ISSN : 0974 - 7427 Volume 6 Issue 3

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Minireview

BCAIJ, 6(3), 2012 [104-109]

Invitro antimicrobial screening and phytochemical profile of *Cucurbitaceae* fruits extracts

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Received: 23rd February, 2012 ; Accepted: 23rd March, 2012

ABSTRACT

Phytochemical screening and anti bacterial activity were carried out in Indian Cucurbitaceae species viz. Cucurbita pepo, Momordica charantia, Coccinia indica. Petroleum ether, aqueous, methanol, and ethanolic crude extracts of fruits were evaluated for antimicrobial activity using the well diffusion method on reference microorganisms. Phytochemical screening revealed the presence of various compounds such as alkaloids, steroids, phenolic compounds,glycosides etc.,. Methanolic extract has the potentive antimicrobial activity against B.Subtilis, and E. coli when compared to other extracts. In conclusion, most of the cucurbitaceae plants contain nearly the same compounds and also exhibited anti microbial activity against B.Subtilis,and E. coli.

KEYWORDS

Cucurbitaceae;
Cucurbita pepo;
Momordica charantia;
Coccinia indica;
E.coli;
B.Subtilis.

INTRODUCTION

The *cucurbitaceae* consists of nearly 100 genera and over 750 species. Although most have Old world origins, many species originated in the New world and at least 7 genera have origins in both hemispheres. There is a tremendous genetic diversity within the family, and the range of adaptation for cucurbit species includes tropical and subtropical regions, arid deserts, and temperate locations. Plants belonging to the *Cucurbitaceae* family include common fruit vegetables such as the cucumber, pumpkin, winter melon, bitter gourd and bottle gourd, as well as, dessert fruits such as the watermelon and honeydew. Cucurbit plants produce male and female flowers. Female flowers need to be pollinated so that fruits will form. Pollination involves the transfer of pollen from the stamens of a male flower to the stigma of a female flower. Pollination brings about the fertilization of the ovules found inside the ovary of female flowers. It is difficult to ascertain whether female flowers are pollinated successfully and the only way is to watch the fruit developmental process. The ovaries of poorly pollinated female flowers may swell initially to give a young fruit but they turn yellow subsequently and are aborted eventually. There are occasions that poorly pollinated flowers continue to develop into deformed-looking fruits. In outdoor gardens where pesticides are used, poor fruit set by cucurbit crops may occur due to the reduction or absence of natural pollinate or populations. Beneficial insects such as bees are attracted to the bright yellow flowers of cucurbits.

A few species are adaptable to production at el-

evations as high as 2000 meters. Cucurbits are a well-recognized source of secondary metabolites. Therefore, cucurbits are among the largest and the most diverse plant families are cultivated worldwide in a variety of environmental conditions. Cucurbits (*cucurbitaceae*) are among the most important plant families supplying humans with edible products and useful fibers. They are associated with the origin of agriculture and human civilization and are among the first plant species to be domesticated in both Old and New world. Luffa acutangula, L. cylindrica etc. are the examples of the cucurbitaceous plants. From literature survey it is noticed that few plants of this family possess ribosome inactivating proteins (such as MAP30, Luffin A and B) and terpenes with immunomodulatory, antiretroviral, anti HIV activities besides other pharmacological actions viz. Antidiabetic, hyperlipidemic, antioxidantant anticancer.

Active components of Cucurbitaceae

Alkaloids, fatty acids, flavonoids, glycosides, lignins, phenols, saponins, steroids, tannins and triterpenoids.

TAXONOMY

TABLE 1 : Kingdom: Plantae; Order: Cucurbitales; Family: Cucurbitaceae;

Common Name	Genus	Species	Binomial Name
Ivy gourd	Coccinia	Indica	Coccinia indica
Bitter gourd	Momordica	Charantia	Momordica charantia
Pumpkin	Cucurbita	Pepo	Cucurbita pepo

Cucurbita pepo Linn

Pumpkin - English; Calbaza - Mexico/Spanish; Elegede - Yoruba; Huiioy - Guatemala[1].

The species, *Cucurbito pepo* is a cultivated plant of the genus *Cucurbita*. It includes varieties of squash and guord[16]. The medium sized plant grown for it fruits and edible seeds. Hence, it is known to be used as human food in Nigeria. Other members of the family are also available. It has been used locally in Eritrea to treat tapeworm and has also been used in other regions of the world to treat the early stages of prostate disorders. Its use in this prostate condition is due to its high zinc content[1]. The seeds and pulp of *C. pepo* is used for medicinal values as it irritates the intestinal tract of parasites and worms. It is also used to treat urinary tract problem and gastritis and to remove tapeworms and roundworms from the intestine. Pumpkin is a gourd like squash of the genus *Cucurbita* and the family Cucurbitaceae, has high antioxidative, antidepressive, anti-helminthic activity and antimicrobial activity[16].

Culture and use of cucurbits or squashes (*Curcubita foetidissima*, *C. pepo*, and *C. lagenaria*) have been traced to more than 10 000 years ago. Although still not widely used by the food industry, squashes are consumed worldwide. Fruits are consumed as vegetables or dessert (pie) and seeds as nuts and, to a lesser extent, as cooking oil. Because of their resistance to drought and the high protein (23-35%) and oil (25-55%) contents of their seeds, squashes have attracted the attention of many growers and plant breeders within the past 50 years. Although many works have been reported on squashes, only a few were related to the naked seed variety. In recent years, Warid et al. (1993) have evaluated 8 lines of naked seed squash from a pool of 100 Austrian lines on the bases of production performance, number of fruit, weight of seeds, and pepo size. Plants produced one to nine fruits weighing 0.47-12.67 kg. The number of seeds per fruit ranged from 16 to 393. Individual naked seed weight ranged from 46 to 223 mg. Naked seeds have the advantage of lacking the seed coat, making them less costly to produce since there is no need for the expensive decorticating process. They therefore may be favored by the oil and nut industries for commercial production[2].

Coccinia indica

Tindora, Tondli - Marathi; Kunduri - Oriya; Ghiloda, Kundru - Malayalam; Kovakka - Tamil; Dondakaya – Telugu[9].

C. indica grows abundantly all over India, Tropical Africa, Australia, Fiji and throughout the oriental Countries[6]. It is cultivated abundantly in india (Assam. Bihar, Orissa Maharashtra, Andhra Pradesh, Tamil Nadu) as a vegetable and its wild form is also found in many parts of India[7]. Fruits are fusiform-ellipsoid, slightly beaked, 2.5-5 by 1.3-2.5 cm marked when immature with white streaks, bright scarlet when fully riped. This plant is traditionally used in various diseases like psoriasis, ringworm, itching, small pox, skin diseases, ulcer, scabies, diabetes, asthma, bronchitis, dysentry, vomiting, cough and cold[14]. Earlier investigation of *Coccinia indica* showed that the crude ex-

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tract has hepatoprotective antioxidant anti-inflammatory and anti-nociceptive anti-diabetic hypolipidemic anti-bacterial and antitussive activities[3]. Pharmacological studies on *Coccinia indica* demonstrated antimicrobial, antilitihic and antioxidant activities. The plant is used as a laxative. It is used internally in the treatment of gonorrhea. Aqueous and ethanolic extracts of the plant have shown hypoglycemic principles[2-4]. It is used in Ayurvedic medicine to treat 'sugar urine.' Ayurvedic medicine is a traditional East Indian healing system. *Coccinia indica* appears to have insulin-mimetic properties, although its effects are not well understood. In one controlled clinical trial of 70 participants, the use of dried herb pellets for 12 weeks was as effective as treatment with chlorpropamide (an oral hypoglycemic drug) for lowering blood glucose levels[10].

Momordica charantia Linn

Bitter gourd - English; Paakharkaai - Tamil; Karela - Hindi and Bengali; Kakarakaya - Telugu; Hagalakayi – Kannada[8]

Momordica Charantia or Bitter Melon, also known as balsam pear or Karela, is a Tropical vegetable, is a common food in Indian cusine and has been used extensively in folk medicine as a remedy for diabetes[13]. Unripe fruits of bitter melon have been found to have blood sugar lowering capacity, similar to that of insulin and can be used to treat patients with diabetes[15]. *Momordica charantia* L. (*Cucurbitaceae*) is a creeping plant native of Asia and found throughout the world[4]. The biological components of MC include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids. The phenolic compounds of MC have been reported to exhibit antioxidant activity. Antioxidant, anti-diabetes, anti-inflammatory, anti-bacterial and anti-cancer effects of MC have also reported. Several diseases and microbial infections such as respiratory infections, bacterial meningitis, sexually transmitted as well as hospital acquired infections, particularly those caused by the members of the family Enterobacteriaceae have shown considerable resistance to a number of antimicrobial agents, such as penicillin, ampicillin, and flouroquinolones among many others. Synthetic antioxidants, such as butylate hydroxyanisole (BHA), teributylhydroquinone (TBHQ), butylated hydroxytoluene (BHT) and propyl gallate (PG) are carcinogenic[11]. The Latin name Momordica means "to bite" (referring to the jagged edges of the leaf, which appear as if they have been bitten). In Ayurveda, the fruit is considered as tonic, stomachic, stimulant, emetic, antibilous, laxative and alterative. Bitter melon has been used in various Asian traditional medicine systems for a long time. Like most bitter-tasting foods, bitter melon stimulates digestion. While this can be helpful in people with sluggish digestion, dyspepsia, and constipation, it can sometimes make heartburn and ulcers worse. The fact that bitter melon is also a demulcent and at least mild inflammation modulator, however, means that it rarely does have these negative effects, based on clinical experience and traditional reports[12] Psoriasis is said to be an immune based skin disorder, but exact cause and pathophysiology of the disease is not clearly understood, however it is said to be a chronic, genetically influenced, immunological based inflammatory disease of the skin[12]. It has numerous uses in popular folk medicine. Its leaves and roots serve as anti-rheumatic, anti-inflammatory, antiseptic and anti-diabetic remedies in Brazil 1,2. In Guatemala, Caribe, Japan and India it has been used in inflammation, diabetes and stomach problems.

PHYTOCHEMICAL EVALUATION[11]

Phytochemical examinations were carried out for all the extracts as per the standard methods[11].

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

a) Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.

b) Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow colored precipitate indicates the presence of alkaloids.

Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's test: Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehlings A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides

b) Legal's test: Extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of phenols

a) Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

a) Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

a) Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for sterols & triterpenoids[5]

a) Libermann-Buchard test: Extract is treated with few drops of acetic anhydride, boil and cool, con. Sulfuric acid is added from the sides of the test tube, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of Steroids and formation of deep red color indicates the presence of triterpenoids[5].

b) Salkowski test: Treat extract in Chloroform with few drops of cone. Sulfuric acid, shake well and allow standing for some time, red color appears at the lower layer indicates the presence of Steroids and formation of yellow colored lower layer indicates the presence of Triterpenoids[5].

PHYTOCHEMICAL CHARACTERS

Petroleum ether, ethanol, aqueous and methanolic

TABLE 2

Phytochemicals	C.pepo				M.charantia				C.indica			
	Solvents				Solvents				Solvents			
	M.E	E.E	A.E	P.E	M.E	E.E	A.E	P.E	M.E	E.E	A.E	P.E
Carbohydrates	+	+	-	-	+	+	+	+	+	-	+	-
Flavonoid	+	+	-	-	-	-	-	+	+	-	-	-
Steroids	-	-	-	+	-	-	-	+	+	-	-	+
Tannins	+	-	-	-	+	+	+	+	+	-	-	-
Phenols	+	-	-	-	-	-	-	-	+	-	-	-
Alkaloids	-	-	+	-	+	+	+	+	+	+	+	-
Glycosides	+	-	+	-	+	+	-	-	+	+	+	-

M.E - Methanol extract; E.E - Ethanol extract; A.E - Aqueous; P.E - Petroleum ether; C.pepo - cucurbita pepo; M.charantia - Momordica charantia; C.indica - Coccinia indica; + = present; - = Absent

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crude extracts of the fruits were tested for phytochemical analysis. Results were presented in TABLE 2

ANTIMICROBIAL ACTIVITY

Microorganisms

The antimicrobial activity of the aqueous and methanolic extracted was tested individually on four different micro organisms: *Escherichia coli*, *Bacillus subtilis*. It was tested by using stokes disc diffusion sensitivity technique and well diffusion methods.

Stokes disc diffusion method

In stokes disc diffusion method, a loop of bacteria from the agar slant stock was cultured in nutrient broth over night and spread with a sterile cotton swap into petri plates containing 10 ml of nutrient agar medium. Sterile filter paper discs (9mm in diameter) impregnated with the plant extract were placed on the cultured plates and incubated at 37°c for 24 hrs. The solvent without extracts served as negative control. Standard antibiotic streptomycin (10µg) was employed as positive control. After 24 hrs of incubation an antibacterial activity was assessed by measuring the inhibition zone. The diameters of the zones of inhibition by the samples were then compared with the diameters of the zones of inhibition produced by the standard antibiotic discs. Each experiment was carried out in triplicate and the mean diameter of the inhibition ones was recorded[8].

Well diffusion technique

Screening of anti bacterial activity was performed by well diffusion technique. The nutrient agar plates were seeded with 0.1 ml of standardized inoculums of each of the four test organisms. The inoculum was spread evenly over plate with loop or sterile glass spreader. The inoculated plates were incubated at 37°c for 20 minutes. After incubation a standard cork order of 6 mm diameter was used to cut uniform wells on the surface of nutrient agar medium and 10µl of the extracts was introduced in the well and incubated at 37°c for 24 hrs and the one of inhibition was measured in millimeter (mm). Mean zone of inhibition and standard deviations were calculated[8].

CONCLUSION

In conclusion, antimicrobial activity is due to the presence of various compounds. Most of the cucurbitaceae plants contain nearly the same compound such as sterols, tannins, glycosides, phenols etc., and also exhibited antimicrobial activity against E.coli and B.subtilis with consistent zone of inhibition. Methanol extraction shows effective antimicrobial activity when compared to other solvents.

ACKNOWLEDGEMENT

We are indebted to Prof. K.Subathra madam Head of the department of biotechnology, Government College of technology, Coimbatore (Tamil nadu) India for constant support and encourage our work successfully.

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