Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

PHYTOCHEMICAL, ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF THE FRUIT EXTRACT FROM *CUCURBITA DIGITATA*

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Received: 25 Feb. 2014 Revised and Accepted: 10 Mar 2014

ABSTRACT

Objectives: The aim of this research was to evaluate the phytochemical, antioxidant and cytotoxic property of the fruit extract from *Cucurbita digitata.*

Methods: The phytochemical screening in fruit extracts from *C.digitata* was performed for carbohydrates, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, phytosteroids, phlobatannins, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests. The total antioxidant capacity was evaluated by an array of experiments such as FRAP (Ferric reducing antioxidant power assay), DPPH(1,1-diphenil-2-picrylhydrazyl), NO (Nitric oxide) and SO (Superoxide) assays. The annihilation activity of free radicals was calculated in % inhibition and absorbance. The cytotoxicity and cell viability was calculated by using MTT (Microculture Tetrazolium Assay) colorimetric assay.

Results: The phytochemical screening showed positive results for flavonoids, quinones, carbohydrates, phytosteroids, glycosides and coumarins. Total contents of 7.44mg/g, 6.78mg/g, 0.89mg/g, 345.5mg/g, 167.5mg/g and 5.56mg/g were respectively obtained for flavanoids, quinones, coumarins, carbohydrates, glycosides and phytosteroids in 100 g of the dry sample. The antioxidant capacity measured was effective in Methanol extract and showed inhibition greater than 58% at150 µg in DPPH (1,1-diphenil-2-picrylhydrazyl), NO (Nitric oxide) and SO (Superoxide) assay and absorbance value 1.8 at 150 µg in FRAP (Ferric reducing antioxidant power assay). For the MTT (Microculture Tetrazolium Assay) cytotoxicity studies, the cell viability was about 55% at 750 µg.

Conclusion: *Cucurbita digitata* fruit extract demonstrated the presence of secondary metabolites with potential antioxidant and cytotoxic activities.

Keywords: Phytochemical, Antioxidant, DPPH, MTT assay, Cucurbita digitata, NO assay, SO assay, FRAP assay, Cytotoxicity.

INTRODUCTION

Cucurbita digitata is a species of flowering plant in the squash family known by the common names fingerleaf gourd and bitter squash [1]. Each member of this species group is native to the Southwestern United States and Northwestern Mexico where they are relatively less known. Each group member is found in hot, arid regions with low rainfall [2]. Species of cucurbits are native in most countries of the world, especially in the tropics, and they are now cultivated in every country, state, and province where crop plants can be grown in the summer (warm temperature) During the last decades a large number of cucurbitacins have been isolated from various plant species belonging to the family C*ucurbitaceae*. Although the roots and the fruits of plant belong to these *Cucurbitaceae species* are very bitter, they have been used as folk medicines in some countries because of their wide spectrum of pharmacological activities such as anti-inflammatory and anticancer effects [3]. Hence in this study the Cucurbita digitata species was selected to analyze its therapeutic importance with respect to phytochemical, Antioxidant and Cytotoxic effects.

MATERIALS AND METHODS

Chemicals and reagents: Chemicals used in the study were of analytical grade and procured from Merck India Pvt. Ltd.

Collection of Sample

Fresh fruits of *Cucurbita digitata* were collected from Tirunelveli, Kanyakumari district of Tamil Nadu. The fruits were washed well, using tap water and distilled water twice and dried in shade for a period of 20 days, at an ambient temperature of 35˚C. After drying the fruits of *Cucurbita digitata* the seeds were separated by cutting them into small pieces to avoid its intercession. The dried samples were ground properly using a mortar and pestle and later using a grinder, to obtain the powdered and fibrous form.

Fig.1: Image of *Cucurbita digitata* Figure 2: It shows the Crossection of the *C.digitata* ripe fruit

Preparation of Extracts

35 grams of the powdered material was extracted first with 95% (v/v) hexane by Soxhlet apparatus, and then the residues were further extracted with dichloromethane separately. Same procedure was repeated for ethyl-acetate, methanol, acetone and water with same type of repeated residues. All the solvents were used based upon their increasing polarity index; the extracts were evaporated to dryness on a water-bath and subsequently distilled and suspended in different solvents for further analysis.

Qualitative Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using the standard procedures as described by Sofowara 1982 [4], Trease and Evans 1989 [5] and Harborne1973 [6].

Antioxidant activity

Ethanol and methanolic extracts of *Cucurbita digitata* were analyzed using assays such as DPPH, NO, SO, FRAP and MTT for evaluating antioxidant attributes.

DPPH free radical scavenging activity

Ability of the extracts to annihilate the DPPH radical (1, 1-diphenil-2-picrylhydrazyl) was investigated by the method of Blois [7] and the annihilation activity of free radicals was calculated in % inhibition using the following formula: % of Inhibition = (A of control – A of Test)/A of control X 100

Nitric oxide scavenging activity

Nitricoxide radical inhibition was estimated by using the principle of Griess Illosvoy reaction (Ebrahimzadeh MA, *et al.,* 2009) [8] in which sodium nitroprusside in aqueous solution (at physiological pH) spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Annihilation activity of free radicals was calculated in % inhibition as under: Inhibition % = (Absorbance of control – Absorbance of sample) X 100 / Absorbance of control

Ferric reducing antioxidant power assay

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain [9]. FRAP assays uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present. At low pH, reduction of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form (intense blue color) can be monitored by measuring the change in absorption at 593nm. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture.

Superoxide anion scavenging activity

Measurement of superoxide radical scavenging activity was done by using standard method [10]. The superoxide anions generated by phenazinmethosulfate (PMN)/ nicotinamid-adenin-dinucleotidphosphat, reduced form (NADPH) system, were detected by the reaction with 2,2'-di-p-nitrophenyl)-5,5'-diphenyl-(3,3'-dimethoxy-4,4'-diphenylene) ditetrazolium chloride (nitro blue tetrazolium – NBT). The annihilation activity of free radicals was calculated in % inhibition as before.

MTT assay for cell viability

MTT assay [11] is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cell survival was calculated using the formula,

Viability % = (Test OD/ Control OD) X 100

Cytotoxicity % = 100 – Viability%

Statistical analysis of all the experiments were performed in triplicate and the results were expressed in mean ± S.D. Student's *t*-test was performed.

RESULTS

All results of phytochemical analysis are showed in the Table 1 and 2. The results of Antioxidant studies using DPPH, FRAP, NO, SO assays are shown in the Figure.3, 4, 5 and 6 respectively. The Cytotoxicity study results are shown in Table 3.

Table 1: It shows the quantity of phytochemical compounds in fruit extract of *Cucurbita digitata*

Phytochemicals	Quantity per gram
Flavanoids	7.44mg/g
Quinones	6.78mg/g
Coumarins	0.89mg/g
Carbohydrate	345.5mg/g
Glycosides	167.5mg/g
Phytosteroids	5.56mg/g

Table 2: It shows the presence of phytochemicals in fruit extract of *Cucurbita digitata*

Phytochemical test	Inference				
	Hexane	Ethylacetate	Acetone	Methanol	water
Carbohydrates	+	+	-	-	++
Tannins test	-	-	-	-	-
Saponin test	-	-	-	-	-
Flavonoid test	-	+	+	++	+
Alkaloid test	-	-	-	-	-
Quinones	-	-	+	++	-
Glycosides test	-	-	-	-	-
Cardiac glycosides test	-	++	-	-	-
Terpenoids test	-	-	-	-	-
Triterpenoids	-	-	-	-	-
Phenols	-	-	-	-	-
Coumarins	-	+	-	++	+
Proteins	-	-	-	-	-
Steroids and Phytosteroids	-	+	-	-	-
Phlobatannins	-	-	-	-	-
Anthraquinones	-	-	-	-	-

Note: [++] means strongly present, [+] means present, [-] means absent

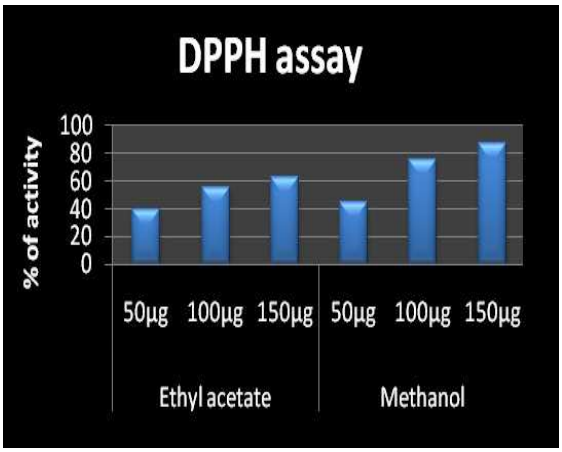

Fig.2: The graph shows the results of DPPH assay

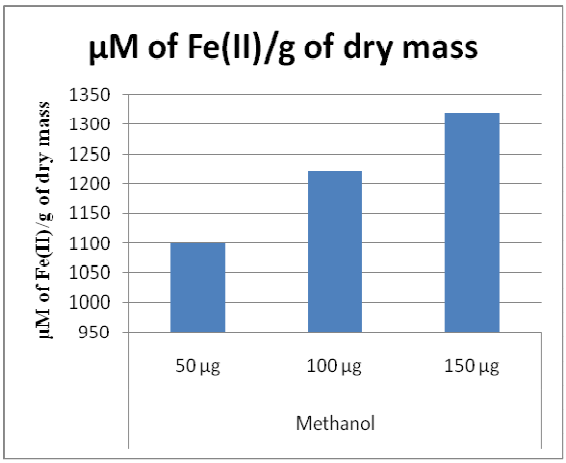

Fig. 3: The graph shows the results of FRAP assay

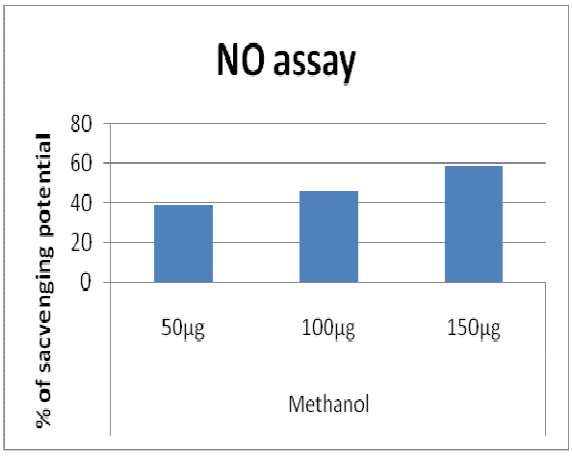

Fig. 4: The graph shows the results from NO assay

DISCUSSION

In the study *Cucurbita digitata* fruit extract showed rich phytochemical property as shown in the Table 2 and the phytochemical screening confirms the presence of flavanoids, quinones, coumarins, carbohydrates, glycosides and phytosteroids with total contents of 7.44mg/g, 6.78mg/g, 0.89mg/g, 345.5mg/g, 167.5mg/g and 5.56mg/g respectively in 100 g of the dry sample given in Table 1 and 2.

The above listed phytochemicals have unique therapeutic effects for a range of illness, for instance; Flavonoids are known to possess curative implications particularly in anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral activities[12].

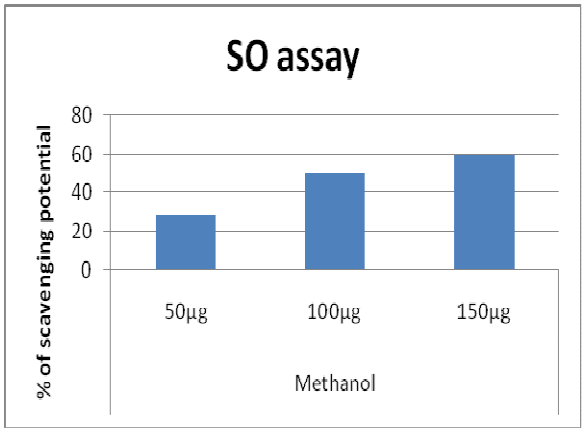

Fig. 5: The graph shows the results from SO assay

Table 3: The table shows the MTT assay results

	250µg	500µg	750µg
% of viability	78.15303	65.06596	54.67018
% of activity	21.84697	34.93404	45.32982

Flavonoids at times is referred to as phytoestrogens which is associated to relief of menopausal symptoms, reduction of osteoporosis, improvement of blood cholesterol levels and lowering the risk of certain hormone-related cancer and coronary heart diseases. Besides, some cardiac glycosides and flavonoids are found to have hypoglycemic activities. High molecular weight steroids showed analgesic properties [13] and are also responsible for the healthy functioning of central nervous system. Coumarins possess blood-thinning, anti-fungicidal and anti-tumorproperties. Indeed Coumarin increases the blood flow in the veins and decreases capillary permeability and exhibits antiapoptotic property [14]. Glycosides contain digitoxin, digoxin and ditoxin which are having strong and direct action on the heart muscles, helping to regain strength during fatigues [15]. Furthermore the glycosides are also significantly diuretic and help in transferring fluids from the tissues and circulatory system to the urinary tract, thereby influencing lowering blood pressure [16]. Hence the above mentioned therapeutic implication are favorable with respect to *C. digitata.* For the radical scavenging activity of the *Cucurbita digitata* fruit extract [17-19] using stable free radical DPPH assay the results showed increasing activity with the highest of 86% of inhibition at150µg for methanol extract than in ethanol (Figure-3). Besides, the flavonoids are a major group of compounds that act as primary antioxidants for free radical scavenging. Thus the antioxidants present in the extract quenches the DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via free radical attack on the DPPH molecule) and convert them to a colourless product (2, 2-diphenyl-1- picrylhydrazyl, or a substituted analogous hydrazine) resulting in a decreasing absorbance at the 517 nm. Similarly the other free radical scavenging tests of Ferric Reducing antioxidant power assay method, NO assay and SO assay shows an increase in the antioxidant activity or free radical scavenging. The increase in activity with increase in concentration of the fruit extract and its absorbance are recorded as shown in charts [figure 4, 5 and 6]. Finally the cytotoxicity test performed using MTT Assay methods shows significant reduction of yellow tetrazolium dye to a purple formazan product, as shown in the table 3. This indicates the ability of the fruit extract to induce cytotoxicity and decrease cell viability [20]. Considering the results obtained, it can be concluded that the plant contains essential phytochemical constituents and possess active antioxidant and cytotoxic property.

CONCLUSION

Medicinal value of plants lies in chemical substances that have a definite physiological / biochemical action on the human body. Diverse phytochemicals have been found to possess a wide range of activities, which help in protection against chronic diseases. Phytochemical screening of fruit extracts of Cucurbita digitata disclosed the presence of contained flavanoids, quinones, coumarins, phytosteroids and cardiac glycosides. The present study suggested that the fruit extracts of Cucurbita digitata could be a potential source of natural antioxidant and thus be useful as therapeutic agents against progressive aging and age-associated oxidative stresses-related degenerative diseases /disorders. Though extensive studies have been carried out on other species of cucurbitaceae on nutrition, anti-inflammatory, antidiabetic and anticancer profile, only a minimal research have been performed in Cucurbita digitata species. Hence, further studies on this plant are called for evaluating the pharmacological remedy towards degenerative disorders and chronic diseases.

ACKNOWLEDGEMENTS

Authors wish to thank Biozone Technologies for providing necessary facilities for conducting the work

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