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Research article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ASSAY OF VARIOUS SEEDS EXTRACT OF *CUCURBITACEAE* FAMILY

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ABSTRACT : The present study deals with the antimicrobial activity and phytochemical screening of seeds extract of five plants of Cucurbitaceae family- *Momordica charantia* (Karella), *Cucumis sativa* (Cucumber), *Praecitrullus fistulosus* (Tinda), *Cucurbita pepo* (Kaddu), *Lagenaria siceraria* (loki) that are commonly available and readily consumed in India. Results of antimicrobial activity revealed that all the seeds extracts were very effective against *Serratia marcescens , E. coli, Streptococcus thermophilous, Fusarium oxysporium, Trichoderma reesei* while some extracts showed no inhibition against *Aspergillus niger (Cucumis sativa), Candida albicans (Praecitrullus fistulosus*, *Cucurbita pepo*, *Lagenaria siceraria*). Phytochemical analysis of these plants confirms the presence of various phytochemicals like tannins, cardiac glycosides, terpenoides, carbohydrates, resins, saponins and phytosterols. While other phtochemicals like alkaloids, flavonoids, glycosides, steroidal terpenes and phylobatamins were found to be absent in all the extracts. These plants can be a source of useful drugs but further studies are required to isolate the active component from the crude plant extract for proper drug development.

Keywords: Phtochemical screening, antimicrobial assay, *Cucurbitaceae*

INTRODUCTION

The use of plants as source of remedies for the treatment of diseases can be traced back to the prehistoric times. There has been a considerable growth in the field of herbal medicine due to its natural origin and lesser side effects. A wide range of medicinal plant parts like root, stem, flower, fruit, twigs exudates and modified plant organs has been used for extraction of raw drugs. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). These chemicals are termed as phytochemicals.

Phytochemicals are bioactive non-nutrient plant compounds that have protective or disease preventive property. The word 'phyto-' is derived from a Greek word *phyto* which means - plant. They confer plants with odour (terpenoids), pigmentation (tannins and quinines), and flavour (capsacin) (Mallikharjuna et al., 2007) and are a part of plant naturally defence system. These bioactive components are said to be responsible for the antimicrobial effects of plant extracts *in vitro*. They are grouped as flavonoids, alkaloids, glycosides, saponins, tannins, terpenoids, carbohydrates, and sterols. Tang et al., (2010) have isolated the antimicrobial sphingolipids from cucumber stem.

Cucurbitaceae is a plant family, also known as gourd family, which includes crops like cucumbers, squashes, luffas and melons. Cucurbits form an important and a big group of vegetables crops cultivated extensively in the subtropical and tropics countries. The family consists of about 118 genera and 825 species (Rai et al., 2008). Plants of this family have many medicinal and nutritional benefits (Gill & Bali, 2011).

Momordica charantia (Karela) commonly known as bittergourd is an economically important medicinal plant belonging to the family Cucurbitaceae. Its fruit extract act as anti-diabetic agent in normal and alloxan-diabetic rats (Kolawole et al., 2011). Ribosomes inactivating protein from M. charantia can be used as antiviral therapy (Puri et al., 2009). It also has antimicrobial, antioxidant activity (Leelaprakash et al., 2011), antitumor activity toward human nasopharyngeal carcinoma cells in vitro and in vivo (Fang et al., 2011).

Cucumis sativa (Cucumber) is a widely cultivated plant of gourd family which is eaten in the unripe, green form. Its fruit extract has shown free radical scavenging and analgesic activities in mice (Kumar et al., 2010), carminative and antacid property (Sharma et al., 2012). Studies of Gill et al., (2009) have shown the antioxidant and anti-ulcer effect of C. sativa in rats. Abiodun & Adeleke, 2010 reported that the seeds of the plant served as good source of protein, fat, minerals and calcium.

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Praecitrullus fistulosus (Tinda) also called Indian round gourd is a squash-like cucurbit grown for its immature fruit, a vegetable especially popular in South Asia. Fruit possess antioxidant property in DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity (Gautam & Shivhare, 2011) and its antihelminthic activity against Pheretima posthuma has been verified by Gautam et al., (2011).

Cucurbita pepo (Kaddu) also known as pumpkin is grown for its fruit and edible seeds. It has white seeds enclosed in a husk. These seeds are chewable and sweet with a little nutty flavour. Its seeds have shown immunosuppressive activity in peripheral mononeuclear blood cells in vitro (Winkler et al., 2005) and antibacterial properties (Obi et al., 2009). Gill & Bali (2011) have shown the anti-ulcer and antioxidant activities of tertacyclic triterpenoids *(cucurbitacins*) extracted from C. pepo seeds. Inhibition of testosterone induced hyperplasia of the prostate in sprague-dawley rats has been verified by the studies of Williams et al., (2006) in pumpkin seed oil.

Lagenaria siceraria (Loki) also known as gourd is a vine grown for its fruit which has light green smooth skin and white flesh. Seeds are cream to brown, compressed, embedded in a white spongy pulp. The extracts of its fresh and dried fruits exhibited appreciably high antioxidant activity (Erasto & Mbwambo, 2009), analgesic activity (Shah & Seth, 2010), antihyperlipidemic (Nainwal et al., 2011) and cardioprotective effect (Fard et al., 2010) in rats.

In the present investigation, we report the phytochemicals screening and antimicrobial property of seeds extract of some of the most commonly available and readily consumed plants in India of Cucurbitaceae family such as *Momordica charantia* (Karela), *Cucumis sativa* (Cucumber), *Praecitrullus fistulosus* (Tinda), *Cucurbita pepo* (Kaddu), *Lagenaria siceraria* (Loki) to indicate which of them can become a new source of natural antimicrobics for pharmaceutical industries.

MATERIALS AND METHODS

Plant material:

Seeds of wild variety of five plants of Cucurbitaceae family - *Momordica charantia* (Karela), *Cucumis sativa* (Cucumber), *Praecitrullus fistulosus* (Tinda), *Cucurbita pepo* (Kaddu), *Lagenaria siceraria*(Loki) were purchased from Grain Market, Sector- 26 Chandigarh.

Preparation of seeds extract:

The seeds of five plants were washed under tap water to remove any preservative sticking onto the surface and air dried overnight. Dried seeds were then crushed using pestle mortar to get fine powder. The seed extracts were prepared in 10:1 ratio of PBS and crushed seeds. Then the seed extracts is homogenized in cold conditions and stored at 5° C further testing.

For antimicrobial or phytochemicals screening, aliquots from each extract were centrifuged at 5000 r.p.m for 4 minutes and supernatant was collected.

Test microorganisms for antimicrobial assay:

Bacterial cultures- *Serratia marcescens* , *E. coli* (gram negative bacteria), *Streptococcus thermophilus* (gram positive bacteria) and Fungal cultures of *Candida albicans, Fusarium oxysporium, Trichoderma reesei, Aspergillus niger* were purchased from Microbial Type Culture Collection Centre (MTCC) at Institute Of Microbial Technology (IMTECH), Chandigarh, India and stored at -20ºC.

Preparation of inoculum:

All the cultures were revived on selective media broth and were given the required incubation conditions specific of each culture. Then these cultures were used for antimicrobial assay.

Phytochemicals screening:

Flavonoids:

To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids. (Njoku & Obi, 2009)

Terpenoids (Salkowski test):

5ml of extract was mixed with 2ml of chloroform and carefully added conc. H_2SO_4 (3ml) to form a layer. A reddish brown coloration at the interface shows positive results for the presence of terpenoids (Edeoga *et al*., 2005).

Cardiac glycosides (Keller-Killiani test):

Crude extract 2mL was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the inter phase indicated the presence of cardiac glycosides (Yadav &. Agarwala, 2011).

Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added an observed for brownish green or a blue-black colouration (Ayoola et al., 2008).

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Steroids (Liebermann Burchard reaction):
a) To 200 mg plant extract add 10 ml chloroform. Take 2ml of this filtrate and add 2ml acetic anhydride and conc. H_2SO_4. Blue green ring indicate steroids (Siddiqui et al., 2009).
b) 2 ml of acetic anhydride was added to 0.5 g of each extract with 2 ml of H_2SO_4. The colour change from violet to blue or green in some samples indicated the presence of steroids (Egwaikhide & Gimba, 2007).

Saponins
About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins (Egwaikhide & Gimba, 2007).

Phlobatanins
About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins. (Njoku & Obi, 2009)

Phytosterols
2ml of acetic anhydride was added to 1ml extract + 2ml conc. H_2SO_4. The colour change from violet to blue or green indicated presence of sterols (Edeoga et al., 2005).

Alkaloids
Extracts (2ml) were dissolved individually in 1%dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.
Mayer's Test: Filtrates were treated with few drops of Mayer's reagent (potassium mercuric iodide). Formation of a yellow cream precipitate indicated the presence of Alkaloids.
b) Wagner's test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish brown precipitate indicated the presence of alkaloids (Roopashree et al., 2008).

Carbohydrates (Molisch's test)
One drop of concentrated sulphuric acid was added to about 1g of the extract, and then three drops of 1% α-napthol in 80% ethanol were added to the mixture without mixing to form an upper phase. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates (Abba et al., 2009).

Glycosides
The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicated the presence of glycosides (Egwaikhide & Gimba, 2007).

Resins
To 0.5 g of plants extract was added 5 ml of boiling ethanol and filtered through Whatman No. 1 filter paper and the filtrate was diluted with 4 ml of 1% aqueous HCl and formation of resinous precipitate indicate the presence of resins (Chukwu et al., 2012).

Procedure of Antimicrobial Assay
Antimicrobial assay was performed by Agar well diffusion method. For each microbe, selective media was prepared, poured into the petriplates and allowed to solidify. Then 100µL of inoculum was spread onto solidified media with the help of sterile spreader. Four wells were made on the surface of solidified media by a cork borer of diameter 0.8cm. Specific concentration of seed extract was added in each well as given in table 1. Then, all the plates were then incubated and observed for the zone of inhibition in mm.

Table 1: Showing concentration of each well

	Crude seed extract (µL)	Sterile distilled water (µL)
Well 1	100	0
Well 2	50	50
Well 3	75	25
Well 4	25	75

RESULTS AND DISCUSSION

Results of phytochemical screening:
Phytochemical analysis of the five plant seeds is presented in Table 2. The medicinal properties of the plant could be attributed to the presence of bioactive compounds in seed extracts under study. All the seed extracts have shown the presence of cardiac glycosides, terpenoids, carbohydrates and saponins.

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Cardiac glycosides have anti-inflammatory activity (Shah *et al.,* 2011), protect against lethal endotoxemia (Matsumori *et al.,* 1997) and are used in cardiac treatment of congestive heart failure. Cichewicz & Thorpe (1996) have reported the membrane disruption and inhibitory effect of terpenoids against fungi and bacteria. Studies have shown that saponins have heamolytic property, induced cytotoxicity effect (Rao & Sung, 1995), expectorant action (Ayoola & Adeyeye, 2010), antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing (Nafiu *et al.,* 2011). Saponins have the property of precipitating and coagulating red blood. These plants are used to stop bleeding and in treating wounds (Okwu & Josiah, 2006). They exhibit foaming properties and cell membrane- permeabilizing properties. Their soapy character is due to their surfactant properties (Noudeh *et al.,* 2010).

Tannins have been found in the extracts of *Cucumis sativa* (Cucumber) and *Praecitrullus fistulosus* (Tinda). Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane (Njoku & Akumefula, 2007). Tannins are potential metal ion chelator, proton precipitating agents and biological antioxidant (Okonkwo & S.I., 2009). Ellagitannins have free radical scavenging activity (Deshpande *et al*., 2007). Phytosterols have been found in the extracts of *Momordica charantia* (Karela), *Cucumis sativa* (Cucumber), *Lagenaria siceraria* (Loki). Phytosterols have a significant hypocholesterolemic effect (Castro *et al.,* 2005). Resins were found in all seed extracts except *Cucumis sativa* (Cucumber). Alkaloids, glycosides, phylobtaninis, flavonoids, steroidal terpenes were found to be absent in all the extracts.

Table 2: Showing the results of phytochemicals screening of seeds extracts used in study.

Phytochemical	***M. charantia*** (Karela)	***C. sativa*** (Cucumber)	***P. fistulosus*** (Tinda)	***C. pepo*** (Kaddu)	***L. siceraria*** (Loki)
Flavonoids.	--	--	--	--	--
Tannins.	--	+	+	--	--
Glycosides.	--	--	--	--	--
Cardiac Glycosides.	++	+	+	+	+
Terpenoids	+	++	+	++	++
Carbohydrates	+	+	++	+	+
Resins	+	--	+	+	+
Saponins	+	+	+	+	+
Alkaloids	--	--	--	--	--
Steroidal Terpenes	--	--	--	--	--
Phytosterol	+	+	--	--	+
Phylobatanins	--	--	--	--	--

+/++ indicates presence of phytochemical., -- indicates absence of phytochemical.

Photochemicals are as antimicrobial compounds, have made great contribution for quick and effective management of plant disease and microbial contamination in several agricultural conditions. The results for antimicrobial activity of different plant extracts under study against bacteria are shown in Figure 1-4. The diameter of zone of inhibition decreased with concentration of plant extract. All the crude seeds extract had shown zone of inhibition against *Serratia marcescens, Fusarium oxysporium*, *Trichoderma reesei* at all concentrations and zone of inhibition decreased in size (mm) with concentration of crude seed extract. At lower concentrations (1:1 and 1:3), crude seed extracts was ineffective to inhibit the activity *Streptococcus thermophilus* and *E. coli*. *Candida albicans* is found to be resistant to all other crude seed extracts except of *M. charantia* and *C. sativa.* Except *C. sativa*, all extracts were able to inhibit the growth of *Aspergillus niger*.

The antimicrobial assay of five seeds extract used in study showed the antibacterial activity against both gram positive and gram negative bacteria and against fungal organisms. This antibacterial potency may be due to the presence of many potent compounds such as flavonoids, terpenes, phenolics and alkaloids etc (Egwaikhide *et al*., 2010).The seeds extract of the plant species under study were found to contain tannins, cardiac glycosides, terpenoids, carbohydrates, saponins, resins and phytosterols. The presence of these phytochemical compounds in these plants enhances their pharmaceutical and therapeutic potentials.

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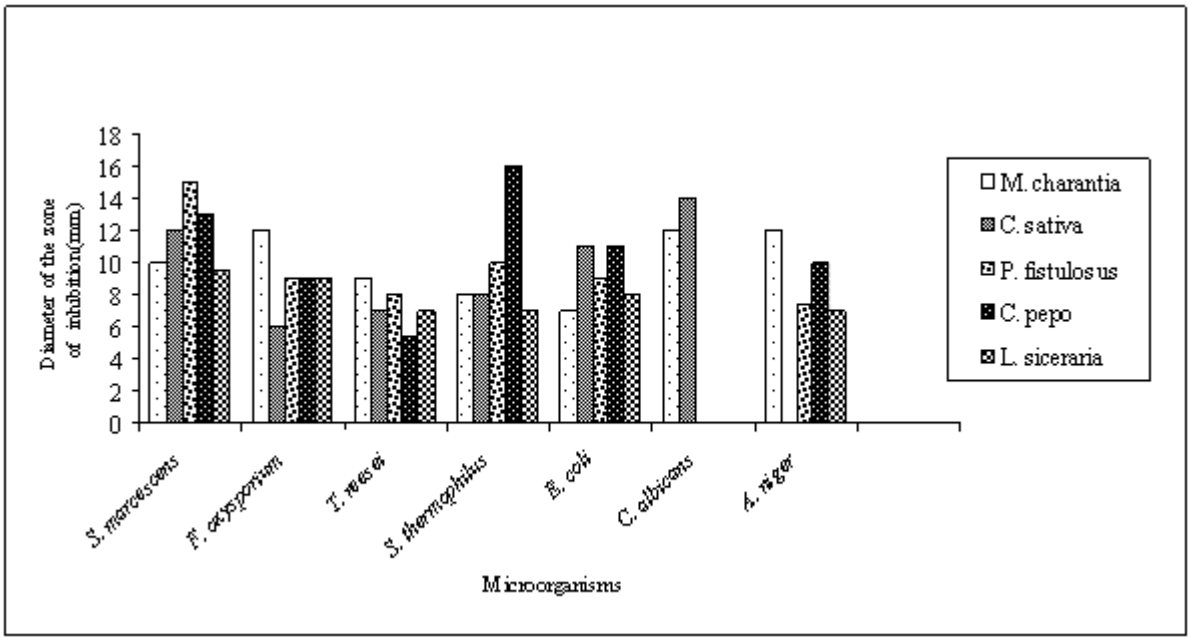

Fig. 1: Graph showing the zone of inhibition against various microorganisms with 100% crude seed extracts of *Cucurbitaceae* family (well 1)

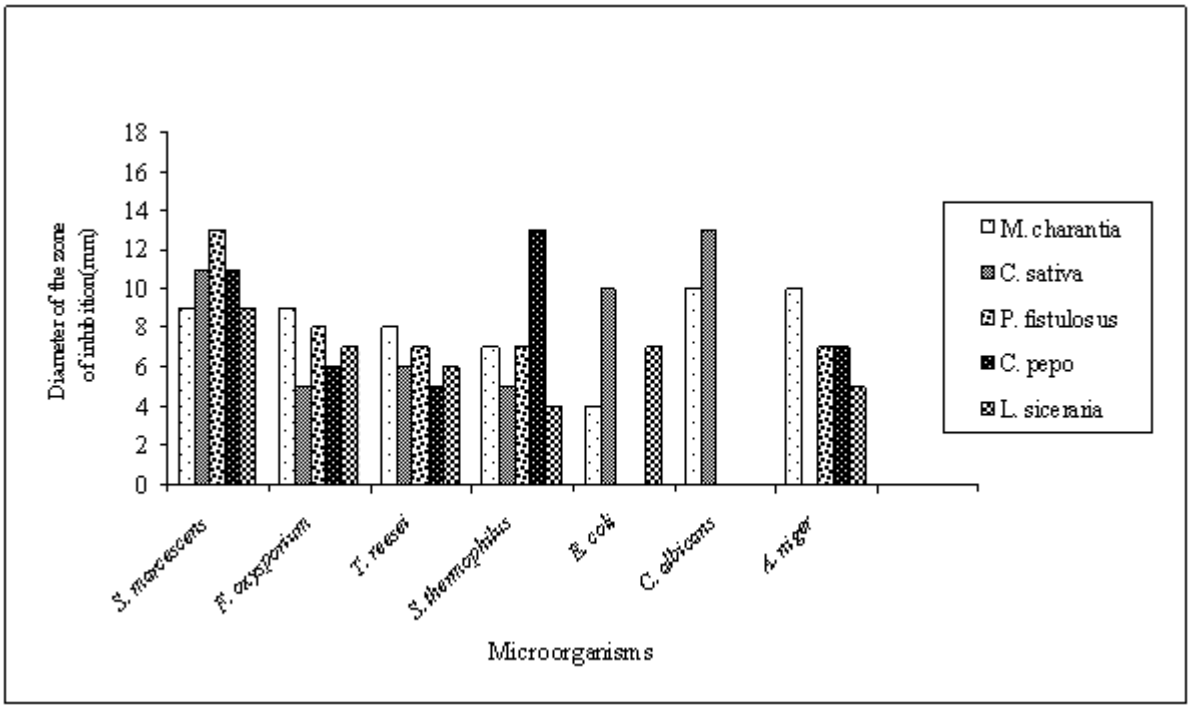

Fig. 2: Graph showing the zone of inhibition against various microorganisms with 75% of crude seed extract of *Cucurbitaceae* family (well 3)

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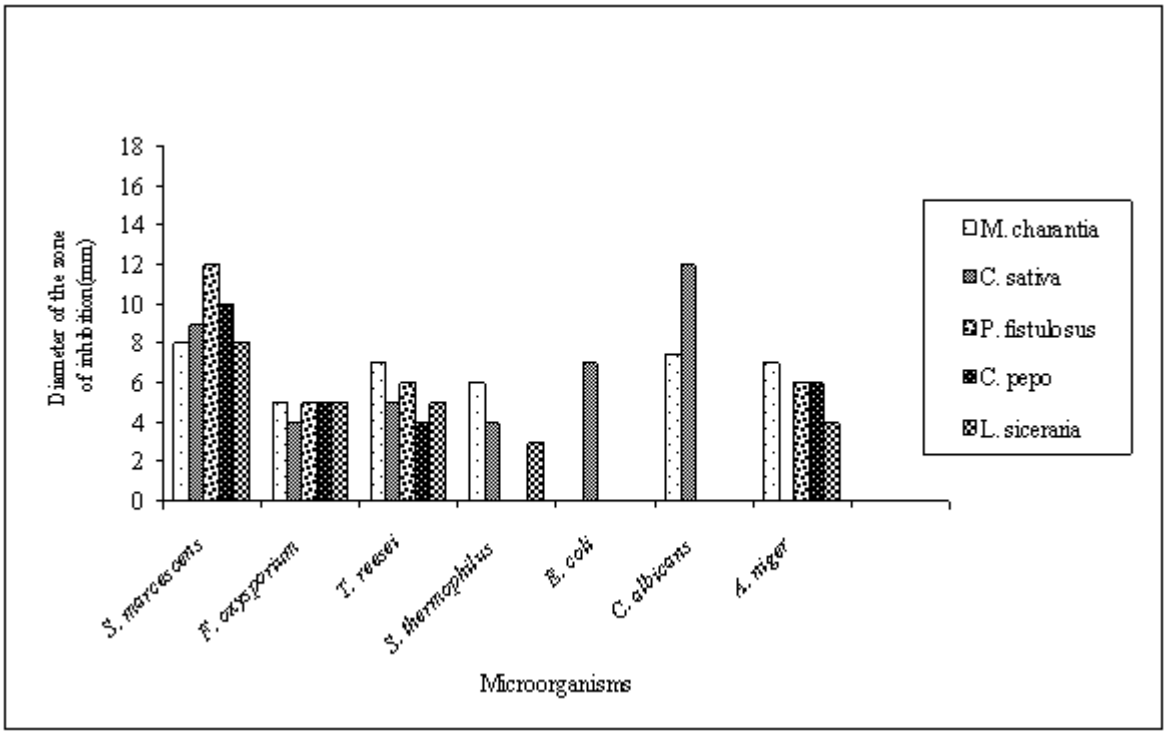

Fig. 3: Graph showing the zone of inhibition against various microorganisms with 50% of crude seed extract of *Cucurbitaceae* family (well 2)

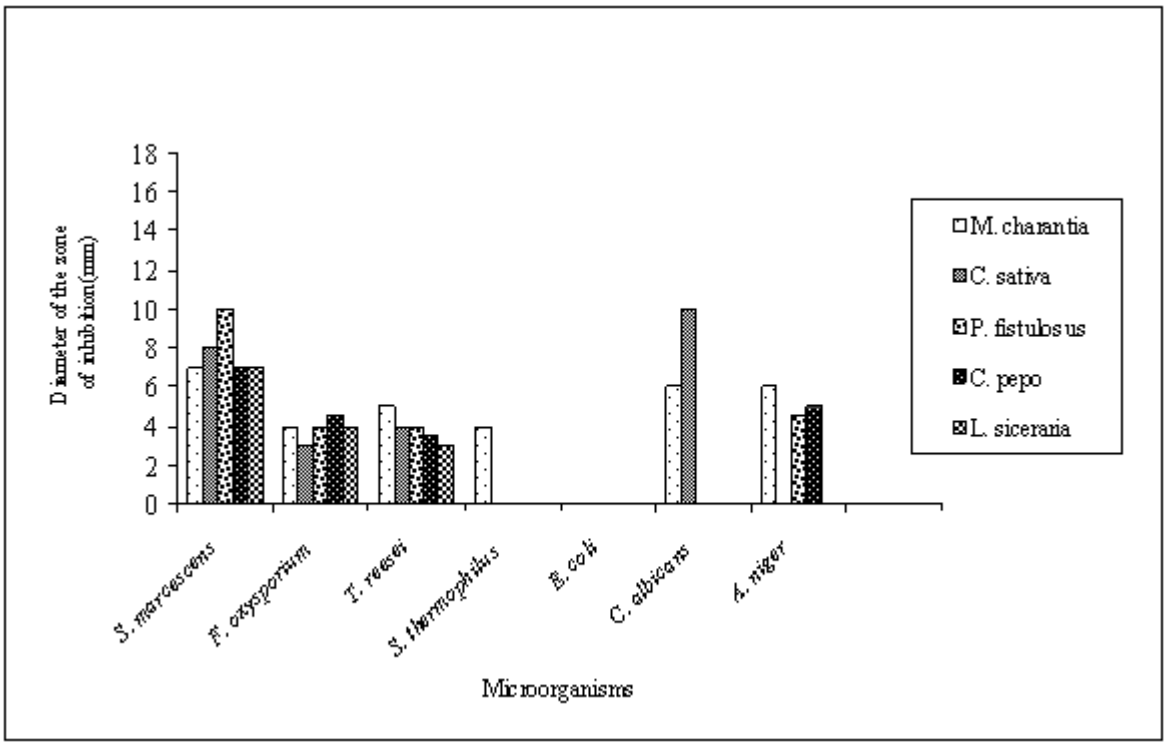

Fig. 4: Graph showing the zone of inhibition against various microorganisms with 25% of crude seed extract of *Cucurbitaceae* family (well 4)

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Thus the secondary metabolites identified in the plant materials used in the study could be responsible for antimicrobial activity exhibited by the seeds extracts of the plants. Their varied occurrences in various plant extracts however indicate that probably, their therapeutic effect(s) are not the direct effect of a single group or compound, but rather that the compounds possibly act in combination to bring about an effect.

CONCLUSION

The present study reveals that these plants under study can be used for the treatment of cancer, congestive heart failure, lowering of cholesterol levels in blood, healing of wounds, endotoxemia etc. since they contain various phytochemicals that are known to treat above mentioned diseases. The demonstration of broad spectrum of antibacterial activity by *Momordica charantia* (Karela), *Cucumis sativa* (Cucumber), and *Praecitrullus fistulosus* (Tinda) may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease, chemotherapy and control. With the evidence of antibacterial and antifungal activities of the extracts of preparations under study, it can be rationally suggested that further work needs to be done to identify the chemical natures of the active principles as well as their modes of actions on bacterial cells and their roles in diseases curing. Further studies are needed with these plants under study to, characterize and elucidate the structure of the bioactive compounds of these plants for industrial drug formulation and to purify proteins from these plants which may act as a drug for the treatment of various diseases.

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