IJPSR (2021), Volume 12, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 January 2020; received in revised form, 09 April 2020; accepted, 12 April 2020; published 01 January 2021

ANTIOXIDANT POTENTIAL OF DIFFERENT PARTS (LEAVES, STEM, FRUIT, SEED, FLOWER AND ROOT) EXTRACTS OF *CUCUMIS MELO* VAR AGRESTIS

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Keywords:

Wild musk melon, Flavonoid, Phenolic, Radical scavenging, *Cucumis melo* subsp. Agrestis

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ABSTRACT: *Cucumis melo* var agrestis (CMA) is belongs to the family of Cucurbitaceae. This plant is also known as wild musk melon it is widely distributed in the coastal areas. Many of the Cucumis species were reported as antioxidants, but there is no report on the antioxidant activity of CMA. The present study is aimed to evaluate the phytochemical analysis and antioxidant activity of different parts (leaves, stem, fruit, seed, flower, and root) extract of *Cucumis melo* var agrestis. The phytochemical screening confirms the presence of alkaloids, flavonoids, tannins, carbohydrates, saponins, glycosides, proteins, and amino acids in all extracts except Seed extract of CMA (SECMA), Fruit extract of CMA (FRCMA), and Flower extract of CMA (FLCMA) absence with tannins and FLCMA, a Root extract of CMA (RCMA) absence with saponins. Fruit extract of CMA (FRCMA) has the lower IC_{50} value for DPPH, ABTS, FRAP, and Phosphomolybdate assay when compared with all other extracts, and also the FRCMA possess higher phenolic and flavonoid content and also its activity is a nearby standard which confirms the higher antioxidant activity of FRCMA. The results of the present study concluded that FRCMA and LCMA has potent antioxidant activity and antioxidant activities are important to healing of many diseases; this research work is important in the future to conduct further studies on fruits of this plant material.

INTRODUCTION: The antioxidants are vital in life because most diseases are caused by free radicals which are scavenged by the antioxidants. These antioxidants inhibit the oxidative stress of the cells. The human systems are producing endogenous antioxidants like glutathione, catalase, superoxidase dismutase [1, 2]. Dietary antioxidants are also used for the management of numerous diseases. Vitamin C, Vitamin A and Vitamin E are potent dietary antioxidants. Several plants are having phytochemicals for its antioxidant property. Phenolic compounds and flavonoids present in plant materials are produced potent antioxidant properties [3]. The antioxidant property of the plant material is mandatory for dealing with several diseases and disorders of the animal kingdom [4, 5]. *Cucumis melo* var agrestis (CMA) belongs to the family of Cucurbitaceae; this herbal is extensively scattered in-country and seaside areas **Fig. 1**. This plant is usually called wild musk melon, kachari, small guard [6].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(1).465-69

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).465-69

MATERIALS AND METHODS:

Collection and Authentication: The entire plant of CMA was collected from the village of Pungavarnatham, Thoothukudi district, Tamil Nadu, India month of December 2017 and verified from Government Siddha Medical College Chennai, voucher specimen no. GSMC/MB-87/18.

Soon after collection, the various CMA parts were cleaned, dried in the shade and crushed to a coarse powder, stored in separate airtight plastic containers until further use.

FIG. 1: WHOLE PLANT OF *CUCUMIS MELO VAR AGRESTIS*

Extraction of Plant Material: The coarsely powdered various parts (leaves, stem, fruits, seeds, flower, and root) of CMA were defatted by using petroleum ether (60-80 °C) and then extracted with 60% methanol for 7 days using cold maceration method and filtered. The extract was further concentrated under vacuum using a rotary vacuum evaporator (Buchi R-V120, Switzerland) at 40°C. The obtained crude extract was weighed and stored at 4 °C for further analysis [7].

Preliminary Phytochemical Analysis: The obtained extracts were subjected to phytochemical evaluation and identified the various plant constituents present in the test sample qualitatively [8-10].

Quantification of Total Phenolics and Flavonoids:

Estimation of Total Phenolics: The total phenolic content of different extracts were estimated using the Folin-Ciocalteau assay method [11, 12]. In this method, 100 µl of extract (1 mg/ml) or standard solution of Gallic acid (10, 20, 40, 60, 80, 100 µg/ml) was added with 50µl of Folin-Ciocalteau reagent and 860 µl of distilled water and the mixture was incubated at room heat for 5 min. After incubation period, 100 µl of 20% sodium carbonate and made up to 2 ml with distilled water; again the mixture was incubated in the dark to complete the reaction for 30 min. The absorbance of the mixture was studied at 765 nm using blank (Distilled water). The total phenolic content was established from the calibration curve of Gallic acid, and it was stated as milligrams of Gallic acid equivalents (GAE) per gram of extract.

Estimation of Total Flavonoids: The total flavonoid contents of different extracts were determined by the Aluminium chloride colorimetric method. In this method, 100µl of different extracts and standard solutions of Quercetin (10, 20, 40, 60, 80, 100µg/ml) and made up to 2 ml with ethanol 2 ml [13, 14]. Then, the obtained mixture was added with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate, and made up to 5 ml with distilled water. After 30 min, the absorbance of the mixtures was studied at 415 nm using blank (Ethanol). The total flavonoid content was estimated from the standard Quercetin calibration curve and it was stated as milligrams of Quercetin equivalents (QE) per gram of extract.

***In-vitro* Anti-oxidant Study:**

DPPH Free Radical Scavenging Assay: The radical scavenging capacity of different extracts was evaluated using DPPH radical. The 0.135 mM DPPH in methanol solution was used for this assay. 1 ml of DPPH solution was mixed with 1 ml of different extracts in methanol in different concentrations ranging 0.02–0.1 mg. Then the mixture was placed in the dark at room temperature 30 min. At the end of the assay, the absorbance was studied at 517 nm. Quercetin was used as standard [15]. The radical scavenging activity was calculated by,

DPPH radical scavenging activity (%) = Absorbance of control –Absorbance of sample ×100 / Absorbance of control

Where, Control is without any sample (Only DPPH with ethanol). Sample is either standard or extracts.

ABTS Radical Scavenging Assay: In this assay 7 mM ABTS solution and 2.4 mM potassium persulfate solution were used [16]. The above two solutions were mixed in equal proportions for the preparation of the ABTS solution. The above mixture was placed at room temperature for 12 h in the dark for reaction [18]. Then the 1 ml of this mixture was diluted with 60 ml of methanol to maintain the absorbance 0.706 ± 0.001 at 734 nm. 1

ml of different extracts was added with 1ml of the ABTS solution and after 7 min, absorbance was studied at 734 nm. The ABTS percentage inhibition was calculated using the formula,

ABTS radical scavenging activity (%) = Absorbance of control –Absorbance of sample ×100 / Absorbance of control

Where, Control is without any sample (Only ABTS with ethanol). Sample is either standard or extracts.

Ferric Reducing Antioxidant Power Assay (FRAP): The FRAP solution contains 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mMHCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The temperature of the solution was increased to 37 °C before use. 0.1 ml of different extracts (1mg/ml) was added with 2.850 ml of the FRAP solution and permitted to place in the dark for about 30 min. Then the yellow color product was formed, which was studied at 593 nm [17]. The trial was achieved in triplicate, standardization curve was designed, with vitamin C (20-100 µg/ml) as standard and FRAP activity of different extracts (µg/ml) were studied as vitamin C equivalents [19].

Phosphomolybdate Assay for Total Antioxidant Activity: The different extracts were liquefied in methanol to get a concentration of 1 mg/ml. About 3 ml of different extracts were added with 0.3 ml of reagent solution (0.6 M Sulphuric Acid, 28mM Sodium Phosphate, 4mM Ammonium molybdate) and the mixture was heated at 95 °C for 90 min using a water bath. After heating, the mixture was cooled and studied the absorbance at 695nm using blank. The trial was achieved in triplicate; a standardization curve was designed by vitamin C (20-100 µg/ml) as standard, and FRAP activity of different extracts (µg/ml) was studied as vitamin C equivalents.

RESULTS AND DISCUSSION: Percentage yield of leaves extract of CMA (LCMA), stem extract (STCMA), fruit extract (FRCMA), seed extract (SECMA), flower extract (FLCMA), and root extract (RCMA) were obtained to be 12.10, 16.85, 18.22, 17.35, 26.95 and 8.73% w/w respectively. The results indicated that the flower has maximum extractive yield compared with other extracts.

Preliminary phytochemical studies confirm the presence of alkaloids, flavonoids, tannins, carbohydrates, saponins, glycosides, proteins and amino acids in all extracts except SECMA, FRCMA, and FLCMA absence with tannins and FLCMA, RCMA absence with saponins. Different phytoconstituents were responsible for antioxidant capacity, so that phytochemical screening is important in the evaluation of antioxidant activity.

TABLE 1: ESTIMATION OF TOTAL PHENOLIC CONTENT IN DIFFERENT EXTRACTS OF CMA

S. no.	Extracts	Total Phenolic Content expressed as Gallic acid equivalent (mg/g)	Total Flavonoid Content expressed as Quercetin equivalent (mg/g)
1	LCMA	17.82	12.93
2	STCMA	4.70	12.89
3	FRCMA	19.82	18.39
4	SECMA	1.76	11.04
5	FLCMA	5.21	8.05
6	RCMA	14.30	12.88

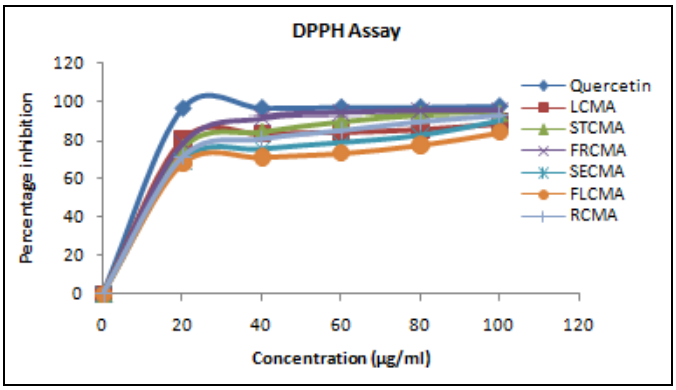

FIG. 2: DPPH SCAVENGING ACTIVITIES OF DIFFERENT EXTRACTS AND STANDARD QUERCETIN

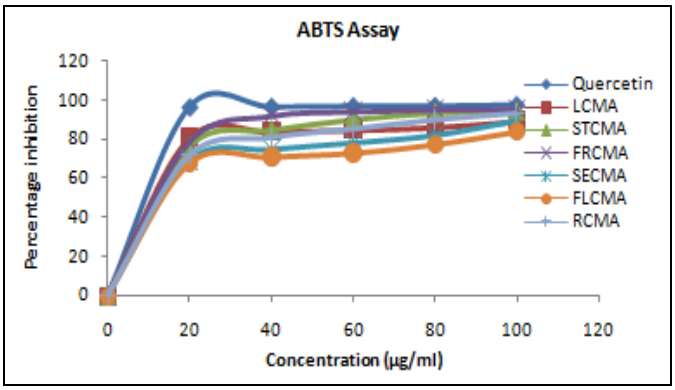

FIG. 3: ABTS SCAVENGING ACTIVITIES OF DIFFERENT EXTRACTS AND STANDARD QUERCETIN

Phenolic and flavonoid content has potent antioxidant potential; thereby total phenolic and flavonoid contents were estimated. Total phenolic content and total flavonoid content of different extracts were calculated from the standard calibration curves of Gallic acid and Quercetin, respectively. Total phenolic content of LCMA, STCMA, FRCMA, SECMA, FLCMA, and RCMA was found to be 17.82, 4.70, 14.82, 1.76, 5.21, and 14.30 mg/g, respectively **Table 1**. Total flavonoid content of LCMA, STCMA, FRCMA, SECMA, FLCMA, and RCMA was found to be 18.39, 12.89, 12.93, 11.04, 8.05, and 12.88 mg/g, respectively **Table 1**.

TABLE 2: DPPH AND ABTS RADICAL SCAVENGING ASSAY IC_{50} VALUES OF EXTRACTS AND STANDARD QUERCETIN

S. no.	Extracts	DPPH assay IC_{50}value of extract (µg/ml)	ABTS assay IC_{50} value of extract(µg/ml)
1	LCMA	18.46	9.04
2	STCMA	19.23	14.69
3	FRCMA	15.60	7.66
4	SECMA	27.31	19.33
5	FLCMA	30.78	17.96
6	RCMA	23.03	15.51
7	Quercetin	5.74	6.50

DPPH assay and ABTS assay were produced the free radicals, which are scavenged by extracts and standard quercetin. The percentage inhibition of DPPH radical and ABTS radical by extracts, standard quercetin are shown in **Fig. 2** and **Fig. 3**. The 50% of an inhibitory concentration of free radicals was calculated for the determination of antioxidant capacity. DPPH assay and ABTS assay IC_{50} values are shown in **Table 2,** and FRCMA had the lower IC_{50} values when compared to other extracts.

TABLE 3: FRAP ASSAY AND PHOSPHOMOLYBDATE ASSAY VALUES OF EXTRACTS IN TERMS OF ASCORBIC ACID EQUIVALENTS (AAE)

S. no.	Extracts	FRAP assay values in terms of AAE (µg/ml)	Phosphomolybdate assay values in terms of AAE (µg/ml)
1	LCMA	29.61	14.50
2	STCMA	28.13	13.81
3	FRCMA	30.77	25.28
4	SECMA	28.24	12.21
5	FLCMA	20.16	13.39
6	RCMA	29.32	15.02

FRAP assay was calculated for reducing the capacity of ferric ions by extracts, and total antioxidant capacity was calculated for reducing the capacity of Molybdenum ion by extracts. FRAP assay and Phosphomolybtate assay were expressed as ascorbic acid equivalent. Values of FRAP assay and phosphomolybdate assay are shown in Table 3. FRAP assay and phosphomolybdate assay revealed that FRCMA was showing increased activities. Fruit extract of CMA (FRCMA) has the lower IC_{50} value for DPPH, ABTS, FRAP, and Phosphomolybdate assay compared with all other extracts and the FRCMA possesses higher phenolic and flavonoid content, and also its activity is a nearby standard which confirms the higher antioxidant activity of FRCMA.

CONCLUSION: The results of the present study concluded that FRCMA and LCMA has potent antioxidant activity and antioxidant activities are important to the healing of many diseases; this research work is important in future to conduct further studies on fruits of this plant material and isolation of active principle.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: We declare that we have no conflicts of interest.

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How to cite this article:
Gopalasatheeskumar K and Kalaichelvan VK: Antioxidant potential of different parts (leaves, stem, fruit, seed, flower and root) extracts of *Cucumis melo* var Agrestis. Int J Pharm Sci & Res 2021; 12(1): 465-69. doi: 10.13040/IJPSR.0975-8232.12(1).465-69.