IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL STUDIES ON FRUIT EXTRACT OF *CITRULLUS COLOCYNTHIS* SCHRADER

Dhore R. K. and Kutemate, N.B..

[*]Department of Botany, Dr. Manorama & Prof. H .S. Pundkar Arts, Commerce &
[**]Science collage, Balapur, District Akola (M.S) India
Department of Botany,G.S.Arts,Science and Commerce Collage,Khamgoan,Dist.Buldhana (M.S) India

ABSTRACT

Citrullus colocynthis Schrader belongs to family Cucurbitaceae. It grows widely in Melghat, Maharastra, India and it has been used in folk medicine by tribals. To evaluate the different medicinal properties of *Citrullus colocynthis* Schrader. The present study attempt to evaluate the preliminary phytochemical screening and antimicrobial studies in fruit extract of conform the presence of various phytochemicals like Carbohydrates, Saponins, Tannins, Flavonoids, Alkaloids, Glycosides, Proteins, Phytosterols, Steroids, Phenols and Terpenoids. The solvent petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate and aqueous extract showed good results. Disk diffusion method was used to study the antibacterial activity against *Salmonella tiphy, Escherichia coli, Streptococcus pyogenes,* antifungal activity against *Candida albicans and* antiprotozoal activity against *Entamoeba histolytica.* The observed sample in different solvent fruit extract showed antibacterial, antifungal, and antiprotozoal activities by the formation of inhibitory zone. It may be attributed to the presence of phytochemical and may be used as antimicrobial agents.

Key Words: *Citrullus colocynthis* Schrader, Melghat, preliminary phytochemical screening, antimicrobial studies.

INTRODUCTION

Melghat is situated in Satpula range. It is located at $21^0\ 26^1\ 45^{11}$ N $77^0\ 11^1\ 50^{11}$E in northern part of Amravati district of Maharashtra state in India. The marvelous by gift of nature is plant medicines which cure many diseases. The plants contain many phytochemicals of therapeutic use. Phytochemicals are biologically active naturally occurring chemical compounds found in plants, which provide health benefits for humans (Hasler and Blumberg, 1999). In many microbial diseases of humans, secondary plant metabolites are the basis of treatment. Hence in the recent era plant medicines are being used in the treatment of various microbial ailments. *Citrullus colocynthis* Schrader belongs to family Cucurbitaceae. Citrullus colocynthis Schrader is monoecious plant with perennial root, creeping stem, diffuse or slender angled

branches, scabrid. Tendrils are simple, slender, and hairy. Leaves are variable, pale green above, ashy beneath, usually deltoid in outline, three lobed, lobed deeply pinnate. Male flowers: Peduncles long villous, Calyx tube broadly campanulate, hairy, long, pale yellow, segments obovate, apiculate. Stamens 3 short, free, anther cohering, one 1-celled, other 2-celled. Ovary rudimentary, glanduliform. Female flowers: Calyx and corolla as in male. 3 legulate stamens, ovoid. Ovary with 3-placentiferous and many compressed ovules are present. Fruits globular, variegated green and white ripe filled with a dry spongy very bitter pulp, epicarp thin. Seed are off white or brown in colour. It is one of important ethnomedicinal plant used by local tribal medicinemen. Plant is traditionally used in treatment of wound healing.

MATERIALS AND METHODS

Plant material:

The whole plant of *Citrullus colocynthis* Schrader was collected in fresh condition from southern part of Melghat, Maharashtra, India. The plant was identified authenticated by well known taxonomist Dr. S. M. Bhuskute Principal, Bhavbhuti Mahavidyalaya, Amgaon, district Gondia, Maharashtra. Voucher specimen has been deposited in the department of Botany, Bhartiya Mahavidyalaya, Amravati, Maharashtra. The fruits were washed under running water and dried under shade, then ground into a fine powder by using blender and stored in plastic bottle at room temperature.

Preparation of extracts:

The extraction of soluble compounds from fruit of *Citrullus colocynthis* Schrader was performed by using the soxhlet's extractor method with various solvents like Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate and Distilled water. 10gms of dried powder were taken in a cone made from Whatman filter paper No.1 and placed into soxhlet's apparatus. 100 ml of above solvents were taken successively, in the round bottom flask attached to the soxhlet's apparatus. A condenser was attached to this setup. Then the whole setup was placed on a heating mantle. The temperature was set in the range where the solvants gets vaporized and rises up to the condenser where it condenses back into liquid. This liquid falls into the plant sample in the cone and extracts certain compounds and falls back into the round bottom flask. This process was continued till all the compounds get's extracted from the sample. The extracts obtained fromthe above process was evaporated and stored in cap glass vials.

Phytochemical analysis:

Preliminary phytochemical screening of samples was carried out with the following methods described by Harborne, J.B. (1973).

Test for Detection of carbohydrates: Molisch's Test: Small Quantity of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil waterextract were taken separately in 10 ml of distil water and two drops of Ethanolic naphthol (20%) and 2ml of concentrated Sulphuric acid were added, formation of reddish violet ring at the junction indicates presence of carbohydrates.

Test for Detection of Saponins: Foam Test: 2 ml of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extract were taken and added equal amount of distilwater and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins (Kumar et al., 2009).

Test for Detection of Tannins: Ferric Chloride Test: Small Quantity of Petroleum ether, Benzene, Acetone Chloroform, Ethyl acetate, Distil water extract were taken separately in water, 2-3 drops of 5% ferric chloride was added. Formation of black or green colour indicates the presence of tannins.

Test for Detection of Flavonoids: Sulphuric Acid Test: A fraction of extract was treated with concentrated sulphuric acid and observed for formation of orange colour.

Test for Detection of Alkaloids: Mayer's Test: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Detection of Glycosides: Sulphuric Acid Test: To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added then few drops of concentrated sulphuric acid were added. Presence of greenish blue colour indicates the presence of glycosides.

Test for Detection of Proteins and Amino acids: Ninhydrin Test: To 2ml of plant extract, few drops of 0.2% Ninhydrin was added and heated for five minutes. Formation of blue colour indicates presence of proteins.
Test for Detection of Steroids and phytosterols: Sulphuric Acid Test: To 1 ml of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.
Test for Detection of Phenols: Ferric Chloride Test: To1 ml of plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicates presence of phenols.
Test for Detection of Terpenoids: Salkowski test : 2ml of plant extract was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. Reddish brown colouration of the interface is formed indicating the presence of terpenoids.

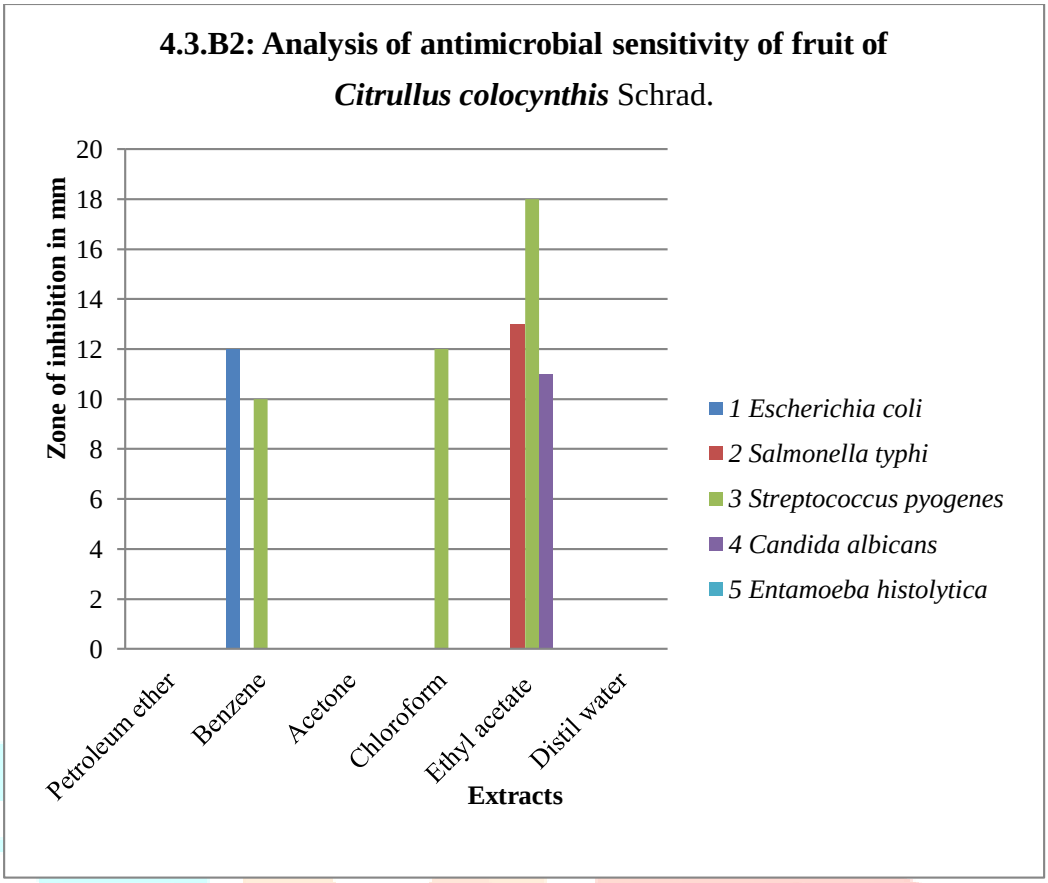
Antimicrobial Activity: The role of medicinal plants is very important in treatment of various diseases in human being. Medicinal plants are the first home remedy that a person relies on for hepatic as well as gastrointestinal troubles. Ginger and garlic are home remedies against gaseous trouble, indigestion etc. Though antifungal and antibacterial drugs control infections but the pathogen become resistant to these allopathic drugs, second chance is the infection gets cured causing other complications and side effects. To prevent patient from drug resistant species and undesirable side effects plant medicine prove to be the best solution. Higher plants have shown to be potential source of new antimicrobial agent (Mitscer, 1987). Especially hepatic and gastrointestinal disorders are challenge before modern medicine, hence scientist are showing great interest for the discovery of new effective plant based drugs for treatment of these disorders.
Collection of Bacterial and Parasitic Isolates: Bacterial isolates of *Escherichia coli, Salmonella typhi, Streptococcus pyogenes* andparasitic isolates of *Entamoeba histolytica,* were obtained from Samruddhi Microbiology Diagnostic Laboratory, Amravati which is run by Dr. S. R. Gulhane (Microbiologist). The isolates were authenticated by biochemical tests as described by Cheesebrough (1985) preserved on potato dextrose agar and nutrient agar respectively and stored at 4^0C until ready to use.
Disc Diffusion Method: The bacterial isolates of *Escherichia coli, Salmonella tiphy, Streptococcus pyogenes, Candida fungus* and parasitic isolates of *Entamoeba histolytica,* were subculture overnight at 37^0 C on potato dextrose agar and nutrient agar plates respectively. Six plate per organism. The suspension of each bacterial and parasitic isolates were prepare as described by John *et al.*(1999) in isotonic sodium chloride solution. Solidified petridishes, for each microorganism for six solvents on Muller-Hinton agar were flooded with the appropriate suspension of bacterial isolates respectively. Sterile 6 mm diameter absorbent filter papers disc (punched out from No.1 Whatman paper) were impregnated with respective solvents of plant extracts namely Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water

Table 1. Preliminary phytochemical screening of *Citrullus colocynthis* Schrader (fruit)**.**

S. No	Secondary metabolite	B_1 Petroleum ether	B_2 Benzene	B_3 Acetone	B_4 Chloroform	B_5 Ethyl acetate	B_6 Distil water
1	Carbohydrates	++	+++	+	+++	++	+++
2	Saponins	+++	+++	+++	+++	+	---
3	Tannins	---	---	---	---	---	+
4	Flavonoids	+++	++	+++	+++	+++	+++
5	Alkaloids)	+	+++	+	++	---	---
6	Glycosides	---	+	+	+++	+	+
7	Proteins	---	---	---	---	---	---
8	Phytosterol & Steroids	++	+++	+++	+++	++	+++
9	Phenols	---	---	---	---	---	+
10	Terpenoids	+++	++	+++	+	++	+++

Table 2. Antimicrobial activity in tuber extract of *Citrullus colocynthis* Schrader (Fruit)

S. N.	Micro-organism	Petroleum ether	Benzene	Acetone	Chloroform	Ethyl acetate	Distil water
1	*Candida albicans*	00	00	00	00	**11mm**	00
2	*Entamoeba histolytica*	00	00	00	00	00	00
3	*Escherichia coli*	00	**12mm**	00	00	00	00
4	*Salmonella typhi*	00		00	00	**13mm**	00
5	*Streptococcus pyogenes*	00	**10mm**	00	**12mm**	**18mm**	00



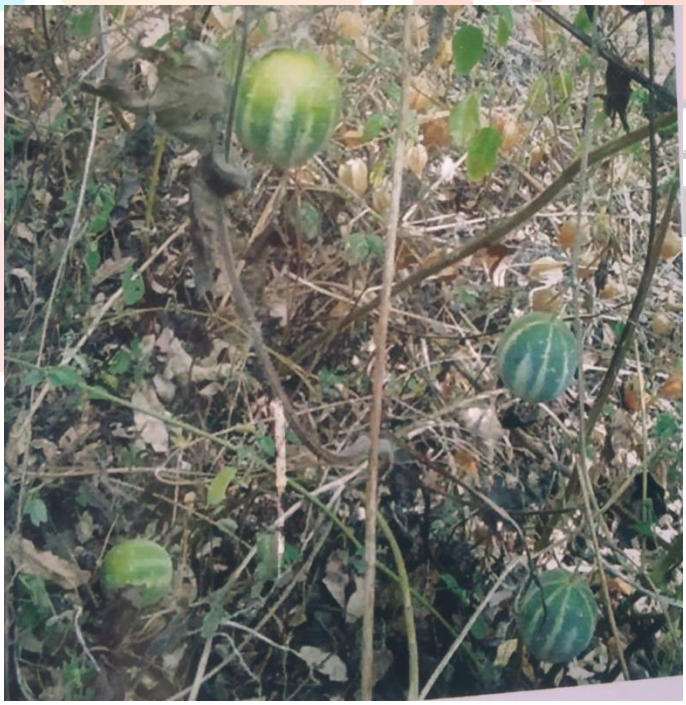
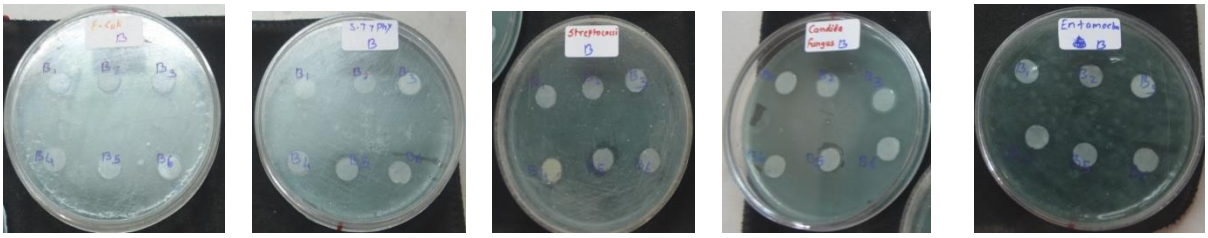


Fig. ***Citrullus colocynthis*** Schrad. (fruit)

Antimicrobial sensitivity test of *Citrullus colocynthis* Schrad. (fruit)



Escherichia coli *Salmonella typh* *Streptococcus pyogenes* *Candida albicans* *Entamoeba histolytica*

extracts and placed on inoculated lawn. Six extracts from each plant parts in ten plants were tested for antimicrobial sensitivity. All the plates were kept for incubation period i.e. 24 hrs. for bacteria and parasites respectively at room temperature. Results were noted down in terms of sensitivity zone around the disc which is measured in millimeter (mm) and results were sequentially recorded in the tabular form.

RESULTS AND DISCUSSION

Table 1 represents various photochemical present in different extracts. The *Citrullus colocynthis* Schrad. (fruit) extract in petroleum ether shows presence of carbohydrate, saponins, flavonoids, alkaloids, phytosterol and terpenoids. While the extract in benzene shows the presence of carbohydrates, saponins, flavonoids, alkaloids, glycosides, Phytosterol and terpenoids. Acetone extract depicts the presence of carbohydrate, saponins, flavonoids, alkaloids, glycosides, phytosterol and terpenoids. The presence of carbohydrates, saponins, flavonoids, alkaloids, glycosides, phytosterol and terpenoids was determined in chloroform extract. The ethyl acetate shows the presence of carbohydrates, saponins, flavonoids, glycosides, phytosterol and terpenoids. Significant amount of carbohydrates, tannins, flavonoids, glycosides, Phytosterols, Phenols and terpenoids were present in aqueous extract of *Citrullus colocynthis* Schrad. (fruit)The benzene extract showed inhibitory activity against *E. coli and S. pyogenes* with a zone of inhibition of 12mm and 10mm respectively. The extract in Chloroform showed positive result *against S. pyogenes* with zone of inhibition of 12mm. Ethyl acetate extract showed the inhibitory activity against *Candida albicans*, *S. typhi* and *S. pyogenes* with maximum zone of inhibition of 11mm, 13mm and 18mm respectively. Flavonoids and terpenoids extracted in all solvent in moderate as well as abundant amount. Terpens have found to inhibit growth of cancerous cells and also decrease micro-organism concentration (Gupta *et al.*2011). The extract in petroleum ether, acetone, and distilled water did not show any antimicrobial activity.

CONCLUSION

The result of present study clearly indicates that, the preliminary phytochemical analysis in fruit extracts of *Citrullus colocynthis* Schrad. (fruit) shows presence of rich amount of metabolites like Carbohydrates, Saponins, Tannins, Flavonoid, Alkaloids, Glycosides, Phytosterol, Steroids, Phenols, and Terpenoids. Fruit extract of *Citrullus colocynthis* Schrad in benzene and ethyl acetate showed good antibacterial activity and it may be due to the presence of phytochemicals and may be used as antimicrobial agents.

REFERENCES

Cheesbrough, M. (1985). Medical Laboratory Manual for Tropical Countries. 17:203-305.

Gupta Neerja, Saxena, G. and Kalra, S.S. (2011). Antimicrobial pattern of certain terpenoids. *International Journal of pharmacy and Bio Sciences*, 1: 87-91.

Harborne, J.B. (1973). Photochemical methods: A guide to modern technique of plant analysis, Chapman and Hills, New York. 1-279.

Hasler, C.M. and Blumberg J.B. (1999). Phytochemicals: Biochemistry and physiology. Introduction, *Journal of Nutrition*,129: 756S–757S.

John, C. Keren, K. and Bradford, K. (1999). Generic protocol to estimate the burden of Shigella diarrhea and dysenteric mortality. *WHO Bulletin.*23 :28-30.

Kirtikar, K.R. and Basu, B.D. (1996). Indian medicinal plants, India, 2 (2):1166.

Kumar, A., Ilavarasn, R., Jayachandran T, Decaraman M., Aravindhan P., Padmanaban N., Krishnan MRV. (2009). Photochemical investigation on a tropical plant. *Pak J Nutri*.8: 83-85.

Mitscer, L.A., Drake, S., Gollopudi, S.R. and Okwute, S.K. (1987). A modern look at folkloric use of anti-infective agents, *Journal of Natural Products*.50 : 1025-1040.