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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC FRUIT EXTRACT OF *CUCUMIS DIPSACEUS*

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ABSTRACT
The antioxidants are vital in life. Because, most of the diseases are caused by free radicals which are scavenged by the antioxidants. Naturally herbals has the antioxidants role with the phytochemicals such as flavonoids, vitamin C, Vitamin E etc., So that, current research is focused on study of antioxidant activity of herbal. *Cucumis dipsaceus* Ehrenb is annual climbing herb, belong to genus Cucumis L., family Cucurbitaceae. Commonly, Hydroalcohol is a suitable solvent for successive extraction of polar and non polar phytoconstituents. Many of the hydroalcoholic plant extract have potent antioxidant activity, since there is no evidence for *Cucumis dipsaceus*. Therefore current research is focused on antioxidant activity of hydroalcoholic fruit extract of *Cucumis dipsaceus* (HAFEC). The antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays. The results of current research revealed that HAFEC has potent antioxidant activity which produced similar effect to standard quercetin. Results concluded that, the antioxidant activity of HAFEC may be due to the presence of phytoconstituents such as flavonoids, triterpenoids, catechin etc., the future direction of this study is to perform in vivo studies and finding of molecular mechanism.

KEYWORDS: Cucurbitaceae, *In vitro*, DPPH, ABTS, flavonoid.

1. 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 prevent the oxidative stress of the cells. The human systems producing antioxidants like glutathione, catalase and superoxide dismutase.[1-6] Scavenging of intracellular free radical creation provides a beneficial approach to prevent oxidative stress associated with diabetic vascular difficulties, neurological disorder, cancer and cardio vascular diseases. Antioxidants acts in different stages, stopping the formation of reactive oxygen species or scavenge the free radicals or increase the antioxidants protection enzyme abilities. Naturally herbals has the antioxidants role with the phytochemicals such as flavonoids, vitamin C, Vitamin E etc., *Cucumis dipsaceus* Ehrenb is annual climbing herb, belong to genus Cucumis L., family Cucurbitaceae.[7,8] This herb is available in forest of Western Ghats, District Coimbatore (Tamil Nadu) and District Mysore (Karnataka), India. Usually, the leaves of *Cucumis dipsaceus* are consumed as a leafy vegetable. Its fruit juice is topically applied to prevent hair loss. This herb is commonly known as Teasel gourd, Arabian cucumber, Hedgehog cucumber, Mullampandri vellari, Pepinodiablito. Commonly hydroalcohol is suitable solvent for successive extraction of polar and non polar phytoconstituents. Many of the hydroalcoholic plant extract have potent antioxidant activity, since there is no evidence for *Cucumis dipsaceus*.[9-12] Therefore current research is focused on antioxidant activity of hydroalcoholic fruit extract of *Cucumis dipsaceus*.

2. MATERIALS AND METHODS
2.1 Collection and authentication of plant
The fruits of *Cucumis dipsaceus* were collected from the surrounding areas of Kalapatti, Tamil Nadu, India, during the month of December-2018 and authenticated from Botanical survey of India (BSI) Southern circle, Coimbatore, Tamil Nadu. The authentication certificate number is No.BSI/SRC/5/23/2019/Tech/3010.

2.2 Preparation of plant material
The collected fruits were cleaned, washed with distilled water, dried under sunshade in dark room, and powdered by using mechanical mixer. After size reduction fruits were sieved under sieve No. 40 and sieve No. 60, stored in airtight container at room temperature.[13]

2.3 Extraction of fruit material

Coarsely powdered fruits of *Cucumis dipsaceus* were extracted with hydroalcoholic (50% v/v Methanol) solvent using Soxhlet apparatus for about 72 h at 40°C. After that the sediment was filtered with Whatman no.1 filter paper (Whatman Ltd, England). The extract was further concentrated under vacuum using rotary vacuum evaporator (Buchan R-V120, Switzerland) at 40°C. The obtained crude extract was weighed and stored at 4^0C for the further analysis.[14]

2.4 Qualitative Phytochemical Analysis of HAFEC

A small quantity of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample.[15-17]

2.4.1 Test for Carbohydrates

Molisch's test

Few drops of Molisch's reagent was added to 2-3ml of filtrate, followed by addition of concentratedsulphuric acid along the sides of the test tube. Formation of violet colour ring at thejunction of two liquids indicates the presence of carbohydrates.

Fehling's test

One ml Fehling's-A (copper sulphate in distilled water) was added to 1ml of Fehling's-B (potassium tartarate and sodium hydroxide in distilled water) solution, boiled for one minute. To this added1ml of filtrate and heated gently. Formation of brick red precipitate indicates the presence of reducing sugars.

Benedict's test

Few ml of filtrate was mixed with equal volume of Benedict's reagent (alkaline solution containing cupric citrate complex) and heated in boiling water bath for 5min. Formation of reddish brown precipitate infers the presence of reducing sugars.

2.4.2 Test for Alkaloids

Small amount of extract mixed with few ml of dilute hydrochloric acid. Shaken welland filtered. Following tests were performed with the obtained filtrate.

Dragendorff's test

A few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added to 2-3ml of filtrate. Orange red precipitate indicates the presence of alkaloids.

Mayer's test

A few drops of Mayer's reagent (potassium mercuric iodide solution) was added to 2-3ml of filtrate.Cream (dull white) precipitate was formed.

Wagner's test

A few drops of Wagner's reagent (solution of iodine in potassium iodide) was added to 2-3ml of filtrate,. Reddish brown precipitate was obtained.

Hager's test

A few drops of Hager's reagent (Picric acid) were added to 2-3mlof filtrate. Yellow precipitate was obtained.

2.4.3 Test for Triterpenoid

Libermann-Burchard test

A small quantity of extract was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green color, infers the presence of phytosterols and formation of deep red color indicates the presence of triterpenoids.

Salkowski test

A small quantity of the extract was treated with chloroform and few drops of concentrated sulphuric acid and allowed to stand for few minutes. Yellow colour at the lower layer indicates the presence of triterpenoids.

2.4.4 Test for Glycosides

Legal's test

1ml of pyridine and 1ml of sodium nitroprusside was added to 1ml of extract. Pink to red color indicates the presence of glycosides.

Keller-Killiani test

Glacial acetic acid was added to 2ml extract, followed by the addition of trace quantity of ferric chloride and 2 to 3drops of concentrated sulphuric acid. Reddish brown colour appears at the junction of two liquid indicates the presence of cardiac glycosides.

Baljet test

2ml of extract was added to sodium picrate solution. Yellow to orange colour formation indicates the presence of glycosides.

2.4.5 Test for Steroids and Sterols

Salkowski reaction

Two ml of extract was mixed with 2ml chloroform and 2ml concentrated sulphuric acid. Shaken well Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

2.4.6 Test for Phenols

Ferric chloride test

1ml of the alcoholic solution of the extract was added to 2ml of distilled water followed by few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

Lead acetate test

Diluted 1ml of alcoholic solution of extract with 5ml distilled water and to this added few drops of 1% aqueous solution of lead acetate. Formation of yellow colour precipitate indicates the presence of phenols.

2.4.7 Test for Tannins
Lead acetate test
A few drop of lead acetatewas added to 5ml of aqueous extract. Formation of yellow or red colour precipitate indicates the presence of tannins.

2.4.8 Test for Saponins
Foam Test
One ml of test sample was diluted with 20ml of distilled water and shaken it in a graduated cylinder for 3minutes. Foam of 1cm after 10min indicates the presence of saponins.

Froth test
Five ml of test sample was added to sodium bicarbonate solution. Aftervigorousshaking the mixture, kept it for 3minutes. A honey comb like froth formation indicates the presence of saponins.

2.4.9 Test for Flavonoids
Alkaline reagent test
A few drop of sodium hydroxide solution was added to the extract. Formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

Shinodas test [Magnesium hydrochloride reduction test]
Alcoholic solution of extract was treated with a small piece of magnesium ribbon and a few drops of concentrated HCl was added and heated. Appearance of crimson red or occasionally green to blue colour infers the presence of flavonoid.

2.4.10 Test for Proteins and Amino Acids
Biuret test
Three ml of test solution was added to 4% sodium hydroxide and few drops of 1% coppersulphate solution. Formation of violet colour indicates the presence of proteins.

Ninhydrin test
A mixture of 3ml test solution and 3drops of 5% Ninhydrin solution was heated in a boiling water bathfor 10min. Formation of purple or bluish colour indicates the presence of free amino acids.

2.5 Estimation of Total Flavonoid content
2.5.1 Reagents
10% aluminium chloride, 1M Potassium acetate.

2.5.2 Preparation of standard
Standard solution of was prepared by adding 10mg of accurately weighed Quercetin in 10 ml of methanol.

2.5.3 Preparation of sample
10mg of the accurately weighed HAFEC was dissolved in 10ml water and used for the estimation.

2.5.4 Procedure
The total flavonoid content of the HAFEC was determined by using Aluminium chloride colorimetric method. To an aliquot of 1ml of extract (1mg/ml) or standard solutions of Quercetin (10, 20, 40, 60, 80, 100 µg/ml) methanol was added separately to make up the solution up to 2ml. The resulting mixture was treated with 0.1ml of 10% aluminium chloride, 0.1ml of 1Mpotassium acetate and 2.8ml of distilled water. Shaken well and incubated at room temperature for 30 minutes. The absorbance was measured at 415nm against blank, where a solution of 2ml ethanol, 0.1ml potassium acetate, 2.8 ml distilled water and 0.1ml of aluminium chloride serve as blank solution. The total flavonoid content was determined from the standard Quercetin calibration curve. And it was expressed as milligrams of Quercetin equivalents per gram of extract.[18]

2.6. *In vitro* Antioxidant Studies
2.6.1 ABTS radical scavenging assay
Reagents
7 mM ABTS
2.4 mM potassium persulfate
Quercetin

Procedure
To determine ABTS radical scavenging assay, the method of[2] was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of quercetin and percentage inhibition calculated as ABTS radical scavenging activity (%) by using following formula ABTS radical scavenging activity (%) = (Absorbance of control - Absorbance of sample)/ (Absorbance of control) × 100.

2.7 DPPH radical scavenging assay
The effect of the extracts on DPPH radical was estimated using the method of.[2] 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 extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm.[19] Quercetin was used as references. The ability to scavenge DPPH radical was calculated by the following equation

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

3. RESULTS AND DISCUSSION

Preliminary phytochemical evaluation of HAFEC was shown in Table l. The results revealed that HAFEC have alkaloids, flavonoids, triterpenoids, saponins, glycosides, proteins and amino acids.

Table 1: Preliminary phytochemical evaluation of HAFEC.

S. No	Phytoconstituents	Observation	Result
1.	Alkaloids	Yellow ppt, Reddish brown ppt, Turbid extract was obtained	Present
2.	Flavonoids	Yellow color Yellow color turns colorless	Present
3.	Tannins	No changes obtained	Absent
4.	Carbohydrates	No changes obtained	Absent
5.	Triterpenoids	Reddish brown coloration at the interface was obtained	Present
6.	Saponins	Presence of froth, Bulky white precipitate was obtained	Present
7.	Glycosides	Bluish green color was obtained	Present
8.	Proteins and amino acids	Pink to red color was obtained	Present
9.	Phenols	No changes obtained	Absent

The total flavonoid content of HAFEC was estimated using standard calibration curve method, the standard curve of quercetin was shown in Figure 1. The linearity of standard curve was R^2 = 0.990. From the standard curve the total flavonoid content was calculated which was mentioned in Table 2.

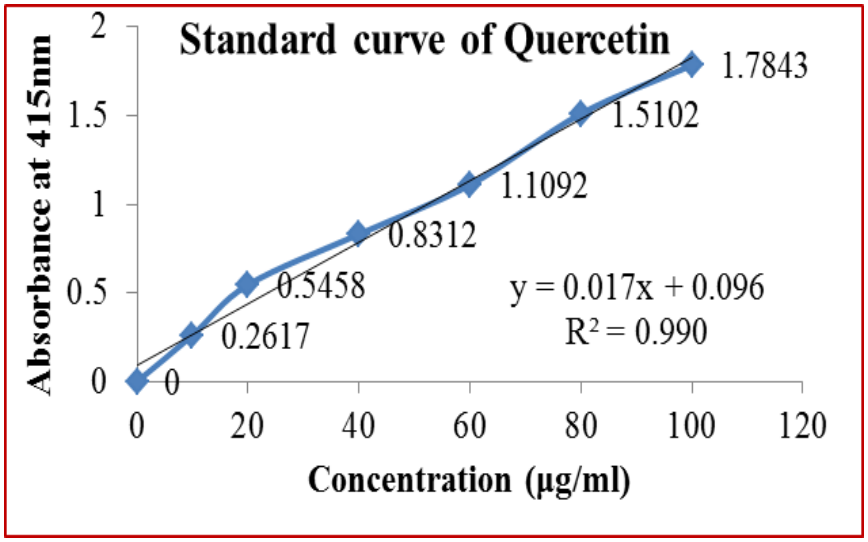
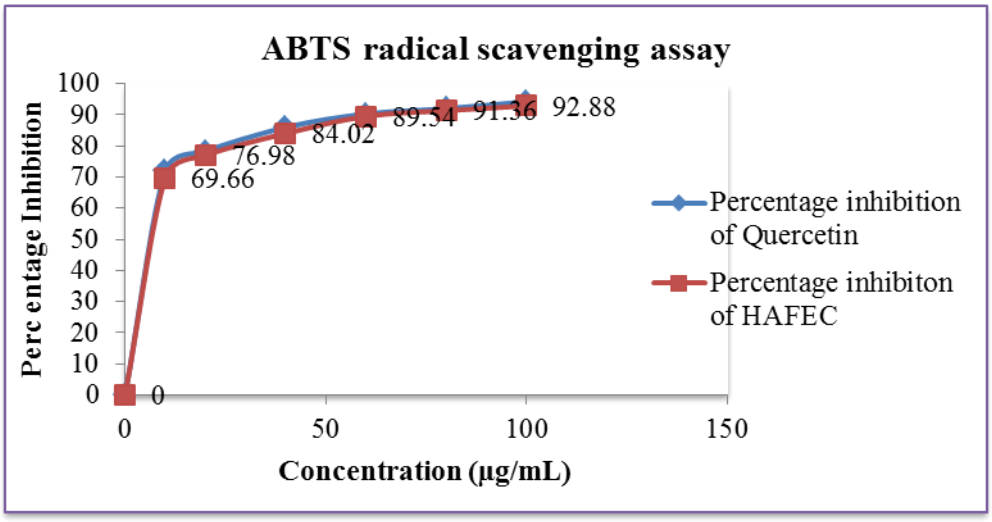


Figure 1: Standard curve of Quercetin.

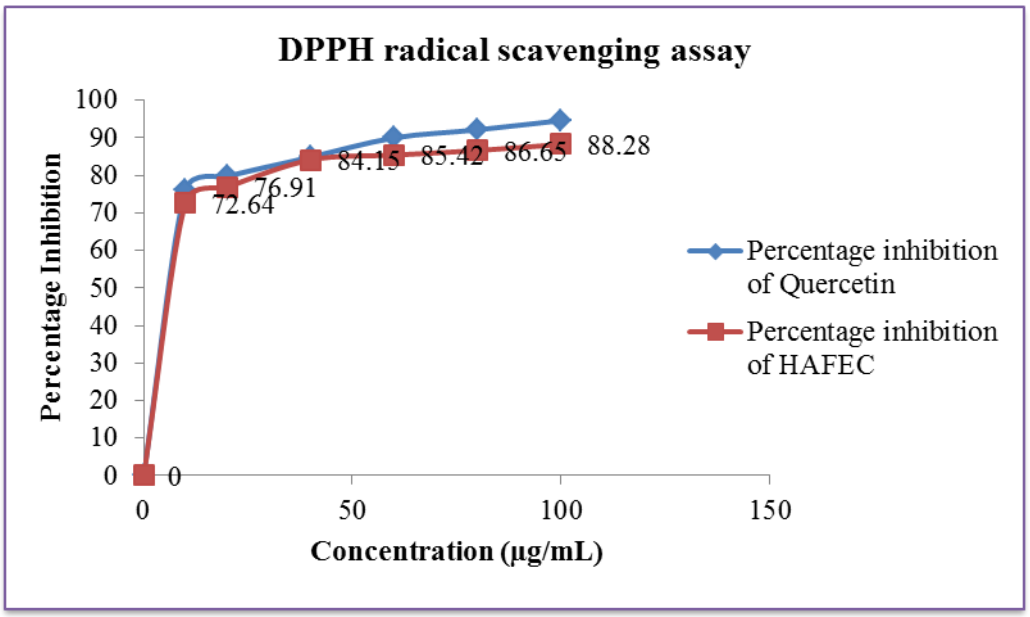
Table 2: Determination of total flavonoid content.

Sample extract (µg/ml)	Weight of dry extract per ml m (g)	Absorbance	Quercetin concentration (µg/ml)	Quercetin concentration (mg/ml)	TFC as Quercetin (mg/g) $A=\frac{C\times V}{m}$
100	0.001	1.2116	65.62	0.06562	65.62

The total Flavonoid content in HAFEC was found to be 65.62 mg/g of extract calculated as Quercetin equivalent.



The percentage inhibition of ABTS radical by HAFEC and quercetin was shown in Figure 1. The 50% of inhibitory concentration of DPPH radicals was calculated for determination of antioxidant capacity. ABTS assay IC_{50} values of HAFEC and quercetin were found to be 8.96 µg/ml and 7.05 µg/ml respectively.



The percentage inhibition of DPPH radical by HAFEC and quercetin was shown in Figure 2. The 50% of inhibitory concentration of DPPH radicals was calculated for determination of antioxidant capacity. DPPH assay IC_{50} values of HAFEC and quercetin were found to be 13.03µg/ml and 9.86 µg/ml respectively.

4. CONCLUSION

The results of current research revealed that HAFEC has potent antioxidant activity which produced similar effect to standard quercetin. Results concluded that, the antioxidant activity of HAFEC may be due to the presence of phytoconstituents such as flavonoids, triterpenoids, catechin etc., the future direction of this study is to perform *in vivo* studies and finding of molecular mechanism.

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