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Phytochemical Analysis and Antioxidant Potential of *Cucumis melo* Seeds

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ABSTRACT- Secondary metabolites or phytochemicals from plants have eminent pharmacological activities such as anti-oxidative, anti-allergic, antibiotic, hypoglycaemic and anti-carcinogenic. These secondary metabolites protect the cells from the damage caused by unstable molecules known as free radicals. They can inhibit oxidation of free radicals in both human body and food system. The food industry uses both natural and synthetic antioxidants to extend shelf life of products. But the application of synthetic antioxidant has been limited due to its carcinogenicity. Recently, research has been focused on fruit materials, which were considered rich source of antioxidant compounds. In this study the phytoconstituents of seed extracts of two varieties of *Cucumis melo* L., namely *Cucumis melo cantalupensis* and *Cucumis melo reticulatus* were studied for their antioxidant property by DPPH free radical scavenging method. During, this investigation, chloroform, petroleum ether, acetone, aqueous, and ethanolic extracts of the fruit seed were made using cold extraction process. Phytochemical study revealed that anthroquinones, quinines, cardiac glycosides, terpenoids, phenols and steroids were present in aqueous extract of both the samples. The total phenolic content of their seed extracts was found to be 8.8 mg GAE/g of dry sample and 9.2 mg GAE/g of dry sample respectively. The phenolic content was found to be linearly proportional to the antioxidant ability of the samples.

Key-words: Antioxidant, *Cucumis melo cantalupensis*, *Cucumis melo reticulates*, DPPH, Phenolic content, Phytochemicals

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INTRODUCTION

Cucumis melo L. belongs to the gourd family of *Cucurbitaceae* [1]. Cucumis melo is sought after for its sweet and refreshing fruit. The flavour and aroma of the fruit is dictated by the amount of volatile organic compounds present in it [2]. The *Cucumis melo* L. genotypes show wide variation in their morphological and biochemical characteristics. Therefore an intraspecific classification system for melons was developed by taxonomists [3]. The American cantaloupe (*var.reticulatus*) and European cantaloupe (*var.cantalupensis*) have similar morphological characters like rapid yellowing of epidermis at maturity, slipped peduncle and orange flesh [4]. The oxygenated environment makes it incumbent for our body systems to evolve efficient ways to detect and scavenge reactive oxygen species at the cellular level [5].

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Over the past decade, antioxidants have achieved prominence in the food industry due to their ability to eliminate free radicals interaction. Each antioxidant however needs to be extensively tested and commercially regulated [6]. Phytochemicals are non-nutrient, bioactive compounds found in fruits, grains and vegetables. The phytochemical analysis, antioxidant potential and total phenolic content of seeds from these two varieties are analysed in this study. Medicinal plants are significant in the production of novel nature centric drugs. The medicinal value of these plants is due to bioactive substances like alkaloids, flavonoids, tannins and phenolic compounds that produce a physiological effect on the human body [7]. Phytochemicals, especially phenolics and flavonoids, act as potent antioxidants and could possess anticancer properties. These plant compounds, when regularly ingested could play a major role in reducing the risk of chronic diseases such as cancer, diabetes, Alzheimer's disease, cardiac failure, strokes, cataracts and some functional declines that accompany aging [8]. This research involves identifying the phytochemical content of two varieties of *Cucumis melo L.* seeds, estimating the total phenolic content as well as their antioxidant property.

MATERIALS AND METHODS

This study was carried out at the Department of Food Process Engineering, SRM University, Kattankulathur, Chennai, India for a period of three months.

Chemicals

Ethanol, acetone, petroleum ether, chloroform, Folin-ciocalteau reagent (Sigma), DPPH (2,2-dyphenyl-1-picrylhydrazyl) (Sigma) and butylated hydroxy toluene (BHT) (Sigma). The solvents and chemicals used were of analytical grade.

Plant Material

The *Cucumis melo var, cantalupensis,* and *Cucumis melo var. reticulates* were collected from the Chengalpattu area in Chennai, Tamil Nadu. The seeds were washed and shade dried for two days. The seeds were then hulled manually and stored at room temperature in an air tight container.

Phytochemical Analysis

Two gm of seeds was weighed and finely grounded. The phytoconstituents in the seeds were extracted using 5 different solvents, namely, ethanol, acetone, distilled water (aqueous), chloroform and petroleum ether. Phytochemical screening of *C. reticulatus* and *C. cantalupensis* to identify the presence of flavonoids, tannins, phenols, terpenoids, saponins, anthocyanins, quinines, cardiac glycosides, glycosides, betacyanins, anthroquinones, cocumarins, alkaloids and steroids were done using standard methods [9].

Antioxidant Activity

The ability of the seed extract to scavenge DPPH ions (2,2-dyphenyl-1-picrylhydrazyl) was measured to determine the antioxidant potential. Qualitative and quantitative analysis was performed by using standard methods [10]. Total 50 µl extract of *Cucumis melo var* seeds was taken in a microtitre plate. Total 100 µl of 0.1% methanolic DPPH was added over the samples and incubated for 30minutes in the dark. The qualitative analysis of sample was performed by observing the discoloration from purple colour. Yellow was considered as a strong positive and pale pink as a weak positive.

Total 100 µl of the *Cucumis melo var* seed sample extract used in the qualitative assay was taken and mixed with 2.7 ml of methanol. 200 µl of 0.1% methanolic DPPH was then added. The suspension was incubated for 30 minutes in the dark. The absorbance maximum was measured using a UV double beam spectrum to scan at 517 nm. The reduction in absorbance was calculated in percentage (% or Inhibition %) using the following formula:

$$\text{Inhibition \%} = \frac{[\text{Absorbance of control (A517)} - \text{Absorbance of sample (A517)}]}{(\text{Absorbance of control (A517)}} \times 100$$

The experimental results were verified by comparing them to the antioxidant activity of synthetic standard butylated hydroxyl toluene (BHT) used. Total 73.5% BHT was used as a positive control [10].

Total Phenolic Content

The total phenolic content was measured using standard protocols [11]. Total 100 µL of the sample aqueous extract was mixed with 0.5 ml of Folin-ciocalteau reagent (1/10 dilution) and 1.5 ml sodium bicarbonate (2% w/v). The mix was incubated in the dark at room temperature for 15 min. The absorbance of blue coloured solution of all samples was measured at 765 nm using a UV spectrophotometer. The results obtained were expressed as mg of gallic acid equivalent (GAE) per gram dry weight of seed extract.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of preliminary phytochemical analysis *C. cantalupensis* and *C. reticulatus* are *ntalupensis* contained saponin, terpenoids, steroids, alkaloids, cadiac glycosides, anthroquinones, and quinones. Ethanol extracts and aqueous solution of *C. cantalupensis* seeds showed the presence of high quinones, terpenoids and phenols concentration, whereas concentrations of anthroquinones, steroids and cardiac glycosides were higher only in aqueous solution. Saponins were present in moderate quantities when extracts of seeds were prepared with petroleum ether and water.

Analysis of *C. reticulatus* samples indicated that quinones and steroids were present in large amounts when all solvents were used except for ether, where they were found in moderate concentrations. Anthroquinones, cardiac glycosides, steroids, and terpenoids were found in moderate proportions for *C. reticulatus* seeds. Quinones, terpenoids and steroids were found to be present in seed extracts of all five solvents used in this experiment for both varieties of melons.

The concentrations of phytochemicals, especially flavonoids and phenols, in the extracts depended on the polarity of the solvents used. The plant extracts in highly polar solvents show higher levels of flavonoids and phenols [12,13].

The total phenolic content estimated using Folin-ciocalteau reagent and the results obtained were expressed in mg of gallic acid equivalent (GAE) per gram dry weight of seed extract. The total phenolic content of *C. cantalupensis* and *C. reticulatus* were found to be 8.8 mg GAE/g of dry sample and 9.2 mg GAE/g of dry sample respectively. Phenols are important plant compounds as they possess the potential to scavenge free radicals due to the presence of a hydroxyl group. Therefore, antioxidant potential of plant material is often attributed to the phenolic content [14]. The total phenolic content of *Cucumis melo* L. seeds were found to be lower than that of other seed extracts such as *Swietenia mahagoni* [15] and pomegranate

cultivars [16].

Table 1: Phytochemical constituents of *Cucumis melo var cantalupensis*

Phytochemicals	Acetone	Ethanol	Aqueous	Chloroform	Petroleum Ether
Tannin	-	-	-	-	-
Saponin	-	-	+	-	+
Flavonoids	-	-	-	-	-
Anthocyanin	-	-	-	-	-
Betacyanin	-	-	-	-	-
Quinones	+	++	++	+	+
Glycosides	-	-	-	-	-
Cardiac glycosides	+	+	++	+	-
Terpenoids	+	++	++	+	+
Phenol	-	++	++	-	-
Cocumarins	-	-	-	-	-
Alkaloid	+	+	+	-	-
Steroid	+	+	++	+	+
Anthroquinone	-	+	++	+	+

[++ present in high concentration; + present in moderate concentration and – absent]

Table 2: Phytochemical constituents of *Cucumis melo var reticulatus*

Phytochemicals	Acetone	Ethanol	Aqueous	Choloform	Petroleum Ether
Tannin	-	-	-	-	-
Saponin	-	-	+	-	-
Flavonoids	-	-	-	-	-
Anthocyanin	-	-	-	-	-
Betacyanin	-	-	-	-	-
Quinones	++	++	++	++	+
Glycosides	-	-	-	-	-
Cardiac glycosides	+	++	++	+	+
Terpenoids	++	++	++	++	+
Phenol	+	-	++	-	-
Cocumarins	-	-	-	-	-
Alkaloid	-	-	++	-	-
Steroid	+	++	++	+	+
Anthroquinone	++	++	++	+	+

[++ present in high concentration; + present in moderate concentration and – absent]

Antioxidant Activity

The antioxidant potential of *Cucumis melo var* seeds was determined using a methanol solution of DPPH reagent. DPPH is a stable free radical that remains unaffected by side reactions like metal ions chelations and enzymatic inhibition. DPPH solution exhibits a deep purple colour with absorption maxima at 517 nm. This colour then fades as free radicals are quenched by antioxidants. The absorbance of the bleached solution decreases and this change in absorbance is used to estimate the antioxidant ability of a substance [17]. The extracts of 2 varieties *Cucumis melo* L seeds were analyzed for their antioxidant potential both qualitatively and quantitatively and these results were found in accordance with each other. The quantitative results were found to be comparable to the synthetic antioxidant BHT (butylated hydroxyl toluene), which was used as the standard. The results of quantitative analysis are summarized in Fig. 1 & Fig. 2.

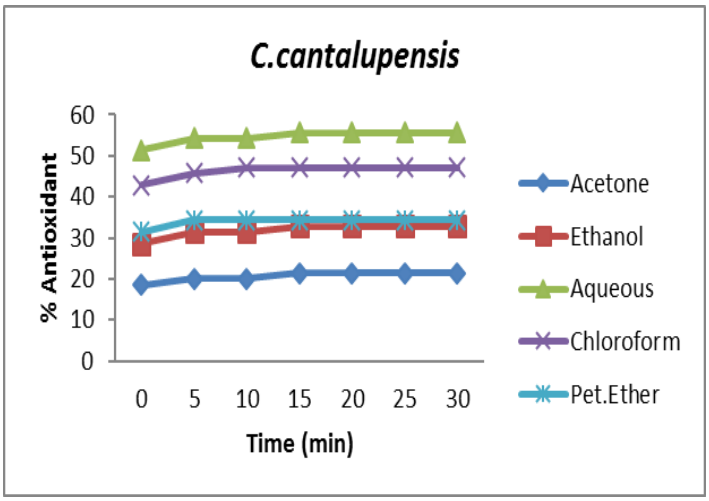

Fig. 1: The antioxidant ability of *Cucumis melo var cantalupensis* measured in % inhibition of DPPH free radicals

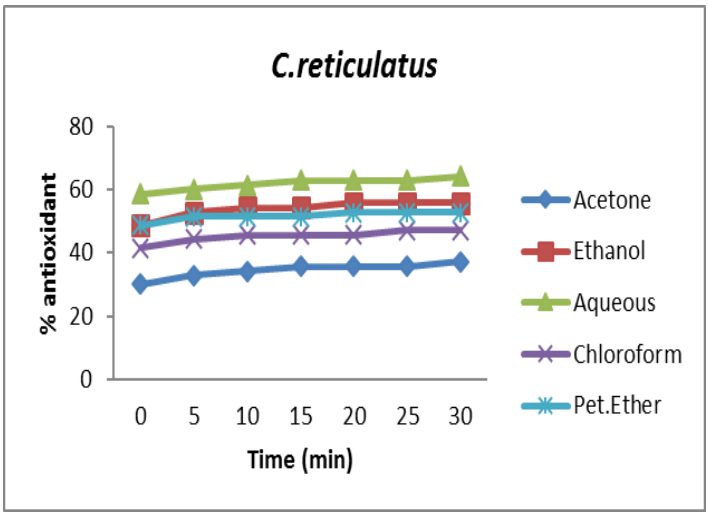

Fig. 2: The antioxidant ability of *Cucumis melo var reticulatus* measured in % inhibition of DPPH free radicals

The maximum antioxidant potential was observed in aqueous extract after 30 minutes of incubation *C. cantalupensis* and *C. reticulatus* that varied from 18.57% to 55.71% and 30% to 64.29%, respectively. *C. reticulatus* seed sample was found to have a higher phenolic content and this shows a linear relationship to the antioxidant ability of *C. reticulatus*. *C. reticulatus* was found to have better free radical scavenging capacity when compared to *C. cantalupensis* seeds and this could be due to the higher phenolic content of *C. reticulatus* seed extract. The free radical scavenging ability of *Cucumis melo var* seeds can be compared to the antioxidant ability of *Erica herbacea L.* and some medicinal plants like *Crocus sativus*, *Taraxacum officinale* and *Tribulus terestris* L. [18,19].

CONCLUSIONS

The present study shown that results of phenolic content and antioxidant activities of *Cucumis melo var cantalupensis* and *Cucumis melo var reticulatus*. Study reveals that the maximum phytoconstituents are present in aqueous extract when compared to other five plant extracts. The antioxidant potential of *Cucumis melo* L. seed varieties were found to be significant though the phenolic content of both samples is low. Phytochemistry of these seeds should be extensively analyzed in order to identify if any other phytconstituents are responsible for the antioxidant ability. The phytochemicals extracted from seeds of the 2 *Cucumis melo var* species studied here can be considered as potential sources of antioxidants. *Cucumis melo* L. seed antioxidant may have potential application in food industry as food stabilizer, for development of new value added product, nutraceutical etc. Further studies need to be carried out in order to identify conclusively, characterize and isolate the bioactive compounds with emphasis on safety and efficiency of these bioactive molecules to make them excellent natural source of antioxidant.

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