In Vitro Antioxidant And Cytotoxic Efficacy Of *Cucurbita maxima*: From The Medicinal Plant Wealth Of Peninsular India

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Abstract : Tropical India and Peninsular India is famous for its rich medicinal plant wealth and the tradition of indigenous system of therapy, Ayurveda. Plants have been used for the cure of various diseases in Ayurveda and is extensively used by the elder generation. The present study is to evaluate the phytochemical composition, and to screen for the in vitro antioxidant and cytotoxicity effects of leaves of *Cucurbita maxima*,a potential plant which is culturally and medicinally significant to the people of Kerala.Among all the extracts methanolic leaf extract of *Cucurbita maxima* showed higher phenolic and flavonoid content and significant DPPH scavenging activity. The in vitro cytotoxicity was tested against Hep G2 (hepatocellular liver carcinoma) in which methanolic extract of *Cucurbita maxima* showed an IC50 108.72 µg/ml at 48hr.Our study confirmed that theleaves of *Cucurbita maxima* has better antioxidant and anticancer properties and hence consuming of its leaves as food will definitely rejuvenate our body.

IndexTerms: **Phytochemical analysis, Antioxidant, Cytotoxicity, Traditional medicinal plants.**

I.INTRODUCTION

Peninsular India is famous for its indigenous medicinal practice, Ayurveda and as the source of several potential medicinal plants.Ayurvedic medicinal system is a well-established medicinal practice in India with a sound literature background originated approximately 5000 years ago. Traditional medicinal plants are endless source for therapeutic drugs for various ailments like antimicrobial, anti-inflammatory, anticancer, antioxidants, antiulcer and so on. The classical Indian textbooks like Rigveda, Yajurveda, Atharvaveda, Charaka Samhita and Susrutha Samhita discuss about various diseases and their treatment, medicinal herbs and their efficiency for removing the ailments.

The World health organisation (WHO) estimated that about 80 percent of the world's population still realize on plant based medicines for their primary health care. This is in fact is a clear indication of the role of medicinal plants in the maintenance of health and treatment of diseases as therapeutic alternatives throughout the world, still in the late 20th and early 21stcentury (WHO, 2002).

The demand on plant based therapeutics is increasing in both developing and developed countries due to the growing recognition that, they are natural products, non- narcotic, easily biodegradable, cause minimum environmental hazards, have no adverse side effects and are easily available at affordable prices. Medicinal plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity known as secondary metabolites. Some of these secondary metabolites are produced are produced for self- defence. Literature reviews show that over the last 20 years, large of secondary metabolites from different plant species have been evaluated for their bioactivities.

As per Ayurveda and the tradition of Kerala, in the Malayalam month of "Karkkidakam" and "Aadi"in Tamil (the Monsoon season of Kerala and Tamilnadu) in olden days our elder people consume various herbal preparations to rejuvenate the body and cure diseases. Elder women in Kerala and Tamilnadu are rich in ethno biological knowledge which has been transmitted from one generation to another. One such knowledge is the traditional cooking recipes of the leaves of various medicinal plants including that of *Cucurbita maxima*.

C. maxima (Pumpkin) belongs to the family Cucurbitaceae. It has received considerable attention in recent years because of the nutritional and health benefits of the bioactive components obtained from its seeds and fruit. Literature reviews show that less focus has been given to explore the bioactive compounds present in the leaves of this plant by researchers and hence this study.

Figure 1: *Cucurbita maxima*

The present study is to evaluate the phytochemicals present, antioxidant and anticancer potentials of *Cucurbita maxima* which is considered to be a potential plant which is culturally and medicinally significant to the people of Kerala in India.

II.MATERIAL AND METHODS

2.1 Plant collection and extraction

The seeds of *Cucurbita maxima* were collected from Kerala Agriculture University, Thrissur, Kerala. The University has released an improved variety "Ambily" in the year 1988. The seeds were seeded properly and the young leaves were used as samples. The leaves were chopped into small pieces and shade dried. The samples are pulverized in an electric blender and the powdered samples are used for further extraction. Extraction was done using the solvents petroleum ether (PE) Chloroform, methanol (ME) and aqueous (AQ). All the extracts were stored in air tight container until use. (Ikram et al., 1984)

2.2 Phytochemical screening

Preliminary phytochemical screening of the four extracts of *Cucurbita maxima* were performed by Trease and Evans, 2002; Harborne, 1999.

2.2.1Test for Carbohydrates

Molisch's Test: To 2ml of plant extract, 1ml of molich's reagent and few drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrate.

2.2.2Test for Tannins

Ferric chloride test: To 1ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.2.3Test for Saponins:

Foam test: To 1ml of plant extract, 5-10 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise.

Formation of 1cm layer of foam indicates the presence of saponins.

2.2.4Test for Flavonoids:

Sulphuric acid test: A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange colour.

2.2.5Test for Alkaloids:

Mayers test: To 2 ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added .Presence of green colour or white precipitate indicates the presence of alkaloids.

2.2.6Test for Anthocyanin and Betacyanin:

Sodium Hydroxide Test: To 2ml of plant extract, 1 ml of 2N sodium hydroxide was added and heated for 5 minutes at 100^0C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

2.2.7Test for Glycosides:

Sulphuric Acid Test: To 2ml of plant extract, 1ml of glacial acetic acid and 5%ferric chloride was added .Then few drops of sulphuric acid were added .Presence of greenish blue colour indicates the presence of glycosides.

2.2.8Test for Proteins and Aminoacids:

Ninhydrin test: To2ml of plant extract, few drops of 0.2%Ninhydrin was addad and heated for 5 minutes. Formation of blue colour indicates the presence of proteins.

2.2.9 Test for Steroids and Phytosterols:

Sulphuric acid test: To 1ml of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid were added .Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.

2.2.10Test for Phenols:

Ferric chloride test: To 1ml of the extract, 2 ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicates presence of phenols.

2.3Antioxidant activity

2.3.1 DPPH assay: free radical scavenging activity

2.3.1.1 Procedure:

The antioxidant activity of *Cucurbita maxima* methanolic leaf extract and standard ascorbic acid were assessed on the basis of the radical scavenging effect of the stable 2,2- diphenyl -1- picrylhydrazyl(DPPH) free radical activity according to the method described by Brand-William et.al., 1995. The ethanol extract with different concentrations (10,50, 100, 200, 400, 600 µg /ml) were prepared using methanol.Ascorbic acid was used as the standard in 1-100 µg /ml solution.0.004% of DPPH solution was prepared in ethanol and 5 ml of this solution was mixed with 5 ml of extract solution and standard solution distinctly. These solution mixtures were kept in dark for 30 minutes. The degree of DPPH purple decolourisation to DPPH yellow indicated the scavenging effectiveness of the extract. The absorbance of the combination was determined at 517 nm using UV –Visible Spectrophotometer and ascorbic acid was served as a positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage of scavenging was calculated as follows:

% DPPH radical Scavenging = Absorbance of control-Absorbance of test sample) X 100 / Absorbance of control.

2.3.2 Cell culture and treatment

Human cancer cell lines HepG2 (hepatocellular liver carcinoma) were obtained from National Centre for Cell Sciences (NCCS), Pune. Cells were maintained in DMEM media supplemented with 10% FBS 100 U/ml penicillin and 100 µg/ml streptomycin with 5% CO2 at 37° C in CO2 incubator. The cultured cells were harvested, counted and used for further assays.

2.3.3 In vitro cytotoxicity assay/Anti-proliferative activity

The cells were seeded in 96 well plates (5000 cells /well) and then kept for incubation for 24 hours at 37°C. Different concentrations of plant extracts were added to the wells. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. After that kept in an incubator for 24 hours at 37°C. Removed the medium completely and added 100µl of MTT reagent to it. Then kept in incubator for 2 hours at 37°C and observed for formazan crystal formation under microscope. The yellowish MTT is reduced to dark coloured formazan by viable cells only. Removed the medium completely and added 100 µl of isopropanol and kept for 10 minutes and incubated at 37°C. The colour developed was quantified with an ELISA plate reader at 570 nm. The cell survival (CS) is expressed as percentage cell survival % CS = (T/C) X 100, where T is the OD of test and C is the OD of control. Cell survival percentage using MTT assay was calculated using the following equation: % cell survival = Absorbance in drug treated wells –Absorbance in blank / Absorbance in control well-Absorbance of Blank X 100

From this the percentage of cytotoxicity was calculated.

III.RESULTS AND DISCUSSION

3.1 Phytochemical analysis

Preliminary qualitative analysis of the four extracts confirmed the presence of phyto constituents like tannin, phenolics, flavanoids, steroids, glycosides, terpenoids, and reducing sugar in various extracts of *Cucurbita maxima*. The methanolic extract was rich with wide range of plant compounds like phenolic, tannin, saponin, flavonoids, terpenoids, glycosides and sugars. The presence of phenolics, tannin, steroids, flavonoids, terpenoids and glycosides were confirmed in the methanolic extract of *Curculiogo orchioides* (Table 1).

Table 1 : Phytochemical analysis of leaves extracts of *Cucurbita maxima*

Sl no	Secondary metabolite	Alcohol extract	Acetone extract	Chloroform extract	p.E	Water extract
1	carbohydrates	+	+	+	+	+
2	saponin	+ (slightly)	-	+	-	+
3	tannins	+	-	+	-	-
4	flavonoids	+	+	+	+	+
5	Alkaloids	+	-	+	-	+
6	Anthocyanide and betacyaninde	+	+	-	+	+

7	Glycosides	-	-	-	-	-
8	protein	-	-	-	-	-
9	Phytosterols and steroids	+	+	+	+	+
10	phenols	+	-	+	+	+

3.2 Antioxidant activity

Free radicals are molecules or atoms that have at least one unpaired electron which usually increases the chemical reactivity of the molecule. Free radicals can react with other molecules to cause cell damage or DNA mutation. Molecules called antioxidants protect against free radical damage and their action permit to ensure a balance between production and destruction of free radicals. Antioxidant capacity of the methanolic extracts were evaluated by DPPH assay. DPPH radical scavenging assay shows that the ethanolic extracts of the plant have potential antioxidant activity which increases with the concentration of the extract with the lowest Effective Concentration which scavenges 50% radical (EC50) of 60 µg/mL (Fig. 1).

Table 2: Percentage of scavenging activity of *Cucurbita maxima* in different extracts

Concentration (µg/ml)	**% of scavenging activity**				
	Ethanol extract	**Acetone extract**	**Petroleum ether extract**	**Chloroform extract**	**Water extract**
100	77.8	66.66	64	65.9	63.63

Table 3: Percentage of scavenging activity of *Cucurbita maxima* in ethanol extract at various concentrations

Concentration(µg/ml)	**% of scavenging activity of Ascorbic acid**	**% of scavenging activity of *cucurbita maxima* (ethanol extract)**
10	40.63	38.6
50	60.59	58.2
100	80.25	77.8
200	87.64	89.2
400	90.26	86.8
600	93.5	91.4

3.3 Anti-proliferative activity

Even though there is remarkable development in the field of molecular mechanism of cancer, the development of chemotherapeutic agents still remains ineffective and costly. 33 Medicinal plants showing potential activity are important sources of bioactive molecules which can be developed as potent chemotherapeutic agents. 34 The cytotoxicity of the plant was measured against human cancer cell lines HepG2 cells using the MTT (3-[4, 5- dimethylthiazol-2-yl]-2, 5- diphenyltetrazolium) assay. Different concentrations (50- 250µg/ml) were used for the assay. 10% DMSO was used as negative control. Methanolic extract showed significant cytotoxicity activity with an IC50 108.72 µg/ml at 48hr. Further studies are required to identify the potential compounds and their mechanism of action.

Table 4: Percentage of cell survival

Concentration(µg/ml) *Cucurbita maxima*(ethanol extract)	**% cell survival**
6.25	74.9
12.5	56.9
50	55.8
100	45.5

IV.CONCLUSION

Methanolic extracts of *Cucurbita maxima* found to be more potential compared to the other extracts which may be attributed to the high phenolic and flavanoid content of the extract. Further studies are required to isolate and characterize the bioactive compounds and their mechanism of action which may lead to the development of novel compounds. The outcome of the present studies indicates the presense of powerful and potential bioactive compounds for the development of new 'leads' to combat various lifestyle diseases of the present era.

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