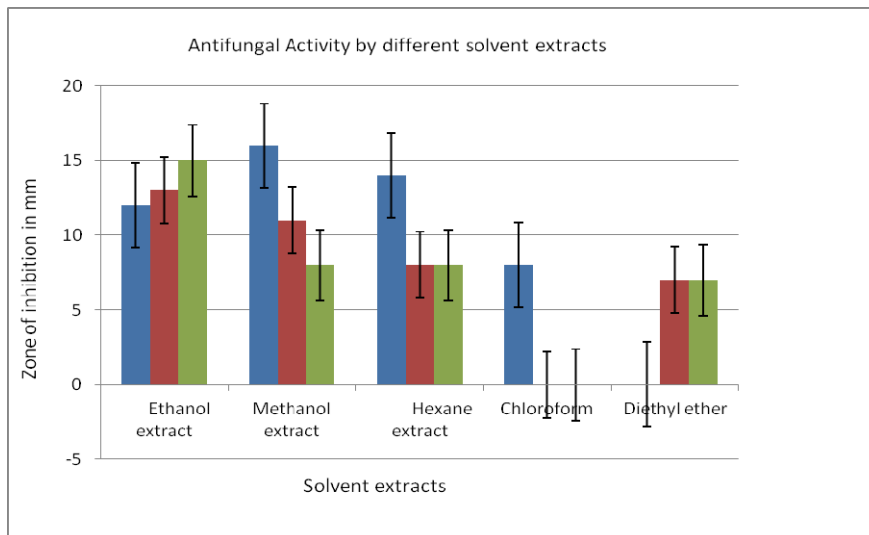


Figure 1: Antifungal activity of three extracts

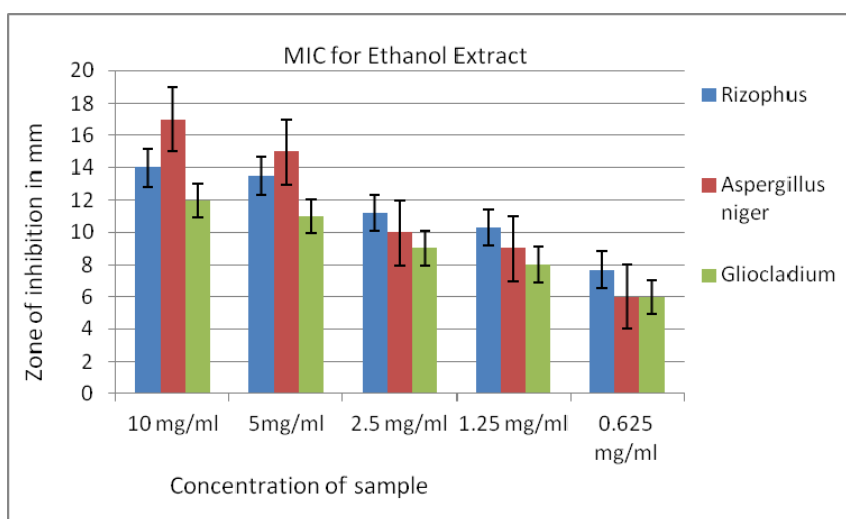


Figure 2: Antifungal and MIC activity of ethanol extracts

The minimum inhibitory concentration was seen at 0.625mg/ml concentration for all the fungal isolates with the ethanolic extract. The zone of inhibition increased with the increase in the concentration of the sample.

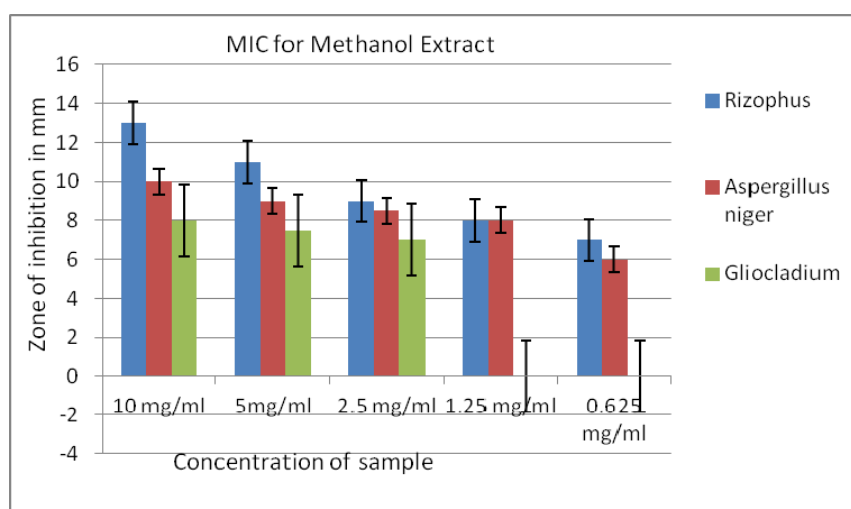


Figure 3: Antifungal and MIC activity of Methanol extracts

Rhizopus and Aspergillus niger showed minimum inhibitory concentration at 0.625mg/ml concentration of the methanolic extract with a zone of 6mm and 7mm respectively.

Gliocladium showed MIC value of 2.5mg/ml with the methanolic extract of the seed sample.

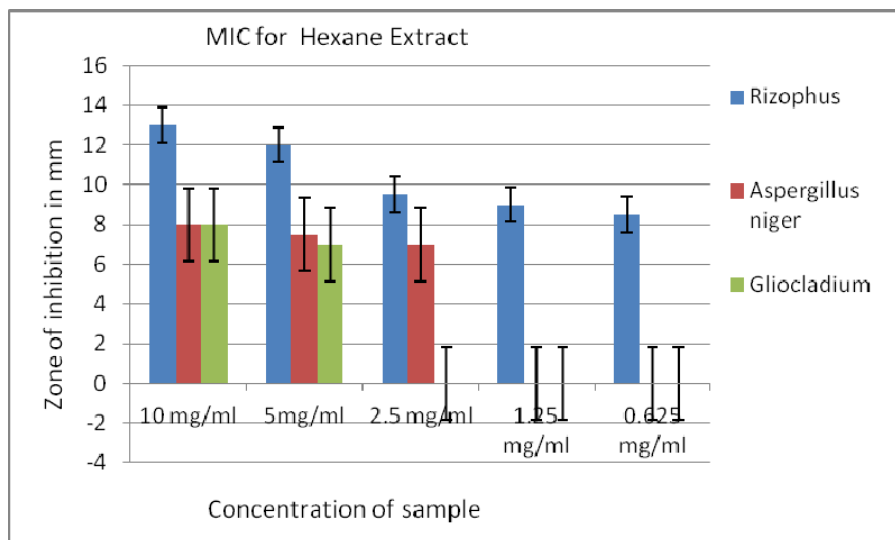


Figure 4: Antifungal and MIC activity of ethanol extracts

The hexane extract showed less antifungal activity and the MIC value was 0.625mg/ml, 2.5mg/ml and 5mg/ml for Rhizopus, Aspergillus niger and Gliocladium respectively.

6. Discussion

Ahmad Oryan et al. 2014, evaluated the differential effects of ethanol extraction of *Citrullus colocynthis* on the blood glucose concentration and pathology of pancreas, liver, lungs, kidney and gastrointestinal tract in the alloxan induced diabetes in rats.

The fruits of *C. colocynthis* have been commonly used as antidiabetic medication in tropical and subtropical countries (Diwan, F.H et al., 2000). This plant has insulinotropic effects (Nmila, R et al., 2000), mild immunostimulating effects (Beneddoud, D et al., 2003), antioxidant activity (Kumar, S et al., 2008) and anticancer activity. Phytochemical screening revealed that *C. colocynthis* extract contains triterpene, flavonoids, tertiary and quaternary alkaloids, glycosides and saponin compounds (Abdel-hassan et al., 2000). One study reported a significant reduction of blood sugar level in diabetic rats that treated with aqueous extract of *C. colocynthis* seeds (Benmehdi, H et al., 2008). Different parts of the plant including seeds, fruit, root, stem, and leaves, used as either aqueous or oil extracts, dried or fresh, are believed to have antidiabetic, antihyperlipidemic (Rahbar A. R et al., 2010; Daradka, H et al., 2007), laxative (Huseini, H. F et al., 2009; Abdel-Hassan I. A et al., 2000), anti-inflammatory, analgesic (Marzouk, B et al., 2010), vermifuge (Rahimi et al., 2012), hair-growth-promoting (Dhanotia, N. S et al., 2009), antibacterial, antifungal (Marzouk et al., 2009), and antioxidant properties.

References

- [1] Abbas, D., G. Simon, R. Ali, N. Hossein, M. Masoud, N. Lutfun and D. Satyajit, 2006. Flavone Cglycoside and cucurbitacin glycoside from *Citrullus colocynthis*. DARA, 14(3): 109-114.
- [2] Abdel-Hassan I. A, Abdel-Barry, J.A. and Mohammeda, S. T. "The hypoglycemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and Alloxan diabetic rabbits," Journal of Ethnopharmacology, 2000; 71, no.1-2, pp. 325–330.
- [3] Abdel-hassan, I.A., Abdel-barry, J.A. and Tariq, M.S. The hypoglycemic and anti hyperglycemic effect of *Citrullus colocynthis* extract in normal and Alloxan diabetic rabbits. J.Ethnopharmacology. 2000; 71 (1-2):325-330.
- [4] Adam, S., M. Al-Yahya and A. Al-Farhan, 2001. Response of Najdi sheep to oral administration of *Citrullus colocynthis* fruits. Small Rumin. Res., 40: 239-244.
- [5] Ahmad Oryan, Mohammad Hashemnia, Ahmad-Reza Hamidi, Adel Mohammadalipour, Effects of hydro-ethanol extract of *Citrullus colocynthis* on blood glucose levels and pathology of organs in Alloxan - induced diabetic rats 2014 by the Asian Pacific Journal of Tropical Disease.
- [6] Beneddoud, D, D., Lalaoui, K, K. and Satta, D. Immunostimulating activity of the hot water-soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galanga* and *Citrullus colocynthis*. J. Ethnopharmacol. 2003; 88(2-3):155-160.
- [7] Benmehdi, H, H., Allali, H., Tabti, B., Djabou, N., Bendiabdellah, A., Lahfa, F. and Djaziri, R. Hypo and antihyperglycaemic effect of *Citrullus colocynthis* L. seeds in normal and Streptozotocin induced diabetic rats. Asian Journal of Chemistry. 2008; 20(4):2711-2718.
- [8] Daradka, H. Almasad, M.M. Qazan, W.S. El-Banna N. M., and Samara, O. H. "Hypolipidemic effects of *Citrullus colocynthis* L. in rabbits," Pakistan Journal of Biological Sciences, 2007; 10, no. 16, pp. 2768–2771.
- [9] Dhanotia, N. S. Chauhan, D. K. Saraf, and V. K. Dixit, "Effect of *Citrullus colocynthis* Schrad fruits on testosterone-induced alopecia," Natural Product Research. 2009; 16, pp. 1–12.
- [10] Diwan, F.H., Abdel-hassan, I.A. and Mohammed, S.T. Effect of saponin on mortality and histopathological changes in mice. Eastern Mediterranean Health Journal. 2000; 6(2-3):345-351.
- [11] Hatam, N.A., D.A. Whiting and N.J. Yousif, 1989. Cucurbitacin glycosides from *Citrullus colocynthis*. Phytochemistry, 28: 1268-1271.

- [12] Huseini, H. F. Darvishzadeh, F.Heshmat, R. Jafariarzar, Z. Raza, M. And Larijani, B. "The clinical investigation of *Citrullus colocynthis* (L.) Schrad fruit in treatment of type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial," *Phytotherapy Research*, 2009; 23, (8), pp. 1186–1189.
- [13] Kumar S, Malhotra R, Kumar D. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharm Rev* 2010; 4(7): 58-61.
- [14] Kumar, S., Kumar, D., Saroha, K., Singh, N. and Vashishta, B). Antioxidant and free radical scavenging potential of *Citrullous colocynthis* L. methanolic fruit extract. *Acta Pharmaceutica*. 2008; 58 (2):215-221.
- [15] Maatooq, G., S. El-Sharkawy, M. Afifi and P. Rosazza, 1997. C-*p*-Hydroxybenzoyl-glycoflavanones from *Citrullus colocynthis*. *Phytochemistry*, 44: 187-190.
- [16] Marzouk, B. Marzouk, Z. Décor R. et al., "Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis* Schrad. From Medenine," *Journal of Ethnopharmacology*.2009; 125, no. 2, pp. 344–349.
- [17] Marzouk, B. Marzouk, Z. Haloui, E. Fenina, N. Bouraoui, A. and Aouni, M. "Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia," *Journal of Ethnopharmacology*. 2010; 128, no. 1, pp. 15–19.
- [18] Nmila, R., Gross, R., Rchid, H., Roye, M., Mauteghetti, M, M., Petit. Tijane, R.G. and Sawaire, Y. Insulinotropic effect of *Citrullous colocynthis* fruit extracts. *Planta Med*. 2000; 66(5):418-423.
- [19] Parinitha M, Srinivasa BH, Shivanna MB. Medicinal plant wealth of local communities in some villages in Shimoga district of Karnataka. India *J Ethnopharmacol* 2005; 98: 307-312.
- [20] Rahbar A. R. and Nabipour, I. "The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia," *Pakistan Journal of Biological Sciences*.2010; 13, no. 4, pp. 1202–1207.
- [21] Rahimi, G. Amin, and M. R. S. Ardekani, "A Review on *Citrullus colocynthis* Schrad, from traditional Iranian medicine to modern phototherapy," *Journal of Alternative and Complementary Medicine*. 2012; 18, no. 6, pp. 551–554
- [22] Seger, C., S. Sturm, M. Mair, E. Ellmerer and H. Stuppner, 2005. ¹H and ¹³C NMR signal assignment of cucurbitacin derivatives from *Citrullus colocynthis* (L.) Schrader and *Ecballium elaterium* (L.) (Cucurbitaceae). *Magn. Reson. Chem.*, 43(6): 489-91.
- [23] Sturm, S., P. Schveider, C. Seger and H. Stuppner, 2009. Analysis of *Citrullus colocynthis* cucurbitacin derivatives with HPLC-SPE-NMR. *Scientia Pharmaceutica*, 77: 254-257.
- [24] Trease and Evans. *Pharmacognosy*. 15 edition, Published by Elsevier, 2005; pg: 266-267