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Research Article

INVESTIGATION OF ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC LEAF EXTRACT OF CUCUMIS MELO VAR AGRESTIS (CUCURBITACEAE) - A WILD EDIBLE PLANT FROM INDIA

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ABSTRACT

Background: Antioxidants acts in different stages, stopping the formation of reactive oxygen species or scavenge the free radicals or increase the antioxidants protection enzyme abilities. Natural products such as herbals and herbal extracts have reported as antioxidant property but there is no report on antioxidant properties of *Cucumis melo var agrestis* (CMA). **Aim and Objectives**: The present study is aimed to evaluate the in vitro antioxidant activity of hydroalcoholic leaf extract of *Cucumis melo var agrestis* (HALEC). **Methods:** The antioxidant activities were evaluated by DPPH assay, ABTS assay, FRAP assay and Phosphomolypdenum assay. Standard quercetin was used for the DPPH radical scavenging assay and ABTS radical scavenging assay and L-Ascorbic acid was used as a standard for FRAP assay and Phosphomolypdenum assay. **Results:** Our results revealed that HALEC has potent antidioxidant potential and it was compared with the standard quercetin and ascorbic acid. **Conclusion**: From the results of this research the HALEC has the potent antioxidant potential and further study needed to isolate the active principles.

Key words: Total antioxidant capacity, Quercetin, Vitamin C, Cucurbitaceae, Mithukku vathal sedi

INTRODUCTION

The antioxidants are important in life because the most of the diseases are caused by free radicals which are scavenged by the antioxidants. The antioxidants are prevents the oxidative stress of the cells. The human systems producing antioxidants like glutathione, catalase, superoxide dismutase [1],[2]. Scavenging of intracellular free radical creation provides a beneficial approach to prevent oxidative stress and the associated diabetic vascular difficulties. Antioxidants acts in different stages, stopping the formation of reactive oxygen species or scavenge the free radicals or increase the antioxidants protection enzyme abilities [5]. Naturally herbals has the antioxidants role with the phytochemicals such as flavonoids, vitamin C, Vitamin E etc., *Cucumis melo var agrestis* (CMA) is belongs to the family of cucurbitaceae, this plant is widely distributed in rural and coastal areas. **(Figure 1)**

Fig. 1: Whole plant of *Cucumis melo var. agrestis*

In Tamil this plant is known as Mithukku vathal sedi or Chukkanchedi, Hindi Kachari, Telugu Dosakaya and English Wild musk melon [6]. Many of the literature says that Cucumis herbals has antioxidant property but since there is no reports on antioxidant property of CMA. Therefore current research is aimed to evaluate the antioxidant activity of hydroalcoholic leaf extract of *Cucumis melo var agrestis* (HALEC).

MATERIALS AND METHODS

Plant Collection and authentication

The fresh leaves of the plant CMA was collected from Pungavarnatham village, Thoothukudi District, Tamil Nadu, India. Month of December 2017 and authenticated by Dr. S. Sankaranarayanan, Department of Medicinal botany, Government Siddha Medical College, Chennai, Voucher specimen No. GSMC/MB-87/18.

Preparation of plant material

The collected leaves were cleaned, washed with distilled water, dried under sunshade in dark room, and powdered by using mechanical mixer. After size reduction leaves were sieved under sieve No. 40 and sieve No. 60, stored in airtight container at room temperature [28].

Extraction of the plant material

200g of finely powdered leaf powder was defatted with 1 L of petroleum ether in a soxhlet apparatus for 48 h, obtained marc was further extracted with 1 L of 60% methanol (600ml of methanol: 400ml of water) in soxhlet apparatus for 48 h, obtained marc was again extracted with water by cold maceration method for 48 h. After extraction the extracts were separately concentrated by distillation and dried at room temperature until

get viscous solid mass. The obtained crude extracts were weighed and stored at 4^0C for the further analysis.[7] Based on the preliminary studies conducted by our lap Hydroalcoholic (60% Methanoilic) extract was used for the evaluation of antioxidant activity [30,31].

***In vitro* anti-oxidant studies**

DPPH free radical scavenging assay

The radical scavenging capacity of the extracts on DPPH radical was evaluated. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02–0.1 mg of the different extracts. The mixture was incubated for a period of 30 min in the dark at room temperature. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercetin was used as standard. [15] The radical scavenging activity was calculated by, DPPH radical scavenging activity (%) = [(AbsControl - AbsSample)]/ (AbsControl)] x 100 where AbsControl is the absorbance of DPPH radical with methanol; AbsSample is the absorbance of DPPH radical and either the sample extract or standard.

ABTS radical scavenging assay

To determine ABTS radical scavenging assay, the stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution [16]. Both the reagents were mixed in equal quantities for preparing the working solution. And allow it to react for 12 h at room temperature in the dark. Further the mixture was diluted by mixing one ml ABTS solution with 60 ml of methanol so that the absorbance becomes 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1000 µl) were allowed to react with (1000 µl) of the ABTS solution for 7 min and the absorbance was read at 734 nm. The ABTS scavenging capacity of the extract was compared with that of quercetin and percentage inhibition was calculated using the formula [(Abs Control- Abs Sample)]/ (Abs Control) x100 where, Abs Control is the absorbance of ABTS radical and methanol, Abs Sample is the absorbance of ABTS radical and sample extract or standard. Percentage inhibition (I %) = (Abs control- Abs sample /Abs control) X 100. Different sample concentrations were used in order to obtain calibration curves and to calculate the IC_{50} values

Ferric Reducing Antioxidant Power Assay (FRAP)

The stock solutions included 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mMHCl, and 20 mM FeCl3•6H2O solution. The working solution was prepared freshly by mixing 25 ml acetate buffer, 2.5 ml of 2,4,6-Tri(2-pyridyl)-s-triazine and 2.5 ml FeCl3•6H2O. The temperature of the solution was raised to 37 °C before use. The plant extracts (100 µL) was allowed to react in the dark with 2.850ml of the FRAP solution for about 30 min. The absorbance of the yellow product was read at 593 nm. The standard curve was linear between 20 to100 µg/ml of FeSO4. Results are expressed in µg/ml of Fe (II) equivalent to quercetin [17, 29].

Phosphomolybdate assay for total antioxidant activity

The total antioxidant activity was determined using the method described by [18] Plant extracts were dissolved in methanol to obtain a concentrations of 20-100 µg/ml. 3 ml of extract was placed in a test tube, 0.3 ml of reagent solution (0.6 M Sulphuric Acid, 28mM Sodium Phosphate, 4mM Ammonium molybdate) was added and the mixture was incubated at 95^0C for about 90 minutes in a water bath. Then the mixture was cooled to room temperature, the absorbance of the each solution was measured at 695nm against blank using UV-Visible spectrophotometer. The experiment was performed in triplicate, calibration curve was plotted, using vitamin C (20-100 µg/ml) as standard and total antioxidant activity of extract (µg/ml) expressed as vitamin C equivalents.

Results

***In vitro* antioxidant studies**

DPPH was produces the free radicals which was scavenged by extracts and standard quercetin. The percentage inhibition of DPPH radical by HALEC and quercetin was shown in **fig. 2**. The 50% of inhibitory concentration of DPPH radicals was calculated for determination of antioxidant capacity. DPPH assay IC50 values of HALEC and quercetin were found to be 18.46µg/ml and 5.74µg/ml respectively.

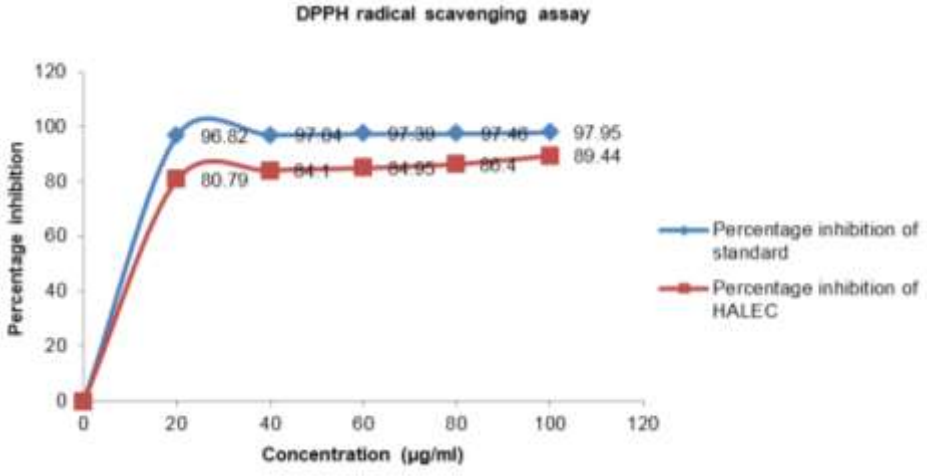

Fig. 2: DPPH radical scavenging activities of HALEC and Quercetin

The percentage inhibition of ABTS radical by HALEC and quercetin were shown in **fig. 3**. The 50% of inhibitory concentration of ABTS radicals was calculated for determination of antioxidant capacity. ABTS assay IC_{50} value of HALEC and Quercetin was found to be 7.04 µg/ml and 6.50 µg/ml respectively.

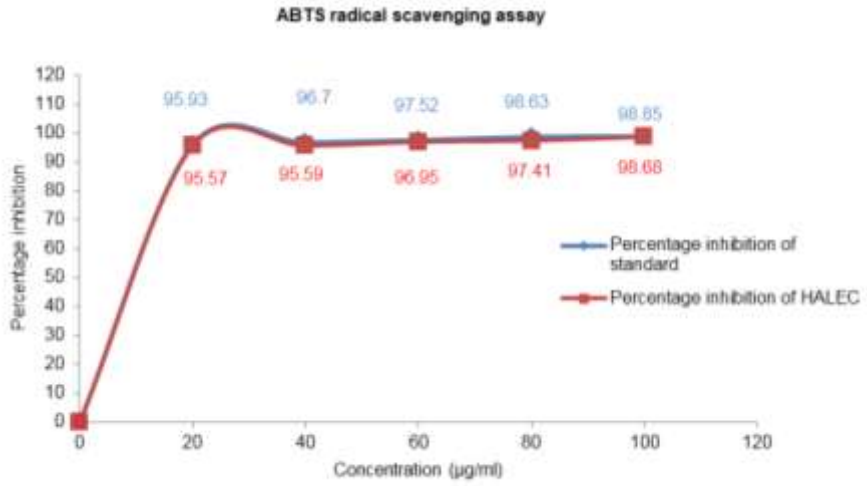

Fig. 3: ABTS radical scavenging activities of HALEC and Quercetin

FRAP assay was calculated for reducing capacity of ferric ions by extracts and total antioxidant capacity was calculated for reducing capacity of Molybdenum ion by extracts. FRAP assay and Phosphomolybtate assay were expressed as ascorbic acid equivalent. Values of FRAP assay and phosphomolybtate assay were shown in **Tables 2 and 3**. The ascorbic acid equivalent of HALEC in FRAP assay and total antioxidant assay increases with concentration dependent.

Table 2: FRAP values of HALEC in terms of Ascorbic Acid Equivalents (AAE)

S. No	Concentration of HALEC (µg/ml)	Vitamin C equivalent (µg/ml)
1	20	18.67
2	40	23.33
3	60	39.52
4	80	43.31
5	100	68.24

Table 3: Total antioxidant capacity of HALEC expressed as µg/mL of ascorbic acid equivalents (AAE)

S. no	Concentration of HALEC (µg/ml)	Vitamin C equivalent (µg/ml)
1	20	12.78
2	40	39.50
3	60	75.01
4	80	103.20
5	100	125.52

DISCUSSION

Free radical and reactive oxygen species are well known inducers of cellular and tissue pathogenesis leading to several human diseases, such as cancer, inflammatory disorders, and diabetes mellitus, as well as in the aging process [25]. Antioxidant activity was evaluated by DPPH, ABTS, FRAP & Total antioxidant assay radical scavenging assays. DPPH and ABTS radical assays have been widely used as reliable methods for determining the free radical scavenging efficacy of the lead molecules. The principle of the DPPH free radical scavenging activity assay is based on the reduction of DPPH radicals in methanolic solution. Due to the presence of an odd electron, DPPH gives a strong absorption maximum at 515 nm. It is generally accepted that substances which are able to donate hydrogen or an electron to DPPH, a synthetic nitrogen-centered stable radical, can be considered as antioxidants and therefore free radical scavengers. ABTS radical assay is the preformed radical monocation of ABTS radical which is generated by oxidation of ABTS radicals with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The antioxidant activity of different lead molecules depends on the number and location of hydroxyl and other functional groups such as carboxyl and phenolic ring system [26]. A simple assay measuring the ferric reducing ability of plasma, the FRAP assay, is presented as a novel method for assessing “antioxidant power”. The reducing properties are linked with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. In the present study, the absorbance of HALEC clearly increased which is due to the formation of the Fe2+- TPTZ complex with increasing concentration as observed. The HALEC showed a significant antioxidant activity. Total antioxidant activity is Phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The DPPH, ABTS radical scavenging assay has displayed highest antiradical activity and FRAP, Total antioxidant assay shows the highest reducing potential of HALEC thereby potent antioxidant activity which was compared with standard Qucertein and Vitamin-C.

CONCLUSION

From the results of this research concluded that hydroalcoholic leaf extract of *Cucumis melo var agrestis* has the potent antioxidant potential. And further studies required for the isolation of active principles present in leaf extract of *Cucumis melo var agrestis*.

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