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Assessment of *in vitro* Antioxidant activity, Total Phenolic and Total Flavonoid in *Careya arborea* Fruit Pulp Extracts

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ABSTRACT

In the current investigation, *Careya arborea* fruit pulp was used to assess total phenolic, flavonoid and *in vitro* antioxidant potential. The ethyl acetate extract (EAE), methanolic extract (ME), water extract (WE) of fruit pulp were studied. Using the Folin-Ciocalteau reagent, total phenolics were measured, and the colorimetric method was used to determine the flavonoid concentration. Antioxidant activity was evaluated using DPPH assay and reducing power by $FeCl_3$. A positive control was ascorbic acid. The fruit pulps from *C. arborea* with the highest phenolic, flavonoid, and antioxidant potential were extracted using ethyl acetate. When compared to a positive control, the *C. arborea* fruit showed a substantial in vitro antioxidant activity ($P < 0.05$). The Finding concluded that the *C. arborea* fruit pulp exhibited antioxidant activity.

KEYWORDS: DPPH free radical scavenging activity**,** *Careya arborea*, Fruit pulp, Total phenolic

INTRODUCTION

The presences of unpaired electrons on atoms, molecules or ions are known as free radical. Chemically, they are unstable and reactive. [1] The body contains free radicals, which are produced during metabolic activities. Bacteria and viruses can be eliminated by free radicals generated by the immune system. They also have a part in the synthesis of hormones, energy, and enzyme activation. Excessive formations of free radical damage tissue Radiation, the sun, medical X-rays, pollution, etc. can all boost the production of extra free radicals. High-fat diets have the potential to promote the production of free radicals. The free radical can be destroyed first line body defense system. Free radicals are neutralized by enzymes such as superoxide dismutase, glutathione peroxides, vitamin C, vitamin E, selenium mineral, and hormone melatonin. In cases of severe or persistent oxidative stress, protection might not be sufficient. Therefore, to keep free radicals in balance, a certain amount of exogenous antioxidants or free radical scavengers is always needed. [2-4] Free radicals are responsible for development of disorders like diabetes, stroke, arteriosclerosis, cancer, the aging process and cardiovascular diseases. [5-7] Different types of Phytoconstituents are found in plants. The phenolic compound is an effective reducing agent [8-9]

Careya arborea Roxb is a member of the Lecythidaceae family, commonly known as wild Guava and Kumbhi (Because the fruits resemble earthen pots). [10] The calyx limb is topped with big, globose, meaty, indehiscent fruits. Many seeds, deeply buried in soft pulp. In Ayurveda, the fruits are erotic and caustic, and they heal kapha. [11] It is found in the Sub-Himalayan region, extending eastward from Jammu to West Bengal, Madhya Pradesh, and Tamil Nadu. [12] Fruits are uses as an astringent, decoction aids digestion, [13] astringent, demulcent, [14-17] edible and aromatic. [18] Fruit powder is used to treat obesity and prevent coughing. Applying fruit pulp to the scalp promotes hair development. [19-20] *Careya arborea* bark has reported Central nervous system depressant, [21] antimicrobial and antioxidant, [22] antidiarrhoeal, [23] hepatoprotective, [24] antitumor, [25] analgesic, [26] and anticonvulsant activity. [27] *C. arborea* fruits pulp remains unexplored. Therefore, the current study was planned to determining their total phenolic components, flavonoid concentration, and antioxidant potential of *C. arborea* fruit pulp

MATERIALS AND METHODS

Plant material

Plant materials in June 2012 of *C. arborea* were procured from Vadodara. Plant was identified and authenticated at Botany Department of The M. S. University, Vadodara. Voucher specimen (DC-CA-2) was kept in our lab's herbarium.

Reagent and Chemicals

Analytical grade reagents and chemicals were all utilized. Folin Ciocalteu reagent, Quercetin, Aluminum chloride, Gallic acid, Potassium acetate, Sodium carbonate α, α Diphenyl – β Picryl Hydrazyl (DPPH), Ascorbic acid were procured from E. Merck (Darmstadt, Germany), Hi-Media lab. Ltd (Mumbai) and Sigma **(**Chemical Co, St. Louis, MO, USA). A Shimadzu UV-1800 was used to record each and every UV-Vis measurement.

Preparation of extracts

About 20 grams of fruit pulp powder were extracted using methanol, water, and ethyl acetate individually for duration of 24 hours. After filtering, the extracts underwent evaporation to concentrate on water bath and dried.

Phytochemical screening [28]

Chemical test were performed for the presence of phytoconstituents such as alkaloid, phenolic, flavonoid, saponin and sterols, by employing chemical tests.

Thin layer chromatographic profile of phenolic compounds [29-30]

The mobile phase used to create the TLC profile of the extracts was $CHCl_3$: ethyl acetate: Formic acid (2:8:0.4) as mobile phase. Alcoholic $FeCl_3$ was sprayed onto the plate. The R_f values of observed compounds were recorded.

Determination of total phenolic content [31]

Folin-Ciocalteau reagent was used to quantify total phenolic (Singleton and Rossi, 1965). The amount of total phenolic was measured in the fruit pulp of *C. arborea* in the methanol extract (ME), ethyl acetate extract (EAE), and water extract (WE). The standard was gallic acid. Methanol was used to take the gallic acid calibration curve.

In a volumetric flask, 1.0 ml of test sample containing 1 mg extract was diluted with 10 ml of distilled water. It was mixed with 1.5 ml of folin ciocalteu reagent. After letting the mixture for five minutes, 4 ml of a 20% sodium carbonate solution was added, and distilled water was used to bring the volume up to 25 ml. After this mixture was left for thirty minutes, and the absorbance measured with a Shimadzu 1800 spectrophotometer at 765 nm of the blue color developed. The calibration curve of gallic acid was used to determine the percentage of total phenolics. Gallic acid percentage was used to express the total phenolic content.

Determination of total Flavonoid content **[32-35]**

The method was performed on methanol extract, ethyl acetate extract and water extract (WE) of *C. arborea* fruit pulp.

Aluminum chloride colorimetric method

The standard utilized was quercetin. 1 mg of Quercetin was dissolved in 100 ml methanol to produce (10µg/ml). Methanol was used to take the quercetin calibration curve. The standard solutions and extracts were separately mixed with 1.5 ml of 95% methanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The absorbance of the reaction mixture was measured at 415 nm in a Shimadzu 1800 spectrophotometer following a 30-minute incubation period at room temperature. The same volume of distilled water was used in place of 10% aluminum chloride in the blank.

The percentage of total flavonoids was calculated from calibration curve. Flavonoid content was expressed as % Quercetin.

DPPH free radical scavenging activity **[36-39]**

Ascorbic acid was dissolved in methanol to get the concentration of 30, 45, 60, 75, 90 and 100 µg/ml. The test samples were dissolved in methanol to give stock solution of 1000µg/ml. 50, 100, 150, 200, 250 and 300 µg/ml and ethyl acetate extract 25,50, 75,100, 125 and 150 µg/ml concentration were prepared with methanol. Accurately 1.3mg of DPPH was dissolved in 1 ml of methanol, the test covered with aluminum foil to protected from the light.

DPPH free radical scavenging activity

A 75 µl DPPH solution was added to 3 ml methanol and the absorbance was measured immediately at 516 nm for control reading. Different concentrations of standard and test samples were diluted with methanol up to 3 ml and added 75 µl of DPPH. Using a Shimadzu 1800 spectrophotometer, the absorbance was measured right away at 516 nm after the addition of the DPPH solution, with methanol serving as a blank. After 30 minutes, the absorbance decreased in the presence of test samples at various concentrations, and the percentage reduction and IC_{50} were calculated using the following formula:

$$\% \text{ Free radical scavenging activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Reducing power by $FeCl_3$ [40-42] ethanol was used to dissolve the extracts and ascorbic acid (standard). The reducing power of methanolic extract and ethyl acetate extracts of C. *arborea* fruits were determined according to the method of Oyaizu (1986). Extracts were mixed with 2.5ml 0.2 M phosphate buffer (pH 6.6) and 2.5ml potassium ferricyanide (1%). After the mixture was incubated at 50 °C for 20 min, 2.5ml of TCA (10%) were added and the mixture was centrifuged at 3000 rpm for 10 min. Supernatant (2.5ml) was mixed with distilled water (2.5 mL) and 0.5 ml ferric chloride (0.1%), kept for 10 min. A similar procedure was used to generate the control, excluding the extracts, and the absorbance at 700 nm was measured and compared to the standard. Improved reducing power is indicated by a higher sample absorbance.

RESULTS AND DISCUSSION

Phytochemical screening

Fruits that underwent phytochemical screening revealed the presence of flavonoids, proteins, sterols, carbohydrates, saponins, and phenolic compounds

TLC profile for phenolics compounds

Phytoconstituents are identified and separated using thin layer chromatography.

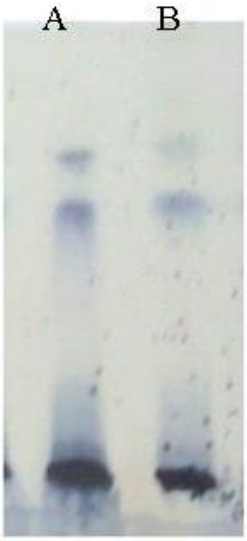

Figure 1.TLC profile of phenolics compounds of *C. arborea* Fruit pulp extracts.

A-Ethyl acetate extract and B- methanolic extract

Total phenolics content

Chemically, Folin-Ciocalteu reagent is phosphomolybdate and phosphotungstate. Under alkaline circumstances, phenolic compounds reduce the phosphotungstate-phosphomolybdate complex to blue reaction products. It measures the amount the quantity of the material required to prevent the reagent from oxidizing. Table 1 presented the findings

Flavonoid content

Aluminum chloride colorimetric method

A colorimetric technique for aluminum chloride, with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones, isoflavons, and flavonols, aluminum chloride (AlCl3) forms acid stable complexes. Furthermore, $AlCl_3$ combines with the ortho-dihydroxyl groups in the flavonoid's A or B ring to generate acid-labile complexe. [38] Results were reported in Table 1.

Table 1: Total phenolic, Flavonoid content and DPPH assay IC_{50} of C. *arborea* Fruit pulp extracts.

Extracts	%W/W Flavonoid compounds	%W/W (phenolic content)	IC_{50}(µg/ml) Value DPPH Assay
Water extract(WE)	0.254±0.012	2.70±0.2804	----
Methyl extract	0.128±0.002	3.956±0.3606	94±0.5292
Ethyl acetate extract(EAE)	0.258±0.011	4.55±0.5292	26.5±0.5
Ascorbic acid(AA)	---	----	53± 0.7638
Values represent mean ± standard deviation (n=3)			

The results indicated that the *C. arborea* fruit pulp methanol and water extract had lower amounts of phenolic and flavonoid compounds than the ethyl acetate extract.

Anti oxidant activity *in vitro*

The methanol and ethyl acetate extracts of *C. arborea* fruit pulp were used to study the *in vitro* free radical scavenging activity and reduction power by $FeCl_3$.

DPPH free radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical and deep violet color. Antioxidant creates a stable, yellow-colored diphenyl picrylhydrazine by reducing DPPH and accepting an electron or hydrogen radical. [39] The free radical scavenging capability of DPPH radical is determined by the decrease in absorbance at 516 nm. The drop in absorbance at 516 nm indicates the DPPH radical's capacity to scavenge free radicals. The activity was represented as a drop in sample absorbance at various concentration points.

The IC50: radical-scavenging activity (concentration in µg required for 50% inhibition of DPPH radical) was calculated from the graph (Figure 1). The greater radical scavenging activity is indicated by the lower IC50 value. The following is the order in which extracts and standards scavenge the DPPH radical: ME > AA > EAE. When compared to methanol, EAE have more radical-scavenging activity (Figure 2). Table 1 presents comparison results between several

extracts of C. arborea fruit pulp with respect to their ability to scavenge free radicals using DPPH. Furthermore, there exists a clear proportionality between the activity in the extracts and the concentration of total phenolics and flavonoid content.

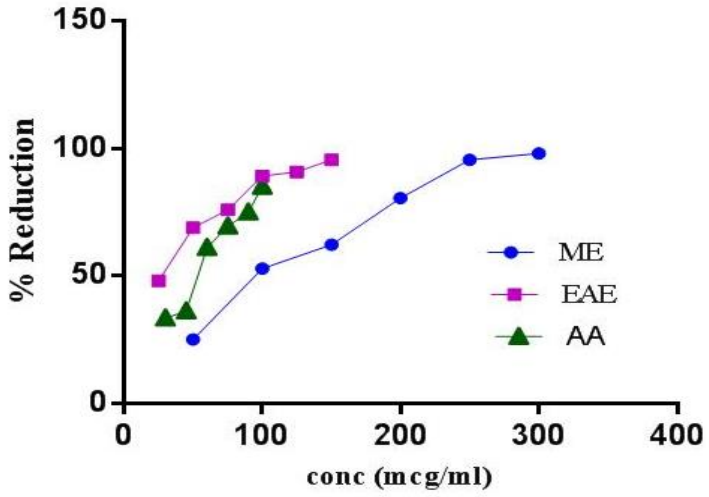

Figure 2: Comparison of DPPH free radical scavenging activity of *C. arborea* fruit pulp.

Where, AA: Ascorbic acid, EAE: Ethyl acetate extract, ME, Methanolic extract

Compared to methanolic extract, the ethyl acetate extract of *C. arborea* fruit pulp has shown a greater capacity for DPPH radical scavenging.

Reducing power by $FeCl_3$

The reduction of $Fe^{3+/}$ferricyanide complex to the ferrous form in presence of antioxidants served as the basis for the reducing activity. The Fe^{2+} forms the Perl's Prussian blue at 700 nm. The reducing power *C. arborea* was studied using the potassium ferricyanide reduction method. As concentration increased, so did the extracts' decreasing power. Compared to the methanol extract of *C. arborea,* the ethyl acetate extract exhibited more activity. The outcome is shown in Figure.3.

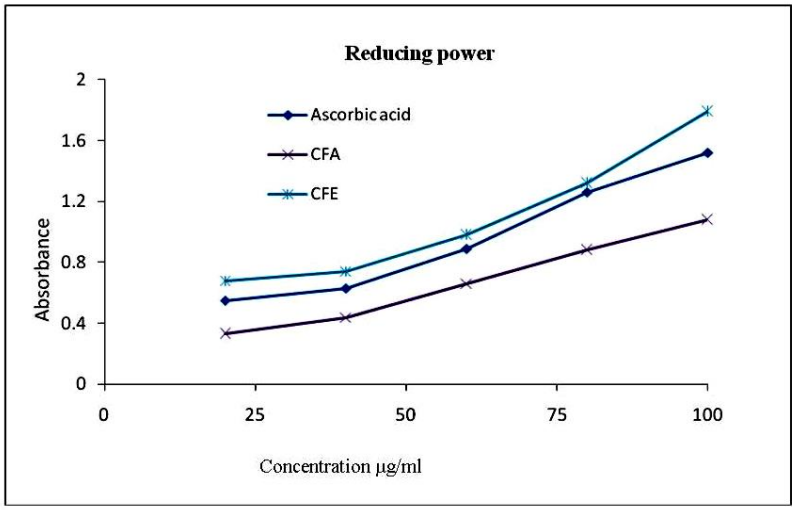

Figure 3. Reductive potential of *C. arborea* fruits

Statistical Analysis

The mean ±SD for each of the three determinations (n = 3) was presented in the results. One way analysis of variance (ANOVA) was used to estimate the variation, and Graph Pad Prism version 6.00 and Microsoft Excel 2007 were used. Value of $p<0.05$ was considered as significant difference.

Conflict of interest statement

We declare that we have no conflict of interest.

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CONCLUSION:

The *Careya arborea* fruit pulp has showed i*n vitro* antioxidant activity when performed using DPPH free radical scavenging assay and reducing power by $FeCl_3$. Greater activity was shown by the ethyl acetate extract. Total phenolic and total flavonoid concentrations in the ethyl acetate extract were found to be higher than in the methanol extract. The study concludes that the antioxidant potential of *C. arborea* fruits was comparable ($P < 0.05$) to standard and that they might be utilized as a natural source of antioxidants.

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