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In vitro antimicrobial screening and phytochemical profile of Cucurbitaceae leaves extracts

P.Santhasheela*, P.Renuka Devi, P.Kaleeswari, A.Ramya, M.Saritha

Department of Biotechnology, Government College of technology, Coimbatore, TamilNadu (INDIA)

E-mail : shanthi_biochem07@yahoo.co.in

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ABSTRACT

The aqueous, ethanolic, methanolic, petroleum ether and chloroform extracts of *Coccinia grandis*, *Citrullus lanatus*, *Momordica charantia*, *Cucurbita pepo*, (Cucurbitaceae) leaves were evaluated for antimicrobial activity against bacterial strains such as *E.coli*, *Bacillus subtilis*. The *in vitro* antimicrobial activity was performed by disc diffusion and well diffusion technique. Extracts were found to contain alkaloids, flavonoids, glycosides and carbohydrates by various phytochemical tests. Of the four extracts tested, aqueous ethanol extract was found to exert consistently better antibacterial activity than methanol extract. In conclusion, most of the cucurbitaceae plants contain nearly the same compounds and also exhibited antimicrobial activity against bacterial strains such as *E.coli*, *B.subtilis*. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Coccinia grandis;
Cucurbita pepo;
Momordica charantia;
Citrullus lanatus,
E.coli;
B.subtilis.

INTRODUCTION

The use of plants and plant products as medicines could be traced back from human civilization. The herbal wealth of India and the knowledge of their medicinal properties have along tradition, as referred in Rig Veda and other ancient literature. The topography of India which is in the tropical belt with its varied climatic zones makes it a vast storehouse of medicinal plants. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (Rojas et al., 2003). Antimicrobials of plant origin have enormous therapeutic potential. The potential

plant antimicrobials are combinations of the secondary metabolites such as alkaloids, steroids, resins, tanins, phenolics, flavonoids, steroids and fatty acids which have a potential effect on the physiological effects on the body^[16]. *Coccinia grandis* L.Voigt, *Cucurbita pepo*, *Citrullus lanatus*, *Momordica charantia* of the family Cucurbitaceae is distributed in tropical Asia, Africa and is commonly found in Pakistan, India, Bangladesh and Srilanka. *Coccinia grandis* is a climber and trailer. Every part of this plant is valuable in medicine and various preparations have been mentioned in indigenous system of medicine for various skin diseases, bronchial catarrh, bronchitis and Unani systems of medicine for ring worm, psoriasis, small pox, and scabies and other itchy skin eruptions and ulcers^[5]. *Momordica charantia*, a climber belonging to family Cucurbitaceae, is commonly known as bitter gourd or

bitter melon in English and kerela in Bengali. Momordica means, to bite (referring to the jagged edges of the leaf, which appear as if they have been bitten). All parts of the plant, including the fruit have bitter taste. In Bangladesh, various medicinal properties are claimed for *M.charantia* that include antidiabetic, abortifacient, anthelmintic, contraceptive, antimalarial and laxative and is used for treatment of dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhea^[4]. *Citrullus lanatus var. citroides* (Wild melon), is used widely in traditional herbal medicine.

The fruit is also diuretic, being effective in the treatment of dropsy and renal stones (Chiej, 1984). The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes (Duke and Ayensu, 1985). It is sometimes used in the treatment of the urinary passages (Grieve and Leyel 1984) bed wetting (Moerman, 1998). The seed is also a good vermifuge and has a hypotensive action. Fatty oil in the seed, as well as aqueous or alcoholic extracts, paralyze tapeworms and roundworms (Chopra 1958). In Northern Sudan is often used for burns, swellings, rheumatism, gout and as laxative (Schippers and Budd, 1997). Cultivated pumpkins are believed to have originated for central Africa as far back as 5500BC and are now grown all over the world (Satyavati et al., 1976). They vary considerably in shape, size and flavour. *Cucurbita pepo* (Pumpkin) has long been used as food and a source of lamp oil, but now, it serves as a raw material of paramedicinal product (Dermarderosian and Butler, 2002). Pumpkin is used as an emollient to soften the dryness of the skin and pimples spots (Duke and Ayensu 1985). Scientific studies mainly refer to Middle East and Asia where cucurbit plants were used actively as herbal remedies have shown tremendous results regarding the use of this botanical family. So the aim of this review focused the cucurbitaceae family for its antimicrobial activity with the help of phytochemical analysis.

Taxonomy

Active components of Cucurbitaceae:

Alkaloids, anthroquinones, fatty acids, flavonoids, glycosides, leucoanthocyanins, lignin's, phenols, reducing sugars, saponins, steroids, tannins and triterpenoids.

Coccinia grandis L. Voigt

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

Common Name	Genus	Species	Binomial Name
Ivy gourd	<i>Coccinia</i>	<i>Grandis</i>	<i>Coccinia grandis</i>
Bitter gourd	<i>Momordica</i>	<i>Charantia</i>	<i>Momordica charantia</i>
Pumpkin	<i>Cucurbita</i>	<i>Pepo</i>	<i>Cucurbita pepo</i>
Watermelon	<i>Citrullus</i>	<i>Lanatus</i>	<i>Citrullus lanatus</i>

Eng: Ivy gourd Oriya: Parwal, Kundru
Kannada: Tondekayi

Mal: Tendli (konkani) Tam: kovakka Tel: Dondakaya

From Botanical description of *coccinia grandis*, it is a dioecious perennial herbaceous vine. Stems are mostly glabrous, produce annually from a tuberous root stock, tendrils simple, axillary. Leaves are alternate, simple, blade broadly ovate, 5-lobed, ((5-9)×(4-9)cm), acute and mucronate at the apex, cordate with a broad sinus at the base. Surfaces are glabrous or scaly, with 3 to 8 glands near the base, margins denticulate, petiole (1-5cm) long. Inflorescence is usually of solitary, axillary flowers. Calyx is of five subulate, recurved lobes (2-5mm) long on the hypanthium. Peduncle is (1-5cm) long. Corella is campanulate, white, (3-4.5cm) long, deeply divided into 5-ovate lobes. Stamens are 3, present as staminodes in female flowers. Ovary is inferior. Fruit is a smooth, bright red, ovoid to ellipsoid berry (2.5-6cm) long. Synonym of botanical name is "cephalandra indica" (Ivy gourd)^[18]

Morphological characters of *Coccinia grandis*

Its perennial herbs with tuberous root stock produce annual stems upto several meters long, which is found spreading on ground and twining around the tress and supports around it. Leaves are triangular or pentagonal in shape. Margin is dentate, upper surface glabrous and attachment of petiole and major vein branching occurs. Apex obtuse, petioles (1-3cm) long and tendrils are unbranched. Flowers are monscious, solitary, rarely in axillary clusters of 2-3, pedicels 10-15mm long. Fruits are slimy in touch, pulpy and ovoid to ellipsoid shape. It is green in colour when young and it turns scarlet red when it ripens (2.5-5cm) long and (1.3-2.5cm) in diameter, glabrous, purple red. The fruit possesses numerous seeds which are ablong (6-7mm), margins thickened.

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Momordica charantia Linn.

Eng: Bitter gourd, Carilla fruit San: Karavellam Hin: Karela, Kareli

Mal: Kaypa, Paval Tam: Pavakkai, Paval, Pakar Tel: Kakara

Bitter gourd or Carilla fruit is a branched climbing annual which is cultivated throughout India. It is a monoecious plant with angled and grooved stems and hairy or villous young parts. Tendrils are simple, slender and elongate. Leaves are simple, orbicular, cordate and deeply divided into 5-7 lobes. Flowers are unisexual, yellow and arranged on 5-10cm long peduncles. Fruits are 5-15cm long with 3-valved capsules, pendulous, fusiform, ribbed and beaked bearing numerous triangular tubercles. Seeds are many or few with shining sculptured surface. The roots are useful in coloptosis and ophthalmopathy. The leaves are useful in vitiated conditions of pita, helminthiasis, constipation, intermittent fever, burning sensation of the sole and nyctalopia. The fruits are useful in skin diseases, leprosy, ulcers, wounds, burning sensation, constipation, anorexia, flatulence, colic, helminthiasis, rheumatism, gout, diabetes, asthma, cough, dysmenorrhoea, impurity of breast milk, fever and debility. Seeds are useful in the treatment of ulcers, pharyngodynia, and obstructions of the liver and spleen. The leaves and fruits are used for external application in lumbago, ulceration and bone fractures and internally in leprosy, haemorrhoids and jaundice (Warrier et al, 1995). The drug improves digestion, calms down sexual urge, quells diseases due to pitta and kapha and cures anaemia, anorexia, leprosy, ulcers, jaundice, flatulence and piles. Fruit is useful in gout, rheumatism and complaints of liver and spleen (Nadkarni, 1954; Aiyer and Kolammal, 1966; Mooss, 1976; Kurup et al, 1979). Kaccoradi taila is an important preparation using the drug (Sivarajan et al, 1994). The seeds give triterpene glycosides, named momordicosides A, B, C, D and E, which are glycosides of cucurbit-5-en-triol, tetraol or pentaol. Leaves and vines give tetracyclic triterpenes-momordicines I, II and III (bitter principles). Immature fruits give several non-bitter and 2 bitter cucurbitacin glycosides. Four of the non-bitter glycosides, momordicosides F1, F2, G and I and the bitter momordicosides; K and L have also been characterized. Fruits, seeds and tissue culture give a polypeptide which contained 17 types of amino acids and showed

hypoglycaemic activity. Fruits also give 5-hydroxy tryptamine and a neutral compound charantin (a steroidal glucoside), diosgenin, cholesterol, lanosterol and sitosterol. Leaf is emetic, purgative and antibilious. Fruit is stomachic, tonic, carminative, febrifuge, antirheumatic and hypoglycaemic. Root is astringent. Fruit and leaf is anti-leprotic. Fruit, leaf and root are abortifacient and anti-diabetic. Leaf and seed isanthelmintic. Seed oil possesses antifeeding and insecticidal properties. Unsaponifiable matter from seed oil exhibited pronounced inhibitory activity against gram negative bacteria. Seed and fruit are hypoglycaemic, cytotoxic and anti-feedant (Husain et al, 1992)^[6]

Other important species belonging to the genus *Momordica* are as follows.

M. dioica Roxb., *M. cochinchinensis*, Spreng. *M. tuberosa* Cogn., *M. balsamina* Linn.

Citrullus vulgaris Schrad. syn. *C. lanatus* (Thunb.) Mats. & Nakai.

Eng: Water melon; San: Tarambuja; Hin: Tarbuj; Ben: Tarbuz

Mal: Thannimathan; Tam: Pitcha, Dharbusini

Watermelon is an extensively climbing annual which is largely cultivated throughout India and in all warm countries. It has thick angular branching stems. Tendrils are bifid, stout and pubescent. Leaves are long, deeply divided or moderately lobed, glabrous or somewhat hairy and hardly scabrous. Petiole is a little shorter than the limb and villous. Calyx-lobes are narrowly lanceolate, equalling the tube. Corolla is yellow within, greenish outside and villous. Lobes are ovate-oblong, obtuse and prominently 5-nerved. Fruit is sub-globose or ellipsoid, smooth, greenish or clouded, often with a glaucous waxy coating.

Flesh is juicy, red or yellowish white. Seeds are usually margined. *C. vulgaris* var. *fistulosus* Duthie & Fuller. syn. *C. fistulosus* has its fruit about the size of small turnip, the seeds of which are used medicinally. The fruit is tasteless when unripe and sweet when ripe. The unripe fruit is used to cure jaundice. Ripe fruit cures kapha and vata and causes biliousness. It is good for sore eyes, scabies and itching. The seeds are tonic to the brain and used as a cooling medicine. An emulsion of the seeds is made into a poultice with the pounded leaves and applied hot in cases of intestinal inflammations (Kirtikar and Basu, 1988). Fruit juice is good in

quenching thirst and it is used as an antiseptic in typhus fever with cumin and sugar. It is used as a cooling drink in strangury and affections of urinary organs such as gonorrhoea; in hepatic congestion and intestinal catarrh. The bitter watermelon of Sind is known as "Kirbut" and is used as a purgative. Seeds yield a fixed oil and proteids; citrullin. Seeds are cooling, demulcent, diuretic, vermifuge and nutritive. Pulp is cooling and diuretic. Fruit-juice is cooling and refreshing (Nadkarni, 1982)^[6].

Curcubita pepo Linn. syn. *Maxima vulgaris* et *P. verrucosus* Moench Meth.

Eng: Pompion, Pumpkin, Vegetable Marrow; San: Karkaru, Kurkaru, Kushmandi

Hin, Ben: Kadimah, Konda, Kumra, Safedkkadu; Mal: Mathan, Matha

Tel: Budadegummadi, Pottigummadi

Pompion or Pumpkin is a climbing herb which is considered to be a native of America and cultivated in many parts of India. The stem and leaves are with a harsh prickly armature. Foliage is stiff, more or less rigid and erect. Leaves are with a broad triangular pointed outline and often with deep lobes. Corolla is mostly with erect or spreading (not drooping) pointed lobes, the tube narrowing towards the base. Peduncle is strongly 5-angled and little or much expanding near the fruit. The fruit is cooling and astringent to the bowels, increases appetite, cures leprosy, 'kapha and vata', thirst, fatigue and purifies the blood. The leaves are used to remove biliousness. Fruit is good for teeth, throat and eyes and allays thirst. Seeds cure sore chests,

haemoptysis, bronchitis and fever. It is good for the kidney and brain. The leaves are used as an external application for burns. The seeds are considered anthelmintic. The seeds are largely used for flavouring certain preparations of Indian hemp, and the root for a nefarious purpose, viz., to make the preparation more potent. The seeds are taeniace, diuretic and demulcent. The fruit is cooling, laxative and astringent. The leaves are digestible, haematinic and analgesic. The other important species belonging to the genus *Cucurbita* is *C. pepo* Duchena, the seeds of which are a popular remedy for tape-worm and oil as a nervine tonic (Kirtikar & Basu, 1988)^[6].

Phytochemical characters

Petroleum ether, chloroform, ethanol, and methanolic extracts of the leaves 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.

TABLE 2

Phytochemicals	Solvents															
	Petroleum ether				Chloroform				Ethanol				Methanol			
	CG	MC	CP	CL	CG	MC	CP	CL	CG	MC	CP	CL	CG	MC	CP	CL
Sterols	+	+	+	+	-	+	-	+	-	+	-	+	+	+	-	+
Tannins	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+
Flavonoids	-	+	-	+	-	-	-	+	+	+	+	+	+	-	+	+
Proteins and amino acids	-	-	+	+	+	+	+	+	+	-	+	+	-	-	+	+
Glycosides	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+
Phenols	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+
Carbohydrates	-	+	-	-	-	+	-	-	+	-	+	-	-	+	+	-
Saponins	-	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Alkaloids	-	+	-	+	+	-	-	+	+	+	-	+	+	+	-	+

CG-Coccinia grandis, MC-Momordica charantia, CP-Cucurbita pepo, CL-Citrullus lanatus, + => Present, - => Absent

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Sterile filter paper discs (9 mm 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^[7].

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^[7].

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. Ethanol extraction shows effective antimicrobial activity when compared to other solvents.

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