



Phytochemical and Biological Activity of Cucurbita Seed Extract

Sumathi Ethiraj¹ and Janarthanam Balasundaram²

¹National Centre for Nanosciences & Nanotechnology, University of Madras,
Guindy Campus, Chennai – 600 025, Tamil Nadu, India

*sumathiethiraj@yahoo.com

²Poonga Biotech Research Centre, Plant Biotechnology Division,
Choolaimedu, Chennai - 600 094, Tamil Nadu, India

kb_janarthanan@yahoo.com

ABSTRACT

The present study was performed to investigate the phytochemical screening, total phenol, tannin content, antioxidant and antibacterial activity from seed extracts of *Cucurbita pepo* and *Cucurbita maxima* (Tindivanam). The phytochemical analysis revealed the presence of active ingredients such as steroids, cardioglycosides, phenols, terpenoids, alkaloids and tannins in the seed extract of *Cucurbita pepo* followed by *Cucurbita maxima*. Gallic acid (GA), Tannic acid (TA) and Butylated Hydroxy Toluene (BHT) were taken as standard in case of total phenol, tannin and antioxidant activity respectively. Total phenol and tannin content were quantitatively estimated which recorded maximum in *Cucurbita pepo* (8.37±0.2 mg Gallic Acid Equivalents (GAE)/g and 20.47 ± 0.37 mg Tannic Acid Equivalents (TAE)/g). The seed extracts were evaluated for antioxidant activities by DPPH (1, 1– Diphenyl -2- picryl - hydrazyl) radical scavenging assay. Among the two species with different solvents used, maximum antioxidant activity was found in the acetone seed extract (84.27±0.19%) of *Cucurbita pepo* followed by *Cucurbita maxima* (67.83 ± 0.37%). Different concentrations of acetone seed extracts were tested for the anti-bacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* using the agar disc diffusion technique. The acetone seed extracts of *Cucurbita pepo* had superior level of antimicrobial activity. The powerful antibacterial effect is attributed to the greater amount of tannins compound in the acetone seed extracts of *Cucurbita pepo*.

Indexing terms/Keywords

Cucurbita sp., DPPH assay; Antimicrobial activity; Phenol, Tannin; Phytochemical screening.

Academic Discipline And Sub-Disciplines

Biotechnology

SUBJECT CLASSIFICATION

Microbiology, Biochemistry

TYPE (METHOD/APPROACH)

Experiment.

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). Most of the people in rural and urban areas of the world are dependent on the medicinal plants for the treatment of infectious diseases. The Ayurvedic and Unani systems of medicines are widely used by the people of Indian subcontinent. In spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous (Raskin *et al.*, 2002). Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Dietary phytochemicals are considered as an effective tool to cure body disorder. They play important roles as therapeutic agents in prevention of many diseases (Kareem *et al.*, 2010). Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity (Ahmed *et al.*, 1999).

Medicinal plants have been used in traditional treatments for numerous human diseases for thousands of years and they continue to be an important therapeutic aid for alleviating the ailments of human kind (Momin and Kadam, 2011). The therapeutic benefits are generally traced to specific plant compounds; specifically due to the active constituents of the plants (Mary *et al.*, 2012). Phytochemical screening of various plants has been reported by many workers (Mojab *et al.*, 2003; Parekh and Chanda, 2008). These studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Hagerman *et al.*, 2008). A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavengers (Evans *et al.*, 1995; Cespedes *et al.*, 2008; Reddy *et al.*, 2008). The crude extracts of herbs, spices and other plant materials, rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Chu *et al.*, 2000).

Tannins have high polyphenolic compounds present in plants, food and beverages. Tannins are soluble in water and polar organic solvents. These tannins are classified as hydrolysable and condensed tannins based on their chemical structure and biological activity (Haslam, 1996; Makkar and Becker, 1998). Both types of tannins are capable of forming strong complexes with certain type of proteins depressing the rate of their digestion (Feeny, 1970). Tannins may also bind to bacterial enzymes or form indigestible complexes with cell wall carbohydrates reducing the cell wall digestibility (Barry and Manley, 1984; Barry *et al.*, 1986; Reed *et al.*, 1990). In recent years, tannins have been investigated to possess high antioxidants (Amarowicz *et al.*, 2004), free radical scavenging (Koleckar *et al.*, 2008), antimicrobial (Ho *et al.*, 2006), gastro protective and anti-ulcerogenic activities (Ramirez and Roa, 2003). Moreover, tannins have been investigated as potent inhibitors of lipid peroxidation in heart mitochondria (Hong *et al.*, 1995) and possess anti-fibrotic effects (Chuang *et al.*, 2011). Due to these therapeutic properties tannins can be used in the treatment of various diseases to improve human health. Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Dietary phytochemicals are considered as an effective tool to cure body disorder. They play important roles as therapeutic agents in prevention of many diseases (Kareem *et al.*, 2010). Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity (Ahmed *et al.*, 1999, Rahman *et al.*, 1999).

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxyxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA (Gutteridge, 1995; Halliwell, 1995). It has been established that oxidative stress is the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, neurodegenerative diseases, immunosuppression and others (Gulcin *et al.*, 2002 ; Devasagayam *et al.*, 2004). The screening of plant products for antibacterial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan *et al.*, 2003). There has been an increasing incidence of multiple resistances in human pathogenic microorganism in recent years (Okunji *et al.*, 1999).

Cucurbita species belongs to family Cucurbitaceae commonly called as "Squash" in English, has been claimed in traditional literature to be valuable against a wide variety of diseases. It is widely cultivated throughout India and in most warm regions of the world, for use as vegetable as well as medicine. Both of its fruits and the aerial parts are commonly consumed as vegetable (Ambasta, 1992). The fruits are sweet, refrigerant, emollient, diuretic, sedative and tonic and are useful in burns, scalds, inflammations, abscesses, boils, migraine and neuralgia (Sharma, *et al.*, 2009). Fruit pulp is used as poultice and supplied on to burns, boils and inflammations. The seeds are used as anthelmintic, antitumor, antihypertensive, anti-inflammatory, diuretic and nervine tonic and are useful in taeniasis, strangury and nervous debility. Seeds also used as abortifacient and insecticidal (Agarwal and Agarwal, 1991). Hence, the present study was performed to investigate the phytochemical screening, total phenol, tannin content, antioxidant and antibacterial activity of the seed extract of *Cucurbita* species.

MATERIAL AND METHODS

Collection of material

The healthy seeds of *Cucurbita pepo* and *Cucurbita maxima* (Figure 1) were collected from Tindivanam, Tamil Nadu. The collected seeds were brought to the laboratory and maintained at Poonga Biotech Research Centre, Plant biotechnology division, Chennai- 600094, Tamil Nadu, India.



Figure 1: Seed separated from *Cucurbita maxima* and *Cucurbita pepo*

Preparation of the seed extract

Preparation of the extracts was done according to a combination of the methods used by Pizzale *et al.*, (2002) and Lu and Foo (2001). About 15g of dried seed fine powder of *Cucurbita pepo* and *Cucurbita maxima* plant materials were extracted with 150 mL acetone, ethanol (75%), chloroform, petroleum ether and aqueous extract for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked for overnight at room temperature. The sample was then filtered through Whatman No.1



paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40° C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10° C.

Phytochemical screening of seed extracts of *C. pepo* and *C. maxima*

The phytochemical screening of seed extracts were assessed by standard method as described by Brinda *et al.*, (1981); Siddiqui and Ali (1997) and Savithamma *et al.*, (2011). Phytochemical screening was carried out on the seed extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the seed extracts tested.

Estimation of total phenol content in seed extracts of *C. pepo* and *C. maxima*

Total phenolic content in the seed extracts was determined by the Folin Ciocalteu colorimetric method (Slinkard and Singleton, 1984). For the analysis, 0.5 ml of dry powdered acetone extracts were added to 0.1 ml of Folin- Ciocalteu reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (Na₂CO₃) was added and the mixture was allowed to stand for 30 min after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of tannin content in seed extract of *C. pepo* and *C. maxima*

Tannins content in seed extract of *C. pepo* and *C. maxima* was estimated by following the method as described by Fagbemi *et al.*, (2005). The acetone seed extracts (1 ml) were mixed with Folin-Ciocalteu's reagent (0.5 mL), followed by the addition of saturated sodium carbonate (Na₂CO₃) solution (1 mL) and distilled water (8 mL). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Different concentrations of standard tannic acid were prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as µg tannic acid equivalent (TAE) per gram of the sample.

Qualitative analysis of Antioxidant activity of *C. maxima* and *C. pepo*

The antioxidant activity of the seed extracts of *C. pepo* and *C. maxima* was determined by following the method as described by George *et al.*, (1996); Assob *et al.*, (2014). 50µL of seed extracts of *C. pepo* and *C. maxima* were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added with the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of *C. pepo* and *C. maxima*

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. Seed extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2005). Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula:

$$\% \text{ DPPH radical scavenging} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test Sample}) / (\text{Absorbance of control})] \times 100}$$

Antibacterial activity of seed extract of *Cucurbita pepo*

The seed extracts from *Cucurbita pepo* plant were used for antibacterial study (Ozkan *et al.*, 2004; Janarthanam and Sumathi 2010). Different concentrations (50, 100 and 150 mg/ml) of the concentrated acetone seed extracts was tested for its antimicrobial activity against strains such as *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The bacterial cultures were grown in Mueller Hinton Agar and Mueller Hinton broth (Himedia) (Lopez *et al.*, 2001).

Antibacterial activity was measured using the standard method of diffusion disc plates on agar (Erturk *et al.*, 2003). Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth Medium (Hi media) for 24 hours at 37°C and plated on Mueller Hinton Agar (Hi media) for agar diffusion experiments. Paper disc (6mm in diameter) were placed on the agar medium to load 20µl of different concentrations of acetone seed extracts of *Cucurbita pepo* were tested. Inhibition diameters were measured after incubation for 24 - 48 hours at 37°C.

RESULTS AND DISCUSSION

In the present study, phytochemical screening was performed with ethanol, chloroform, petroleum ether, acetone and aqueous seed extracts of *Cucurbita pepo* and *Cucurbita maxima*. The acetone seed extracts of *Cucurbita pepo* were rich in terpenoids, quinones, saponins, Cardiac glycosides, steroids, phenols, tannins and alkaloids followed by other extracts (Table 1 & 2).



Table 1: Phytochemical screening from seed extract of *Cucurbita pepo*

Phytochemicals	Seed extract of <i>Cucurbita pepo</i>				
	Aqueous	Ethanol	Acetone	Chloroform	Petroleum ether
Tannin	-	-	+	-	-
Saponin	+	-	+	-	-
Flavanoid	-	-	-	-	-
Quinones	++	++	++	+	+
Glycosides	-	-	-	-	-
Cardio glycosides	+	+	+	++	+
Terpenoids	+	++	++	+	+
Phenol	+	+	++	+	+
Coumarins	-	-	-	-	-
Steroids	+	++	++	+	-
Alkaloids	++	-	+	-	-
Anthocyanin	-	-	-	-	-
Betacyanin	-	-	-	-	-

Key: + = Positive ; ++ = Strong Positive ; - = Negative

Table 2: Phytochemical screening from seed extract of *Cucurbita maxima*

Phytochemicals	Seed extract of <i>Cucurbita maxima</i>				
	Aqueous	Ethanol	Acetone	Chloroform	Petroleum ether
Tannin	+	+	+	-	-
Saponin	-	-	-	-	-
Flavanoid	-	-	-	+	-
Quinone	+	+	++	+	+
Glycosides	-	-	-	-	-
Cardio glycosides	+	+	+	+	+
Terpenoids	+	+	+	+	+
Phenol	+	+	+	+	+
Coumarins	-	-	-	+	-
Steroids	+	+	++	+	+
Alkaloids	+	+	+	-	-
Anthocyanin	-	-	-	-	-
Betacyanin	-	-	-	-	-

Key: + = Positive ; ++ = Strong Positive ; - = Negative

Phytochemical constituents such as tannins, phenols, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many micro-organisms, insects and herbivores (Britto and Sebastian, 2011). Preliminary screening of phytochemicals may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development (Doss, 2009). Tannin compounds present in *E. littorale* inhibit the growth of many fungi, yeasts, bacteria and viruses (Chung *et al.*, 1998). The presence of alkaloids and saponins in leaf extract, the biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities (Stary, 1998). Saponins have properties of precipitating and



coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and haemolytic activity (Sodipo *et al.*, 2000). Traditionally saponins have been extensively used as detergents, surface active agents, industrial applications as foaming and also have beneficial health effects (Shi *et al.*, 2004). Plant steroids are known important for their cardiotoxic activities and also used in nutrition, herbal medicine and cosmetics.

Phenolics are the most widespread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers. Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992). In our study, total phenol content (TPC) of *Cucurbita pepo* and *Cucurbita maxima* seed extracts was estimated by using Folin-Ciocalteu colorimetric method and represented in terms of gallic acid equivalent (GAE). The result of the present study showed that the phenol contents of the acetone seed extracts in terms of Gallic acid equivalent were between 5.21 ± 0.1 mg GAE/ g to 8.37 ± 0.2 mg GAE /g. Total phenol content of *Cucurbita pepo* acetone seed extract was found to be maximum (8.37 ± 0.2 mg GAE/ g) followed by *Cucurbita maxima* (5.21 ± 0.1 mg GAE/ g). (Table 3).

Table 3: Estimation of Total phenol content from seed extract of *C. pepo* and *C. maxima* (Tindivanam)

S.No	Plant sample	Total phenol content (mg GAE/g)
1	<i>Cucurbita pepo</i>	8.37 ± 0.2
2	<i>Cucurbita maxima</i>	5.21 ± 0.1

It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Evans *et al.*, 1995). Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds (Zheng and Wang, 2003; Chinnici *et al.*, 2004; Huang *et al.*, 2009).

The result of the present study recorded highest Tannins content in the seed extracts of *Cucurbita pepo* and *Cucurbita maxima* and the tannins content was expressed as μ g tannic acid equivalent (TAE) per gram of the sample. The optimum yield of tannins was found to be 20.47 ± 0.37 mg TAE / g dry weight from seed extract of *Cucurbita pepo* followed by 14.3 ± 0.42 mg TAE / g dry weight from seed extract of *Cucurbita maxima* (Table 4).

Table 4: Estimation of Total tannin content from seed extract of *C. pepo* and *C. maxima* (Tindivanam)

S.No	Plant sample	Total tannin content (mg TAE /g)
1	<i>Cucurbita pepo</i>	20.47 ± 0.37
2	<i>Cucurbita maxima</i>	14.3 ± 0.42

The results corroborates with the findings of Singh *et al.*, (2012) who has reported maximum yield Tannins from ethanolic extract of *Artemisia absinthium*. Tannins are the natural polyphenolic compounds which can influence the nutritive value of different food stuffs utilized by human and other animals. Tannins also have large influence on the phytochemical and phyto-therapeutical value of medicinal plants. Various methods have been used to increase the extraction efficiency of tannins from different medicinal plants for their use in pharmaceutical field (Cobzac *et al.*, 2005). Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes (Salah *et al.*, 1995).

The seed sample of *Cucurbita pepo* and *Cucurbita maxima* were used for antioxidant studies. Analysis on different extraction of acetone, ethanol (75%), petroleum ether, chloroform and aqueous extract showed the presence of antioxidants. 100 μ l of seed extracts were estimated for free radical scavenging activity using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. The samples were observed for the colour change from purple to yellow and pale pink were considered as strong positive and weak positive respectively (Table 5).

Table 5: Qualitative analysis of antioxidant activity from seed extract of *C. pepo* and *C. maxima* (Tindivanam)

S.No	Extractions	<i>Cucurbita pepo</i>	<i>Cucurbita maxima</i>
	BHT (standard)	+++	+++
S1	Aqueous	+	+
S2	Ethanol	+	+
S3	Acetone	++	+
S4	Chloroform	-	-
S5	Petroleum ether	-	-

Among five different solvent seed extracts of *C. pepo* and *C. maxima*, the acetone seed extract of *Cucurbita pepo* collected from Tindivanam recorded the most effective DPPH radical scavenging activity (84.27 ± 0.19 %) followed by *Cucurbita maxima* (67.83 ± 0.37 %) (Figure 2). *Cucurbita pepo* value being very close to synthetic antioxidant (BHT) as

positive control (98.36±1.4%). The seed sample of *Cucurbita pepo* and *Cucurbita maxima*, acetone seed extracts recorded higher percentage of free radical scavenging activity followed by ethanol, aqueous, chloroform and petroleum ether.

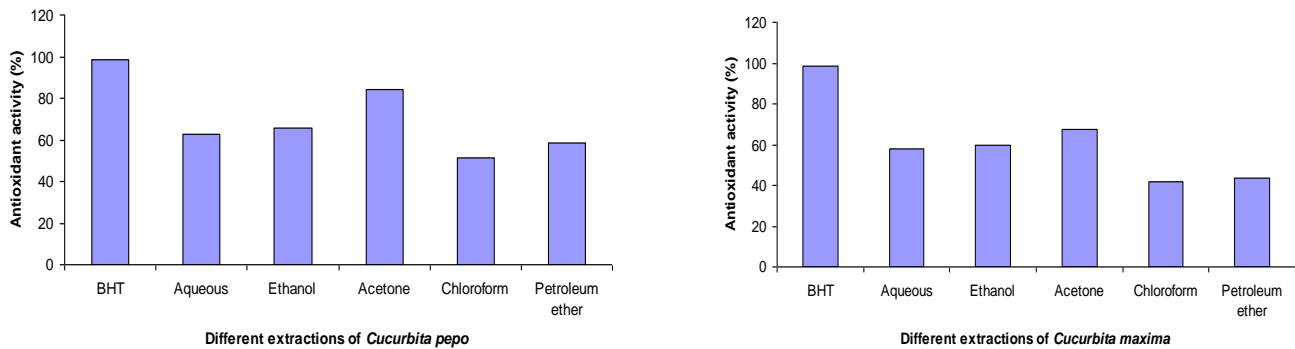


Figure 2: Quantitative analysis of antioxidant activity from seed extracts of *C. pepo* and *C. maxima* (Tindivanam)

The data presented in Table 6, indicate that the seed extracts of *Cucurbita pepo* inhibit the growth of some microorganism to various concentration. The concentrations of 50mg/ml - 150mg/ml acetone seed extract showed antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and inactivity against *Escherichia coli*.

Table 6: Antibacterial activity from acetone seed extracts of *Cucurbita pepo* (Tindivanam)

Inhibition Zone in diameter (mm)*			
Micro-organisms	Concentrations of extract		
	50 mg/ml	100 mg/ml	150 mg/ml
Acetone extract (seed)			
<i>Bacillus subtilis</i> (MTCC No. 10224)	-	10.0±0.2 mm	12.3±0.2 mm
<i>Bacillus cereus</i> (MTCC No. 10211)	-	12.6±0.1mm	16.0±0.2 mm
<i>Pseudomonas aeruginosa</i> (MTCC No. 14676)	-	10.6±0.4 mm	14.3±1.0 mm
<i>Staphylococcus aureus</i> (MTCC No. 9542)	-	11.3±0.1 mm	13.9±0.1 mm
<i>Escherichia coli</i> (MTCC No. 1563)	-	-	-

The maximum clear zone of inhibition was found at 150 mg/ml of acetone seed extract of *Cucurbita pepo* (Figure 3). In seed extracts, there is no zone of inhibition was found in lower concentration (50 mg/ml).

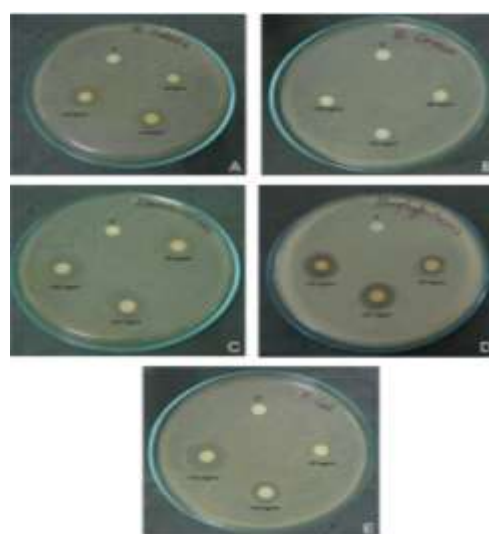


Figure 3: Antibacterial activity from acetone seed extracts of *Cucurbita pepo* (Tindivanam)

Antibacterial activity of seed extract of *Cucurbita pepo* against *Bacillus subtilis* (A), *Bacillus cereus* (B), *Pseudomonas aeruginosa* (C) *Staphylococcus aureus* (D) and *Escherichia coli* (E)



Similar results were obtained on acetone extracts from leaves of *Sida acuta* and *Acalypha wilkesiana* which exhibited antibacterial activity (Oboh *et al.*, 2007 ; Gotep *et al.*, 2010). The antimicrobial activities of acetone extract may be due to the presence of tannins, triterpenoids and phenols. Tannins have been known to form irreversible complexes with prolene rich protein resulting in the inhibition of cell wall synthesis (Mamtha *et al.*, 2004). Thus from our findings, it is concluded that the acetone extracts from dry powdered seed of *Cucurbita pepo* had superior level of antimicrobial activity. The powerful antibacterial effect is attributed to the greater amount of tannins compound in the acetone seed extracts of *Cucurbita pepo*.

In conclusion, phytochemical composition, total phenol, tannin content, antioxidant activity and antibacterial activity of medicinal plants are very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present communication attempts to assess the status of phytochemicals, total phenol, tannin content, antioxidant activity and antibacterial activity, in the seed extract of *Cucurbita pepo* to improve the health status of people and also to use it in the nutraceuticals products of commercial importance. Thus from our findings, it is concluded that the acetone extracts from dry powdered seed of *Cucurbita pepo* had superior level of antimicrobial activity. The powerful antibacterial effect is attributed to the greater amount of tannins compound in the seed extracts of *Cucurbita pepo*.

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