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Extraction and antioxidant activity studies of *Cucurbita moschata* extracts

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ABSTRACT:

The extraction, phytochemical constituent analysis and antioxidant activity analysis of *Cucurbita moschata* is reported. The solvents such as water, ethanol-water, ethyl acetate and methanol were utilized to optimize extraction process arrive at extract with higher yields and better antioxidant potency. Polyphenol content determination indicates that the water extract was found to have highest percent of polyphenols of 11% and Ethanol-water extract contains about 06%. Water extract was proved to have highest content of flavonoids of 6%. This is followed by ethanol-water which is of 3%.DPPH free radical inhibitory activity studies indicate that water extract was found to exhibit IC_{50} value of 390 µg/ml and ethanol water extract has the IC_{50} value of 435 µg/ml. Both the extracts have proved to be moderately active at inhibiting the activity of DPPH. Thus, the present investigation has provided significant information on the phytochemical contents and antioxidant activities of various solvent extracts of *Cucurbita moschata*. The results clearly indicate some major findings in the quest of the search for potential antioxidants that can be of very useful to mankind. The results permit for further studies in this direction leading to isolation and characterization of individual molecules responsible for the antioxidant activities of the extracts.

KEYWORDS: *Cucurbita moschata*, antioxidant, polyphenols, flavonoids

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INTRODUCTION

Cucurbita is a genus in the gourd family Cucurbitaceae first cultivated in the Andes and Mesoamerica and now used in many parts of the world. Itincludes species grownfortheir fruit andedible seeds (the squashes, pumpkins andmarrows, and the chilacayote), as well as some species grown only as gourds. Thesegourds (and other squashes) come in many colors, including blue, orange, yellow, red,and green[1]. They have Bicol lateral vascular bundles. Many North and Central Americanspecies are visited by specialist pollinators in theapid group Eucerini, especially thegenera *Peponapis* and *Xenoglossa*, and these bees can be very important for fruit set. *Cucurbita* species are used as food plants by the larvae of some Lepidoptera species, including cabbage moths, *Hypercompe indecisa*, and turnip moths. Cucurbit in is found in *Cucurbita* seeds[2].

Cucurbita species are often used as food, either for their fruit or the seeds lying within. The winter varieties have thick, inedible skins, and so store well. They are also very sweet. Summersquash, on the other hand, have a very thin skin, which can be eaten. The seeds inside can beground into a flour or meal, roasted and eaten whole, made into pumpkinseed oil, or otherwiseprepared[3].*Cucurbita moschata* is a species originating in either Central America or northern SouthAmerica. It includes cultivars of squash andpumpkin. *C. moschata* cultivars are generallymore tolerant of hot, humid weather than cultivars of *C. maxima* or *C. pepo*. They alsogenerally display a greater resistance to disease and insects, especially to the squash vine borer[4].Pumpkin (*Cucurbita moschata*) is defined as fruit botanically although commonlyregarded as vegetable in consumer terms. Flesh and seeds of pumpkin are commonlyused for culinary and medicinal purposes. Carotenoids are the natural plant pigmentsresponsible for the orange colour of pumpkin[5]. The three species of pumpkin (*Cucurbita pepo, C. maxima and C. moschata*) consisted of beta-carotene (0.06-7.4 mg/100g, alpha-carotene (0-7.5 mg/100g) and lutein(0-17 mg/100g)[6]. Similarly, *cucurbita moschata* consisted of both β-carotene and lycopene. The aim of the present study is to evaluate degradation as well as release of the bioactive compounds (total phenolic, beta-carotene and lycopene) and free radical scavenging activity of pumpkin (Cucurbita moschata) as affected by different cooking (boiling and stir-frying) methods[7].

The use of dietary plants and herbal preparations as alternative medicine has recently received considerable attentionin the United States and Europe. There is estimation that 12.1% of adults in the United States used herbal medicines in 1997[8]. In 2001, $17.8 billion was spent on dietary supplements, 23.6% of it for herbal remedies[9]. In addition, in America, herbal medicines are regulated as dietary supplements and hence can be marketed without prior approval by the Food and Drug Administration (FDA) [10]. In developing countries–all over the world–80% of population

continues to use traditional medicine in primary medical problems[11]. In the past decade, research has been focused on scientific evaluation of dietary plants and preparations of plant origin. Pumpkin is one such plant that has been frequently used as functional food or medicine. The pumpkin belongs to the family Cucubitaceae. It is comprised of *Cucurbita moschata, C. Pepo, C. Maxima, C. Mixta, C. Ficifolia* and *Telfairia occidentalis* Hook. Three of these, *Cucurbita pepo L., Cucurbita maxima Duchesne*, and *Cucurbita moschata* Duchesne represent economically important species cultivated worldwide and have high production[12-14]. In Austria and adjacent countries, pumpkins have been grown for production of oil for about 3 centuries[15]. Several reviews were described from different points of view. Paris provides an entire overview of the classification of various types of squash and pumpkins within the species C. pepo[16]. A comprehensive description of fruit of both wild and domesticated forms of Cucurbita and a critical reviews on physiological aspects of productivity and quality in squash and pumpkins were provide by Decker-Walters and Walters[17] and J. Brent Loy[18], respectively. Pumpkin is a dicotyledonous seed vegetable and consists of a flexible succulent stem with trifoliate leaves, an annual climber growing to 0.6 m by 5 m at a fast rate. At maturity it gives rise to flowers and fruits, which have numerous seeds. Because embryo dry material is 40 to 50% lipids[19-21]and 30 to 37% proteins[22,23], pumpkin seeds are a high-energy source andare consumed throughout the world with increasing inpopularity. Because the seed coat comprises about 20% of the seed weight of *C. pepo*[24], and in *C. maxima*, even a much larger proportion of the seed, new technologies were sought to utilize in oil seed pumpkins. At about the turn of the twentieth century, a thin seed coat variant was discovered and subsequently applied in oil seed pumpkins because of the greater efficiency in oil recovery. In addition, pumpkin seeds are also a good source of the elements K, P, Fe and β-carotene[25,26].Pumpkin is cultivated throughout the world for use as vegetable as well as medicine. It has been used traditionally as medicine in many countries such as China, Yugoslavia, Argentina, India, Mexico, Brazil and America[27,28]. Some of its common uses in most countries are for diabetes and treating internally as well as externally for management of worms and parasites. However, it is commonly consumed as vegetable.

Its popular medicinal uses have focused research so farand the last few decades that have been carried out on *cucurbita moschata*, using modern tools, and credited pumpkin with antidiabetic, ant hypertension, antitumor, immunomodulation, antibacteria, anti-hypercholesterolemia, intestinal anti-parasitic, anti-inflammationand antalgic. It was found that technologies such as germination and ferment could reduce ant nutritional materials and affect the pharmacological activities of *cucurbita moschata*.

Natural antioxidants such as α-tocopherol and L-ascorbicacid are widely used because they are seen as being safer and causing fewer adverse reactions, but their antioxidant activities are,

however, lower than those of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT). Hence, the need exists for safe, economic antioxidants with high activity from natural sources to replace these synthetic chemicals. The antioxidant compounds present in edible plants have recently been promoted as food additives because they display little or no toxic side effects.

The number of antioxidant compounds synthesized by plants as secondary products, mainly phenolics, serving in plant defense mechanisms to counteract ROS in order to survive, is currently estimated to be between 4000 and 6000[29]. A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants[30]. In fact, to counteract deleterious action of ROS, phenolic compounds, naturally distributed in plants, are effective[31]. Because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts[32]. To find new natural sources of active compounds, we studied the antioxidant potential of different extracts of *cucurbita moschata.*

MATERIALS & METHODS

Materials:

1, 1 - Diphenyl – 2 - picrylhydrazyl was procured from Sigma-Aldrich India Company. Ascorbic acid, Gallic acid, Vanillin, Phloroglucinol and Methanol were purchased from S. D. Fine Chemicals. All other solvents are of AR grade and distilled before use. Distilled water was employed for all the experiments. *Cucurbita moschata* seeds were sourced from Amruthkesari Depot Bangalore. The seeds were shade dried and powdered into 100 mesh size and was stored at room temperature in an airtight container.

Reflux Extraction:

Hot water extract preparation:

Hot water soluble polar compounds can be extracted by this method. In this case, cold water insoluble compounds but soluble in hot water can be extracted. 1: 10 proportion of material to solvent was taken for extraction and the extraction is carried out at boiling temperature of water (100^0 C) with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and centrifuged to remove any un-dissolved material. The extract is then concentrated to 1/5 volume on the concentrator and dried completely. Thus prepared extract is stored in airtight bottles.

80% Ethanol extract preparation:

Ethanol-water soluble polar compounds can be extracted by this method while the proteins and polysaccharides get precipitated. Here too, 1: 10 proportion of material to solvent was taken for

extraction and the extraction is carried out at boiling temperature of ethanol (65^0 C) with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and centrifuged to remove any un-dissolved material. The extract is then concentrated to dryness. Thus prepared extract is stored in airtight bottles.

Methanol extracts preparation:

Methanol soluble polar compounds can be extracted by this method while the proteins and polysaccharides get precipitated. Here too, 1: 10 proportion of material to solvent was taken for extraction and the extraction is carried out at boiling temperature of Methanol with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and centrifuged to remove any un-dissolved material. The extract is then concentrated to dryness. Thus prepared extract is stored in airtight bottles.

Ethyl acetate extracts preparation:

Ethyl acetate soluble compounds can be extracted by this method while the proteins and polysaccharides get precipitated. Here also, 1: 10 proportion of material to solvent was taken for extraction and the extraction is carried out at boiling temperature of Ethyl acetate with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and centrifuged to remove any un-dissolved material. The extract is then concentrated to dryness. Thus prepared extract is stored in airtight bottles.

DPPH Assay:

1, 1 - Diphenyl – 2 - picrylhydrazyl (Oxidized form) is a stable free radial with Purple color. In the presence of an antioxidant which can donate an electron to DPPH, the purple color which is typical to free DPPH radical decays, and the change in absorbance at 520 nm is followed which can be measured spectrophotometrically. Dissolved 39.4mg of DPPH in 100ml of methanol to get concentration of 1mM stock.Stored in dark bottle at 4°C until its use. The working concentration of DPPH in the assay was 0.14mM. Methanol (50%) was prepared by diluting methanol 1:1 with de-ionized water. Ascorbic acid standard Stock I (Conc. 200µg/ml) was prepared by dissolving 2 mg of ascorbic acid and make up to a volume of 10ml with de-ionized water. For making standard graph of ascorbic acid 2, 4, 6, 8, 10µg/ml concentration range was used. The DPPH assay was carried out by using modified method of Brand-Williams[33], in brief, to a 860µl of 50% methanol / ascorbic acid / test sample with various concentrations, added 140µl of 1mM DPPH, mixed and incubated at 37^o C for 30min. Read the absorbance at 520 nm against 50% methanol blank by spectrophotometer, a control reaction is carried out by without test sample addition. Colour Correction contains the same concentration of the test sample in methanol without DPPH. The anti-oxidant activity was measured

with reference to the standard ascorbic acid absorbance values. The actual absorbance is taken as the absorbance difference of the control and the test sample and IC_{50} values were determined.

Polyphenol Assay:

Phenolic compounds in alkaline condition (sodium carbonate) dissociate to yield a proton and phenolate anion, which is capable of reducing Folin-ciocalteu reagent. FC reagent is an oxidizing agent comprised of heteropolyphosphotungstate-molybdate. Sequences of one or two electron reduction reaction lead to blue color species. The blue colored product is a mixture of the 1-, 2-, 4-, and 6-electron reduction products in the tungstate series $P_2W_{18}O_{62}^{-7}$ to $H_4P_2W_{18}O_{62}^{-8}$ and the 2-, 4- and 6-electron reduction products in the molybdate series $H_2P_2Mo_{18}O_{62}^{-6}$ to $H_6P_2Mo_{18}O_{62}^{-6}$. Folinciocalteu reagent (0.1N) was prepared by diluting1:20 with commercially available FC Reagent with distilled water to get the required concentration. Sodium carbonate (7.5%) was prepared by dissolving 7.5 gm. of sodium carbonate in 100ml of de-ionized water. Gallic acid (standard) stock I (Conc. 0.1 mg/ml) was prepared by dissolving 1mg of Gallic acid in 10 ml with 50% Methanol. For making standard graph of Gallic acid concentration range of 2 - 20 µg/ml was used. The assay was carried out by Using Singleton, V., Rossi, J. A. Jr, method[34]. In brief, to a 200 µl of 50% Methanol / Standard / test sample with various concentrations, added 1000 µl of FC reagent, mixed and incubated at RT for 5min. added 800µl of 7.5% sodium carbonate, mixed and incubated at RT for 30 minutes. Read the absorbance at 750 nm against blank by spectrophotometer, Color correction was given with the same concentration of the test sample in 50% Methanol without FC reagent.

Flavonoids Assay:

Vanillin, an aromatic aldehyde condenses with the flavon-3-ols and oligomers to form soluble pigments in acidic medium with an absorbance maximum at 500 nm, which can be detected by UV-VIS spectrophotometer. Vanillin Reagent (1%) was prepared by dissolving 1gm of crystallized vanillin in 100 ml of 70% Conc. H_2SO_4 (Prepared fresh). Conc. H_2SO_4 (70%) was prepared by diluting 70 ml on Conc. H_2SO_4 in 100ml De-ionized water. Methanol (50%) was prepared by diluting 1:1 with de-ionized water.

Phloroglucinol (standard) stock I (Conc. 1mg/ml): Dissolved 10mg of Phloroglucinol and made up to a volume of 10 ml with 50% Methanol, then centrifuged at 12,000 rpm for10min. Stock II: Diluted to a conc. to yield 0.1mg/ml with 50% Methanol. For making standard graph of Phloroglucinol, 1 – 10 µg/ml concentration range was used. The Flavonoid assay was carried out by using Swain, T. and Hillis, W. E method[35]. In brief, to a 400µl of distilled water / Positive control / test sample with various concentrations, added 800µl of 1% vanillin reagent, mixed and incubated at RT for 15 minutes. Read the absorbance at 500 nm against blank by spectrophotometer. Color correction was

given with the same concentration of the test sample in distilled water without vanillin reagent. The Flavonoid content in the phyto-extracts was measured with reference to the standard Gallic acid values.

Extraction Yields:

Table – 1: The percentage yield of the extracts with various solvents.

Sl. No.	Extraction Solvent	% of Yield
1.	Water	21.25
2.	Ethanol – Water (80:20)	09.00
3.	Methanol	03.00
4.	Ethyl acetate	04.75

The solvents such as water, Ethanol-water, Ethyl acetate and Methanol were utilized to optimize extraction process so as to arrive at extract with higher yields and better antioxidant potency. It was observed that water provides highest yield among all the solvents followed by ethanol-water. Ethyl acetate and methanol yielded very relatively lower amounts of extracts. Water with ethanol were also selected as the extraction solvents since both are commonly used in the food industry in a variety of ways. The extraction yield is highly valued because a low extraction yield means a lower productivity despite high antioxidant potency. The extraction yields were expressed in terms of the solid content in the dried product per soluble solid content in plant material used on a dry basis. Table 1 showed the extraction yields of the various extracts from seeds. Despite the low values obtained for the extraction yields, the antioxidant contents found were good, indicating that the extraction was efficient. Nevertheless, a relationship between the extracted mass and the corresponding total phenolics and flavonoids were not observed in all cases. Most of the phenolic or polyphenolic compounds in nature have antioxidative activities, e.g. tocopherols, flavonoids and derivatives of cinnamic acid, phosphatidic and other organic acids.

Polyphenol Content:

Table – 2: The polyphenol content of the extracts with various solvents.

Sl. No.	Extraction Solvent	Reflux Extract
1.	Water	11.0%
2.	Ethanol – Water (80:20)	06.0%
3.	Methanol	*ND
4.	Ethyl acetate	*ND

*ND-not determined because of solubility problem

Polyphenol content was determined by Singleton method. It was observed that the water extract was found to have highest percent of polyphenolsof 11% and Ethanol-water extract contains about 06%.Hence it can be concluded that both water extract and Ethanol-water extract could be highly potential in terms of health beneficial physiological effects. The polyphenol contents of other

extracts such as methanol, ethyl acetate could not be determined as they were sparingly soluble in aqueous medium and pose potential solubility problems.

Flavonoids Content:

Table – 3: The Flavonoid content of the extracts with various solvents.

Sl.No.	Extraction Solvent	Reflux Extract
1.	Water	6.0%
2.	Ethanol – Water (80:20)	3.0%
3.	Methanol	*ND
4.	Ethyl acetate	*ND

*ND-not determined because of solubility problem

It is evident from above data that water extract is proved to have highest contents of flavonoids of 6%. This is followed by ethanol-water which is of 3%.The water extract could be potent extract in terms of flavonoid contents which could elicit very useful health beneficial activities contents of other extracts such as methanol, ethyl acetate could not be determined as they were oily in nature and sparingly soluble in aqueous medium and pose potential solubility problems.

DPPH Activity:

Table – 4: The DPPH activity of the extracts with various solvents. The IC_{50} values are expressed in µg/ml

Sl.No.	Extraction Solvent	DPPH Activities (IC_{50})
1.	Water	390µg/ml
2.	Ethanol – Water (80:20)	435µg/ml
3.	Methanol	*ND
4.	Ethyl acetate	*ND

*ND-not determined because of solubility problem

DPPH a stable free radical with a characteristic absorption at 520 nm, was used to study the radical scavenging effects of ethanol extracts. The decrease in absorption is taken as a measure of the extent of radical scavenging. The radical-scavenging activity (RSA) values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of $DPPH^{\cdot}$ solution in the absence of extract at 520 nm.It is observed from the results that water extract was found to exhibit IC_{50} value of 390 µg/mland ethanol water extract has the IC_{50} value of 435 µg/ml. Both the extracts have proved to be moderately active at inhibiting the activity of DPPH.

CONCLUSION:

The present investigation aims at the extraction, phytochemical constituent analysis and antioxidant activity analysis of *cucurbitamoschata*. The solvents such as water, Ethanol-water, Ethyl acetate and Methanol were utilized to optimize extraction process arrive at extract with higher yields

and better antioxidant potency. It was observed that water provides highest yield among all the solvents followed by ethanol-water. Ethyl acetate and methanol yielded very relatively lower amounts of extracts.

Polyphenol content determination indicates that the water extract was found to have highest percent of polyphenols of 11% and Ethanol-water extract contains about 6%. Hence it can be concluded that both water extract and Ethanol-water extract could be highly potential in terms of health beneficial physiological effects.

It is evident from above data that water extract is proved to have highest contents of flavonoids of 6%. This is followed by ethanol-water which is of 3%.The water extract could be potent extract in terms of flavonoid contents which could elicit very useful health beneficial activities contents of other extracts such as methanol, ethyl acetate could not be determined as they were oily in nature and sparingly soluble in aqueous medium and pose potential solubility problems.

The results of DPPH free radical inhibitory activity studies indicate that water extract was found to exhibit IC_{50} value of 390 µg/ml and ethanol water extract has the IC_{50} value of 435 µg/ml. Both the extracts have proved to be moderately active at inhibiting the activity of DPPH.

Thus, the present investigation has provided significant information on the phytochemical contents and antioxidant activities of various solvent extracts of *cucurbitamoschata*. The results clearly indicate some major findings in the quest of the search for potential antioxidants that can be of very useful to mankind. The results warrant for further studies in this direction leading to isolation and characterization of individual molecules responsible for the antioxidant activities of the extracts from very common plants that are available in our neighborhood.

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