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Research Article ISSN: 2321-5038

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND LIFE SCIENCES
www.pharmaresearchlibrary.com/ijrpls

Phytochemical and Antimicrobial screening of various Extracts of Water melon (*Citrullus vulgariss* L).

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Received: 22 August 2014, Accepted: 30 October 2014, Published Online: 24 November 2014

Abstract

Water melon (*Citrullus vulgariss* L) is both a fruit and a vegetable and plant of a vine-like herb. It is one of the most important fruits worldwide because of its high consumption, year round availability and a major contents of health protective bioactive compounds.It consists of high content of lycopene, vitmin C, vitmin B1, vitmin B6, vitmin A, β-carotene, other phytoconstituents like polyphenols e,g quercetin, kaemferol, naringenin, sugars, aromatic amino acids and sulphur containing amino acids, phytosterols, starch and nutrients e.g P, S, K, Ca, Fe, Mg etc and had good health protective effects. The objectives of the present work is to search antimicrobial activities. Based on this a new series of constituents had been planned to extract by Ethanol (EL), Chloroform (CM), Acetone (AE), Ethyl acetate (EA) and Ethyl acetoacetate (EAA) from watermelon (endocarp). The *in-vitro* antimicrobial activity of various extracts of water melon were carried out by using agar diffusion method using bacterial cultures *Staphylococcus aureus* ***(****ATCC 9144), Bacillus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853,) Escherichia coli (ATCC 25922****)* and fungal cultures *Aspergillus niger (ATCC 9029), Aspergillus flavus (ATCC 204304), Candida albicans (ATCC 10231)*. By observing it was found that most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. The extracts were active against all the tested microorganism for anti bacterial activity with range of MIC values for *S.aureus* (MIC: 15-39 µg /ml), *E.coli* (MIC: 16-38 µg /ml) ,*P.aeruginosa* (MIC:15-39 µg /ml) and *B.subtilis* (14-39 µg /ml). The extracts were active against all the tested microorganism for anti fungal activity with the range of MIC values for *A.niger* (MIC :17-39 µg/ml),*A.flavus* (18-37 µg/ml) and *C.albicans* (16-35 µg/ml).

Keywords: *Citrullus vulgaris* L, Phytoconstituents, Lycopene, Anti bacterial Activity, Anti fungal Activity, MIC etc.

Contents

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Manuscript ID: IJRPLS2314

PAPER-QR CODE

1. Introduction

Watermelon (*Citrullus vulgaris*, family Cucurbitaceae) is a vine-like (scrambler and trailer) flowering plant originally from southern Africa. Its fruit, which is also called *watermelon*, is a special kind referred to by botanists as a pepo, a berry which has a thick rind (exocarp) and fleshy center (mesocarp and endocarp). Pepos are derived from an inferior ovary, and are characteristic of the Cucurbitaceae. The watermelon fruit, loosely considered a type of melon, although not in the genus Cucumis has a smooth exterior rind (usually green with dark green stripes or yellow spots) and a juicy, sweet interior flesh (usually deep red to pink, but sometimes orange, yellow, or white) **[1].** A watermelon contains about 6% sugar and 91% water by weight. As with many other fruits, it is a source of vitamin C. The amino-acid citrulline was first extracted from watermelon and analyzed **[2].** Watermelons contain a significant amount of citrulline and after consumption of several kilograms, an elevated concentration is measured in the blood plasma; this could be mistaken for citrullinaemia or other urea cycle disorders **[3].**

Watermelon rinds, usually a light green or white color, are also edible and contain many hidden nutrients[but most people avoid eating them due to their unappealing flavor. They are sometimes used as a vegetable **[4].** In China, they are stir-fried, stewed or more often pickled. When stir-fried, the skin and fruit is removed, and the rind is cooked with olive oil, garlic, chili peppers, scallions, sugar and rum. Pickled watermelon rind is also commonly consumed in the Southern US. Watermelon juice can be made into wine **[5].** Watermelon is mildly diuretic **[6]** and contains large amounts of carotenoids **[7].** Watermelon with red flesh is a significant source of lycopene. Preliminary research indicates the consumption of watermelon may have antihypertensive effects [**8].**

The literature survey revealed that the various extracts of watermelon were possessed a wide range of pharmacological activities Anticancer, Antiobesity Antidiarrhoeal, Antioxident Antibacterial, Antihypertensive, Antiasthamatic, Antitubercular, Immunomodulatory, Antihypercholesterolemic Wounds healing, Renal calculus inhibitors, Antisylorotic, Antiatherogenic, Antiviral. The number of infections which are caused by multi drug resistant gram positive and gram negative pathogens and viruses are life threatening for human being.Infections caused by these organisms pose a serious challenge to the scientific community and need for a effective therapy has lead for novel antimicrobial agents

2. Materials and Methods

Drugs and chemicals

Standard drug Tetracyclin (Antibacterial) and Ketoconazole were purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from Institutional Store and were of AR grade.Bacterial cultures *Staphylococcus aureus (ATCC 9144), Bacilus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853,) Escherichia coli (ATCC 25922)* and fungal cultures *Aspergillus niger (ATCC 9029), Aspergillus flavus (ATCC 204304), Candida albicans (ATCC 10231)* were provided by the Biotechnology Lab of the CLBMCP, Chennai and maintained on Nutrient agar slant and fungal strains were maintained on Sabouraud dextrose broth at 4^0C.

Extraction [9]

Weigh 50 g of fruits of ***Citrullus vulgaris*** (can be mashed to prepare a paste) into a 500 ml round-bottomed flask. Add 200 ml of methanol . Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 250 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. Here the bioactive constituents are extracted with various solvents and using same extraction procedure. The paste of water melon extracted with Ethanol (EL), Chloroform (CM), Acetone (AE), Ethyl acetate (EA) and Ethyl acetoacetate (EAA) from watermelon and followed same procedure.

Preliminary Phytochemical Screening [10, 11, 12, 13]

Preliminary Phytochemical Screening has to be carried out for the identification of reducing sugars, pentoses, disaccharides, polysaccharides, proteins and amino acids phytosterols, polyphenols and carotenoids etc.

Evaluation of Antimicrobial Activity by paper disc diffusion method [14, 15, 16]

The sterilized (autoclaved at 120^oC for 30 min) medium was inoculated (1mL/100mL of medium) with the suspension [10^5 cfu m/l (colony forming unit per milliliter)] of the microorganism (matched to McFarland barium sulphate standard) and poured in Petridish to give a depth of 3-4mm. The paper impregnated with the test compounds (50, 100,150 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1hr at RT and incubated at 37 ^{o}C for 24 hr for anti-bacterial and antifungal activities respectively. Tetracyclin (100 µg/disc) and Ketoconazoloe (100 µg/disc) was used as a standard.

Determination of MIC by agar streak dilution method [17]

MIC of the various extracts of water melon were determined by agar streak dilution method. A stock solution of the synthesized compounds (100µg/ml) in Dimethyl formamide was prepared and graded quantities of the test

compounds were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the compounds was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately10^5 cfu m/l and applied to plates with serially diluted compounds in Dimethyl formamide to be tested and incubated at 37^{o}C for 24hr. for bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate.

3. Results and Discussion

The phytochemical screening of various extracts of water melon were carried out by using standard procedure and following bioactive compounds were found which had been shown in **Table-1.** The presence bioactive compounds in various extracts of water melon (EL, CM, AE, EA and EAA) were confirmed by their specific qualitative chemical confirmatory tests.

Table 1: Phytochemical Screening of various extracts of Water melon ***(Citrullus vulgaris)***

Phytoconstituents	Presence in Extracts
Reducing sugars	EL(+), CM(+), AE(+), EA(-), EAA(-)
Pentose	EL(-), CM(-), AE(-), EA(-), EAA(-)
Disaccharides	EL(+), CM(+), AE(+), EA(-), EAA(-)
Starch	EL(+), CM(+), AE(+),EA(-), EAA(-)
Glycogen and dextrin	EL(-), CM(-), AE(-), EA(-), EAA(-)
Aromatic amino acids	EL(+), CM(+), AE(+), EA(-), EAA(-)
Tyrosine, Tryptophan, Arginine, citruline	EL(+), CM(+), AE(+), EA(-), EAA(-)
Sulphhur containing and α-amino acids	EL(+), CM(+), AE(+), EA(-), EAA(-)
Phytosterol	EL(-), CM(-), AE(-), EA(+), EAA(+)
Polyphenols	EL(-), CM(-), AE(-), EA(+), EAA(+)
Carotenoids	EL(+), CM(+), AE(+), EA(-), EAA(-)
Flavanoids	EL(-), CM(-), AE(-), EA(+), EAA(+)
Alkaloids	EL(-), CM(-), AE(-), EA(-), EAA(-)
Glycosides	EL(-), CM(-), AE(-), EA(-), EAA(-)

Ethanol (EL), Chloroform (CM), Acetone (AE), Ethyl acetate (EA) and Ethyl acetoacetate (EAA), Presence(+) and Absence (-). The zone of inhibition of various extracts of water melon (*Citrullus vulgaris)* was compared with the standard drug tetracycline for the anti bacterial activity and Ketoconazole for the Anti fungal activity and Minimum Inhibitory Concentration (MIC) of various Extracts of C. vulgaris for bacteria and fungi were shown in **Table-1, 2, 3, and 4 .**

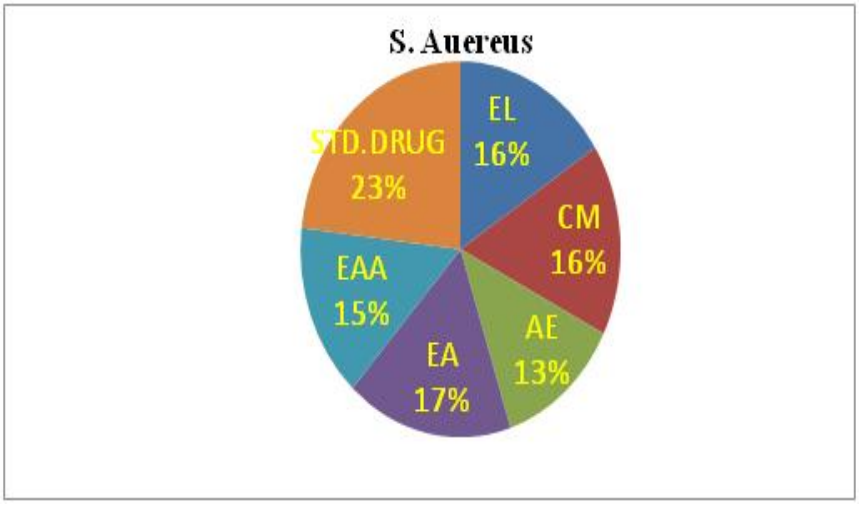

Figure 1: Percentage of zone of Inhibition of various extracts of *C. vulgaris* at concentration of 100 µg /disc against S. aureus.

Table 1: The zone of Inhibition various Extracts of water melon (*Citrullus vulgaris)* (mm) for Anti bacterial activity

Name of The Extracts	S. Auereus			E.coli			P.aeruginosa			B.subtilis		
	Concentration (µg/disc)											
	50	100	150	50	100	150	50	100	150	50	100	150
EC	18	33	39	17	32	38	15	31	37	15	27	37
CM	19	34	40	18	33	39	16	33	38	14	28	38
AE	15	26	30	14	25	29	14	26	31	13	22	29
EA	20	35	41	21	34	41	19	34	40	17	30	38
EAA	17	31	38	16	29	38	16	29	36	14	27	36
Tetracyclin	27	48	57	28	49	58	29	46	55	27	46	56

Control: DMF Standard Drug-Tetracyclin

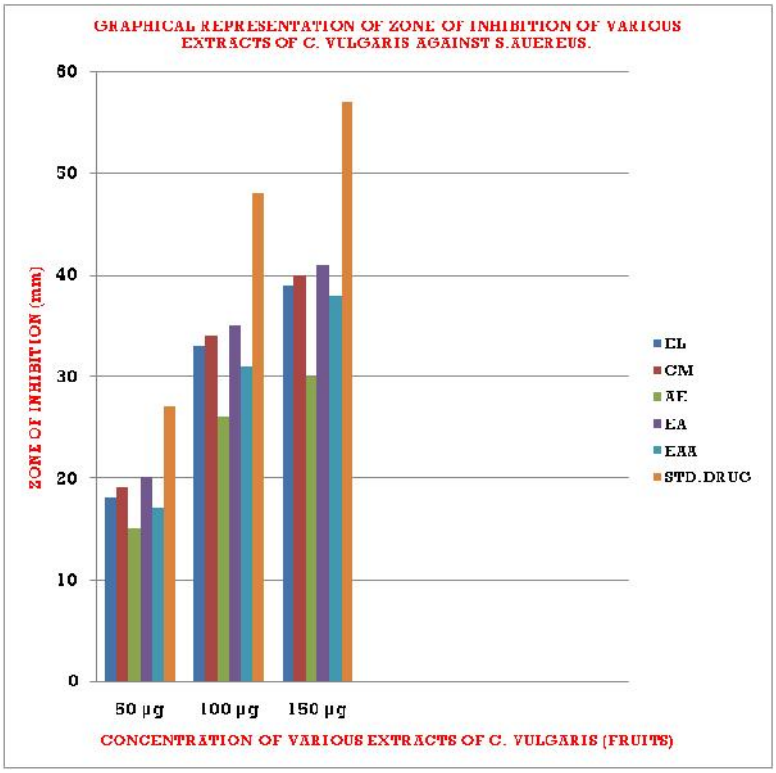

Figure 2: Graphical Representation of Zone of Inhibition of various extracts of C. vulgaris against S.auereus.

Table 2: Minimum Inhibitory Concentration of various Extracts of C. vulgaris (Bacteria)

EXTRACTS	MINIMUM INHIBITORY CONCENTRATION (µg/ml)			
	S.aureus	E.coli	P.aerug	B.subt
EL	36	31	32	35
CM	15	20	29	31
AE	33	32	15	33
EA	17	16	39	14
EAA	39	38	35	39
Tetracyclin	1.2	1.2	1.2	1.2

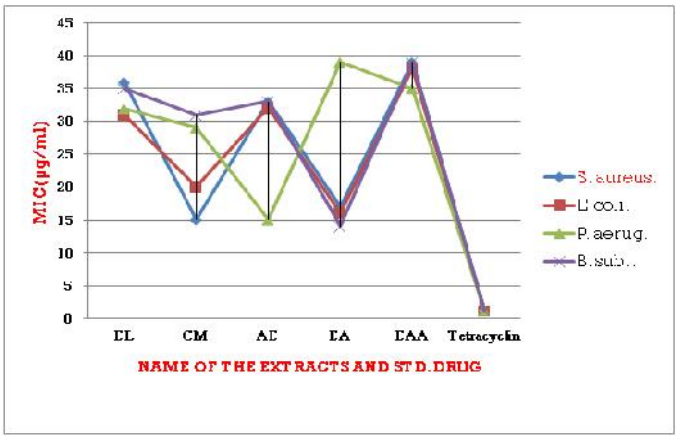

Figure 3: MIC of various Extracts of C.vulgaris (Fruit) for Bacteria

Table 3: The zone of Inhibition various Extracts of water melon (*Citrullus vulgaris)* (mm) for Anti fungal activity

Name of The Extracts	A. niger			A.flavus			C.albicans		
	Concentration (µg/disc)								
	50	100	150	50	100	150	50	100	150
EL	19	20	23	20	20	22	18	20	22
CM	18	19	21	19	19	21	17	19	21
AE	17	16	19	16	18	19	16	19	20
EA	20	22	24	21	22	23	19	21	23
EAA	17	14	15	16	16	17	15	16	19
Ketoconazole (100µg/ml)	38	38	38	35	35	35	36	36	36

Control: DMF, Standard – Ketoconazole

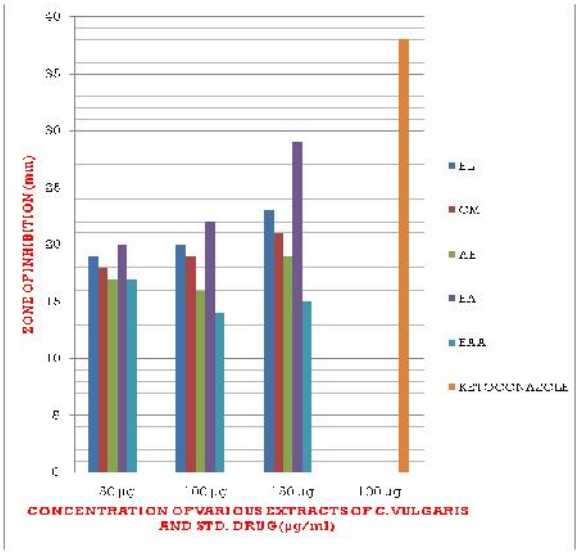

Figure 4: Graphical Representation of Zone of Inhibition of various extracts of C. vulgaris against A.niger

Table 4: Minimum Inhibitory Concentration of various Extracts of C. vulgaris (Fungi)

Extracts	Minimum Inhibitory Concentration (µg/ml)		
	A.niger	A.flavus	C.albicans
EL	17	36	32
CM	35	31	16
AE	34	40	30
EA	25	18	21
EAA	39	37	35
Ketoconazole	6.1	6.1	6.1

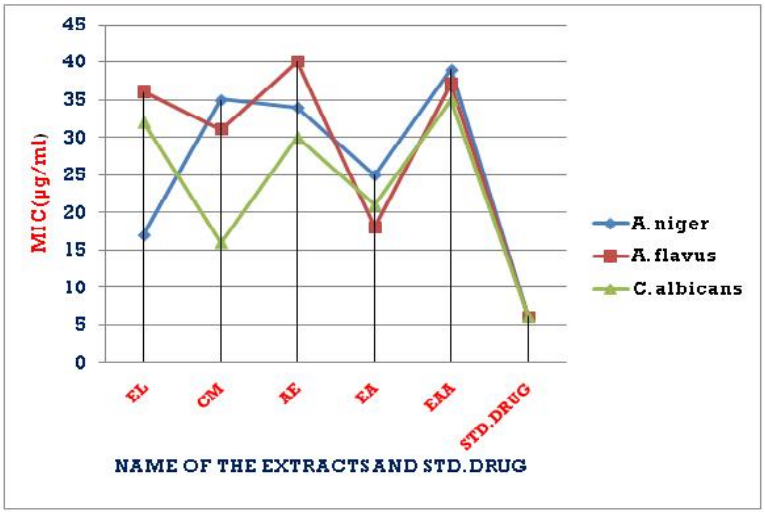

Figure5: MIC of various Extracts of C.vulgaris (Fruit) for Fungi.

4. Conclusion

By observing it was found that most of the extracts (EL, CM, AE, EA and EAA) exhibit moderate to good antibacterial activity with an range of MIC between 15-39 µg/ml and antifungal activity with an range of MIC between 16-39 µg/ml. It was found that the extracts CM, EA and EAA were exhibited good antibacterial activity and the extracts EA, EL and CM were exhibited good antifungal activity.

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