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RESEARCH ARTICLE

In vitro antioxidant activity of *Cayratia mollissima* - an endangered species of Western Ghats of India

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ABSTRACT

India's tropic climate is versatile to grow colorful Vegetables and Fruits. The colourful fruits and vegetables are loaded with Vitamins, minerals, and Phytochemicals. Amongst the Various Fruits and Vegetables, one of the rarest and endangered fruit is *Cayratia mollissima,* which is used by a community of people called Havyaka Brahmins on a special occasion called Aati Amavasya. Using DPPH and Ferric reducing power as an antioxidant model, the antioxidant potential of aqueous and ethanol extracts of *Cayratia mollissima* fruit were found out to explore its health benefits. The findings from this *In vitro* study revealed that *Cayratia mollissima* fruit extracts could serve as Oxygen radical inhibitors. Evaluation of TAC-Total Anti-oxidant Capacity of the extracts were done using FRAP and DPPH assay method. To measure a drug's potency, the Half-maximal inhibitory concentration (IC_{50}) is broadly used. The TAC of aqueous extract and ethanol extract through the FRAP assay method is IC_{50}=354.25 µg and IC_{50}=189.56 µg respectively. The TAC of aqueous extract and ethanol extract through the DPPH assay method is IC_{50}=184.94 µg and IC_{50}=163.88 µg respectively.

Keywords: *Cayratia mollissima,* Ethanol extract, aqueous extract, DPPH assay, FRAP assay

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INTRODUCTION

India is known for its affluent culture of officinal herbs, fruits, vegetables, and spices which includes 2000 species plus, and has a vast geographical arena (Ali et al., 2003). But only very few have been assessed for their potential medicinal value chemically. Through this study, we have tried to study one such unexplored fruit *Cayratia mollissima* which is reported to be an endangered fruit. *Cayratia mollissima* is a climber plant which has 3-foliate leaves with small green-whitish flowers and produces greenish white berries, usually grows at forest perimeters ("*Cayratia mollissima* (Wallich.) Gagnep".). According to the Taxonomy, *Cayratia mollissima* belongs to the family Vitaceae, order Vitales, and genus *Cayratia* (https://en.wikipedia.org/wiki/Cayratia_mollissima). *Cayratia mollissima* also known as *Vitis mollissima* and Kaanakallate (Tulu/Kannada) is distributed in the natives of Chikkamagalur, Hassan, Shivamogga, South Kanara, Kerala specifically at Idukki, Kannur, Kasaragod, Kollam, Palakkad, Thrissur of India. This fruit is specially used by the rural people of Southwest

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India during an occasion called Aati Amavasya. Due to the Human body's normal metabolic process, free radicals are engendered and they play a double role as injurious and beneficial species. Over production of Reactive Oxygen scavenging radicals may contribute to cell and tissue damage and metabolic disorders (Valko et al. 2007). As mentioned by (Leong et al. 2008) Reactive Oxygen Scavenging radicals are uncharged molecules, which consists of one or multiple unpaired electrons due to which they are highly unstable and may harm to other molecules by extorting electrons from them to accomplish stability. Anti-oxidant plays a crucial role in defending the disease conditions by reducing the reactive oxidative damage of Cells engendered by Oxygen Radical (Huda et al. 2009). Recent explorations intimates that the fruit-source antioxidants may have therapeutic essentiality in free radical-attributed disorders such as Type II Diabetes Mellitus, Malignant Neoplastic Disease, Neurodegenerative disease- relating to nerves, Vascular diseases, arthritis- Inflammation in bones and joints, and the aging process.

MATERIALS AND METHODS

Assembling and Extirpate of the fruit *Cayratia mollissima*

The *Cayratia mollissima* fruits were collected from a farmer of Dhaigoli, a small village belonging to Kasargod in Kerala, India. The fresh fruit material was extracted with ethanol and distilled water.

Ethanol Extirpate of *Cayratia mollissima*

50gm of fruit that is harvested was immediately taken for extraction process using 250ml of ethanol (C_2H_5OH) solvent solution using soxhlet's apparatus, which is continuously circulated in the hot percolation process. It will then get concentrated to fresh mass by the method of distillation; the concentrated residue so obtained was stored for further analytical purpose.

Aqueous extirpate of *Cayratia mollissima*

The fruit sample was macerated with distilled water (H_20) for 24Hrs at room temperature. It was then filtered and the solvent was eliminated by the process of distillation. Further, to which the extirpate was stored in a desiccator. According to (Alam et al, 2002) the extirpate procedure was performed.

Antioxidant Assay

The extract's antioxidant activity were determined with two various methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and Ferric reducing antioxidant power (FRAP) methods. Then the inhibition % and Relative % of reducing power were observed through the above mentioned assay.

DPPH (2,2-diphenyl-1-picrylhydrazyl) **radical scavenging assay**

The fruit extract's obtained from ethanol and aqueous were analysed for free radical scavenging ability were tested using techniques such as (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay as depicted by Desmarchelier. et al. (1997) and Blois. (1958). Oxygen radical scavenging power of extracts of the crushed fruits were observed by decolourised methanol solution of 2,2-diphenyl-1-picrylhydrazyl solution. In the presence of antioxidants, 2,2-diphenyl-1-picrylhydrazyl springs up purple colour in methanol solution and fades to one of the shades of yellow colour. 0.1mM of DPPH (2,2-diphenyl-1-

picrylhydrazyl) solution in methanol (CH_3OH) was made, out of which 2.4 ml of this solution was assorted with 1.6 ml. of ethanol and aqueous extract at different concentrations of 25–400 µg/ml separately. The reaction mixtures were left in the dark at Retention Time of 30 min after swirling thoroughly. At 517 nm using a Labman UV Visible Spectrophotometer, the absorbance of the mixture was measured. The percentage of inhibition through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was estimated by the below mentioned equation:

Percent inhibition by DPPH (2,2-diphenyl-1-picrylhydrazyl) **radical scavenging activity of *Cayratia mollissima***

$$= (A_0 - A_1)/A_0 \times 100$$

A_0 - Control sample's absorbance
A_1 - Concentrated extract's absorbance

Then the percentage of inhibition is plotted versus the various titer of the extracts. From the graph, IC_{50} (half maximal inhibitory concentration) value was computed. The experiments were repeated thrice for each concentration. The stronger the DPPH radical scavenging activity, the reaction mixtures' absorbance reduces. The results are disclosed in Fig. 1.

Ferric reducing antioxidant power - FRAP (from Fe^{3+} to Fe^{2+}) assay

The total antioxidant potential-TAC of the sample can be determined by ferric reducing ability (from Fe^{3+} to Fe^{2+}) of plasma FRAP - Ferric reducing antioxidant power) technique as explained by Benzie and Strain et al. (1999) as a measure of antioxidant power. As a result, the ferric ion (Fe^{3+}) will be minimized to ferrous ion (Fe^{2+}); by a potential antioxidant, the latter forms a blue complex (TPTZ), which will get more prominent at the absorbance level of 593 nm. The FRAP (Ferric reducing antioxidant power) reagent was prepared by fusing the solutions acetate buffer, solution of TPTZ in HCl, and 20 mM $FeCl_3$ in the ratio of 10:1:1 (v/v/v). With 2 ml of freshly prepared reagent, 0.5 ml of various concentrations (25, 50, 100, 200, and 400 µg/ml) of extirpates and standard solution were mixed. At a 50˚C water bath, the reaction mixture were mixed thoroughly for the incubation period of 30 min. At 700nm, the reaction mixture's absorbance observed.

Relative % of reducing power of *Cayratia mollissima* =

$$\frac{\text{(As - Ac)}}{\text{(A max. - Ac)}} \times 100$$

As- Sample's absorbance
Ac- Control's Absorbance
Amax- Standard sample's maximum Absorbance.

After 20 minutes, the samples of various concentrations (25, 50, 100, 200, and 400 µg/ml) absorbance were observed at 593 nm. Analyses were performed in triplicates. As explained by Siju et al. (2010), the stronger reducing power was pertained by the enhanced absorbance of the reaction mixture.

RESULTS

Fig. 1 shows the % inhibition of Ethanol and aqueous fruit extract by DPPH **(**2,2-diphenyl-1-picrylhydrazyl) Free radical scavenging activity. The standard used was Ascorbic acid. The percentage of Inhibition by ethanol extract range from 22.08 - 75.01 at different concentrations (25µl-400µl). Wherein the aqueous extract reports the percentage of Inhibition from 27.29 – 64.79 at different concentrations. The IC_{50} value of the Standard sample, Ascorbic acid is 45.36 µg/ml. The IC_{50} value of the test samples for ethanol extract is 163.8 µg/ml and for aqueous extract, it was 184.94 µg/ml

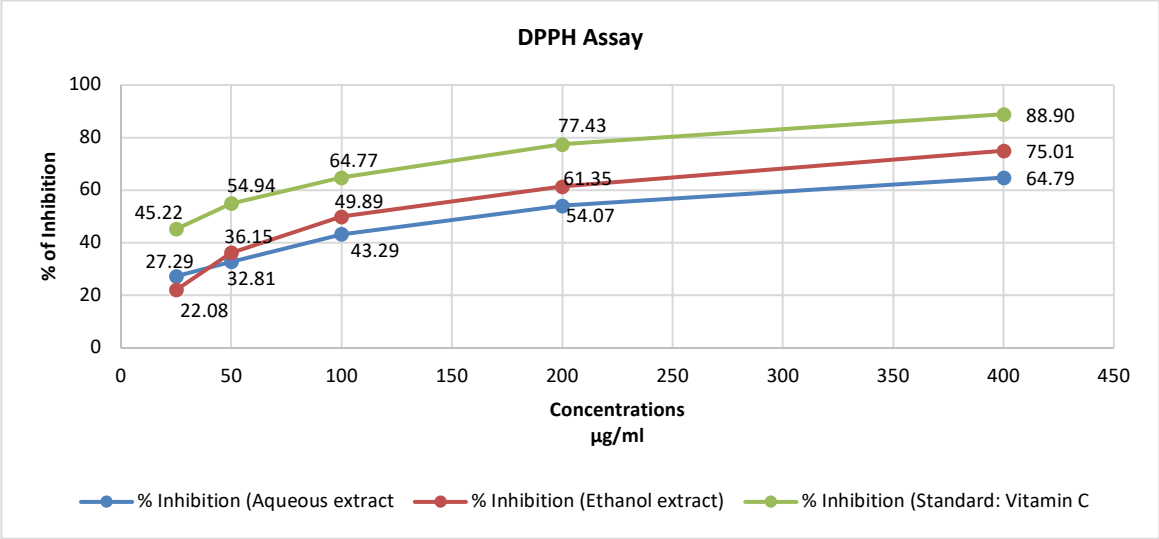

Fig. 1: Percentage Inhibition by Aqueous and Ethanol extract of *Cayratia mollissima*

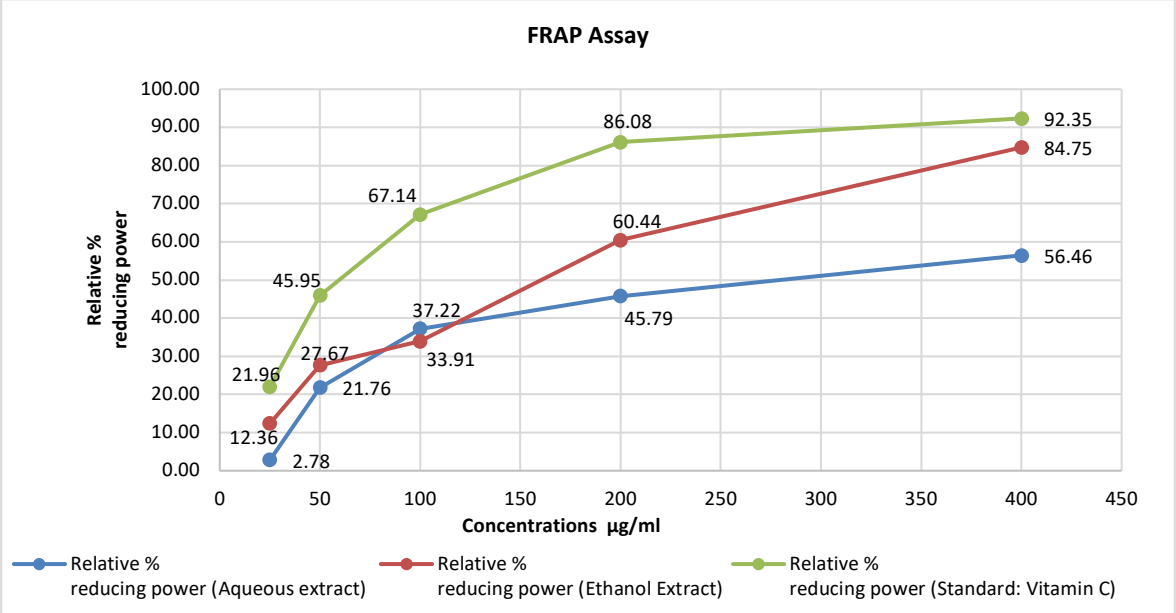

Fig 2: Relative % reducing power by Ethanol and Aqueous extract of *Cayratia mollissima* by FRAP assay

Fig. 2 explains the relative % reduction of the power of Ethanol and aqueous fruit extract by FRAP activity. The standard used was Ascorbic acid. The percentage of Inhibition by ethanol extract range from 12.36 - 84.75 at different concentrations. Wherein the aqueous extract reports the percentage of Inhibition between 2.78 - 56.46% at different concentrations. The IC_{50} value of the Standard sample, Ascorbic acid is 54.40 µg/ml. The sample showed significant antioxidant activity at various concentrations. The IC_{50} value of the test samples for ethanol extract is 189.56 µg/ml and for aqueous extract, it was 354.25 µg/ml.

CONCLUSION

Results obtained by techniques which are *In vitro* in natures indicate that *Cayratia mollissima* fruit extracts are essential origin of pure anti-oxidants which are natural, which can help in prevention of the progression of diseases that are caused due to the presence of free radicals. Amongst the Ethanol and Aqueous extract, the ethanol extract of *Cayratia mollissima* is highly potent. The constituents that are accountable for the anti-oxidant activity is indefinite currently. Hence, further examination would be required for isolating and identifying the antioxidant compounds present in *Cayratia mollissima*.

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