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An approach to Anti-oxidant Potential of *Mangifera indica Linn*. leaves by *In-vitro* models.

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ABSTRACT

Oxidative stress caused by free radicals contributes to chronic diseases such as diabetes, cancer, and cardiovascular disorders. Antioxidants prevent oxidation by neutralizing free radicals. Plants are a natural source of antioxidants, and *Mangifera indica* leaves contain bioactive compounds like polyphenols, flavonoids, and mangiferin, which exhibit strong antioxidant activity. This study aimed to evaluate the *In vitro* antioxidant potential of *Mangifera indica* leaves through phytochemical screening, hydrogen peroxide free radical scavenging, and the phosphomolybdenum assay. The crude extract was prepared by successive Soxhlet extraction using 70% ethanol. Antioxidant activity was measured using the phosphomolybdenum method (total antioxidant capacity) and hydrogen peroxide scavenging assay. Absorbance was recorded at 695 nm and 700 nm, with ascorbic acid as the standard. The extract displayed a concentration-dependent increase in antioxidant activity, though lower than ascorbic acid. Absorbance values ranged from 0.053 to 0.934 for the extract and 0.102 to 1.151 for ascorbic acid across 31.25–1000 µg/mL. This trend indicates notable antioxidant potential. The ethanolic extract of *Mangifera indica* leaves shows significant antioxidant activity, which increases with concentration.

Keywords: Antioxidant, *Mangifera indica Linn,* Hydrogen peroxide, Phosphomolybdenum method.

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INTRODUCTION

Antioxidant is a substance that prevents the oxidation of other molecules. Oxidation is a chemical process that transfers electron or hydrogen from substances to an oxidizing agent. Oxidation reactions can produce free radicals [1]. Free radicals can be named as molecules or molecular fragments containing one or more unpaired electrons and these unpaired electrons confer a considerable degree of reactivity upon a free radical. Free radicals are mainly generated from mitochondria when in the process of generation of radicals in a living system.[2] Oxidative stress is a prime risk factor associated with the pathogenesis of several chronic diseases. Oxidative stress can damage carbohydrate, protein, lipids and DNA in cells and tissues these leads to various de- generative diseases such as diabetes, cancer, cardio-vascular diseases etc. [3]. Antioxidants are classified based on different attributes the first attribute is based on the function they are primary and secondary antioxidants. The second attribute is based on enzymatic and non-enzymatic antioxidants [1].

Despite the ancient nature of tradition, medicinal plants continued to serve as the cornerstone of traditional or indigenous health systems since they have been used for ages to treat and cure a wide range of illness and enhance personal health and well-being [4]. Since, ancient period, plants are being used for the development of new drugs or as a phytomedicine for the treatment of diseases. Even the World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious, safe, less toxic, available and reliable natural resource [5]. According to WHO 85% of population in most developing countries depend upon traditional medicines. Plants are the best source of active secondary metabolites which are essential to humans to treat many diseases. A various herbal formulations are currently available in market equally competing allopathic medicine. Herbal medicines are gaining popularity worldwide due to their safe remedial virtue, especially in treating refectory diseases, antioxidant properties and efficacy in palliation, immune modulation and prophylactic [6].

Systematized medicinal texts like Ayurveda, Unani, Siddha are the main sources of information for the preparation marketed herbal products [7]. Mango (*Mangifera indica L.*) is a juicy stone fruit belongs to the family of *Anacardiaceae* in the order of sapindales and its grown in many parts of the world, particularly tropical countries. It is the national fruit of India and Philippines and the national tree of Bangladesh. Over 1000 mango varieties are available worldwide, out of the available varieties, only a few are grown on commercial scales and traded [8]. Mango leaf has been found to possess several pharmacological benefits owing to its phytochemical's composition. According to numerous studies, mango leaves and fruit possess antioxidant, anti-inflammatory, gastro-protective, anti-diabetic, cardio protective, antitumor, wound healing, anti-pyretic, antibacterial, anti- spasmodic, anti-carcinogenic, anti-viral, hepatoprotective, immunomodulatory and anti-dysentery properties [9].

Mangifera indica is widely selected for antioxidant activity studies for several valid reasons because, it contains high levels of polyphenols, flavonoids, carotenoids, ascorbic acid and xanthones. Key compounds includes mangiferin, quercetin, gallic acid and other compounds known for potent free radical scavenging properties. Its traditional use in Ayurveda medicine, along with growing scientific evidence supporting it's health benefits, makes it a promising candidate for natural antioxidant research [10].

MATERIALS AND METHODS

The selected leaf material was identified and authenticated by a botanist, Chitradurga Karnataka.

Fresh leaves were collected and the leaf part was dried separately at room temperature and pulverized. The powder obtained is subjected to Soxhlet extraction with the 70% ethanol solvent. The extract was taken for phytochemical studies and evaluated for antioxidant activity using Hydrogen Peroxide free radical scavenging assay and Total antioxidant capacity by Phosphomolybdenum assay [11].

Total Antioxidant Capacity by Phosphomolybdenum assay [12]

The total antioxidant capacity of the *Mangifera indica* leaves extracts was evaluated by the Phosphomolybdenum method. 0.3 mL of extract is combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the

reaction solution are incubated at 95^{0}C for 90 minutes. Then, the absorbance of the solution is measured at 695 nm using a UV-Visible spectrophotometer against blank after cooling to room temperature. Ethanol (0.3 mL) in the place of extract is used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve is prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) with ethanol.

Hydrogen peroxide (H_2O_2) Radical Scavenging Activity [13]

Hydrogen peroxide scavenging ability of ethanolic extract of *Mangifera indica* leaves was determined according to the method of 0.2-1.0 ml of test sample (10mg/10ml) is taken in different test tubes to which 1ml of H2O2 is added. The tubes were incubated for 5 minutes at room temperature. After 5 minutes, 2 ml of potassium dichromate, acetic acid reagent is added and the tubes are incubated for 10 minutes at room temperature. The absorbance value of the reaction mixture is recorded at 700 nm. Blank containing the phosphate buffer without the plant extract and a standard is also calculated as

% scavenging [H2O2] = [A control: A test/A control] ×100

Where A control is the absorbance of the control, and A sample is the absorbance of the sample.

RESULTS

Preliminary phytochemical screening of *Mangifera indica Linn*. leaves.

The Preliminary Phytochemical screening of leaves of *Mangifera indica Linn.* was screened and the results revealed the presence of alkaloids, flavonoids, tannins, triterpenoids, cardiac glycosides, phenols and reducing sugar by the phytochemical tests. The results are shown in Table 1.

Table 1: Phytochemical screening of *Mangifera indica Linn*. leaves extract

SL. NO	Phytoconstituents	Results
1.	Carbohydrates	+
2.	Proteins	+
3.	Amino Acids	+
4.	Steroids	+
5.	Triterpenoids	+
6.	Glycosides	+
7.	Alkaloids	+
8.	Saponins	+
9.	Tannins	+
10.	Flavonoids	+

Positive (+), Negative (-)

Total antioxidant capacity by Phosphomolybdenum assay

The antioxidant potential of the sample was evaluated using the Phosphomolybdenum assay, which measures the total antioxidant capacity based on absorbance values. This method provides a reliable indication of the electron-donating ability of the compounds present in the sample. Absorbance increases with concentration for both standard and test, indicating a dose-dependent antioxidant capacity. The standard consistently shows higher absorbance values than the test, suggesting it has greater antioxidant potential. At lower concentrations (31.25–125 µg/mL), both samples exhibit minimal activity, but the difference widens notably beyond 250 µg/mL. The test sample reaches a maximum absorbance of 0.934 at 1000 µg/mL, while the standard reaches 1.151 at the same concentration. This indicates that the test has significant antioxidant potential, it is less potent compared to the standard. The overall trend confirms the reliability of the assay for evaluating antioxidant capacity. The results obtained were tabulated in Table 2 and analysed for comparison with the standard across different concentrations (31.25–1000 µg/mL).

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Table 2: Results of total antioxidant capacity by Phosphomolybdenum assay

SL NO	Concentration (µg/ml)	Absorbance of standard	Absorbance of extract
1	31.25	0.102	0.053
2	62.5	0.166	0.151
3	125	0.250	0.192
4	250	0.656	0.501
5	500	0.991	0.792
6	1000	1.151	0.934

Hydrogen Peroxide radical scavenging assay

The hydrogen peroxide radical scavenging assay was systematically performed to illustrate the concentration-dependent antioxidant activity of both the test sample and the standard. This structured presentation of data enables a clear comparison of the scavenging potential across various concentrations. The results demonstrate a concentration- dependent increase in percentage inhibition for both the test sample and the standard. For the test sample, % inhibition rise from 27.26% at 250 µg to 60.60% at 1500 µg, while the standard showed an increase from 29.29% to 72.10% over the same concentration range. Absorbance values decreased correspondingly with increasing concentration, indicating enhanced scavenging activity at higher doses. The control absorbance remained highest (0.099), confirming the absence of radical scavenging activity in the control setup. Overall, the standard consistently exhibited stronger hydrogen peroxide radical scavenging activity than the test sample at equivalent concentrations, further supporting its higher antioxidant potency. The results confirm the reliability and effectiveness of this assay in evaluating radical scavenging potential of plant extracts. The results are tabulated in table 3.

Table 3: Results of Hydrogen Peroxide free radical scavenging assay

Sl. No.	Concentration	% inhibition of extract	% inhibition of standard
1.	250	27.26%	29.29%
2.	500	35.4%	38.38%
3.	750	42.4%	50.50%
4.	1000	52.52%	61.61%
5.	1500	60.60%	72%

DISCUSSION

Antioxidant activity is the ability of a substance to prevent or delay damage to cells caused by unstable molecules called free radicals or reactive oxygen species (ROS) [1]. These antioxidants work by neutralizing free radicals, which are highly reactive and can cause oxidative stress and cellular damage linked to various chronic diseases, including cardiovascular diseases and cancer [2]. Antioxidant activity is typically found in natural compounds like flavonoids and polyphenols from foods such as fruits and vegetables, and can be measured through various experimental assays, though it's best obtained from whole foods rather than high-dose supplements, which can be harmful.

Antioxidants activities from plants sources have attracted a wide range of interest across the world in recent times. This is due to growing concern for safe and alternative sources of antioxidants. *Mangifera indica Linn.* commonly used herb in ayurvedic medicine. Although review articles on this plant are already published, but this research article is presented to give anti-oxidant effect of selected plant, which were performed widely by different methods. In the present study, ethanolic extract of *Mangifera indica Linn.* leaves exhibited significant antioxidant activity and it was carried using *In-vitro* models, like total antioxidant activity and free radical scavenging assay by using UV spectrophotometer.

The phosphomolybdenum antioxidant assay measures a sample's total antioxidant capacity (TAC) by quantifying its ability to reduce molybdenum (VI) to molybdenum (v), which forms a stable green phosphomolybdate complex at acidic P^H. Its role is to provide a total measure of antioxidant compounds like phenolics, vitamins, and carotenoids in plant extracts and other samples, allowing for the assessment of their overall antioxidant activity and potential health benefits [14].

In a hydrogen peroxide (H_2O_2) scavenging assay, the role of H_2O_2 is to serve as a free radical to be quenched by a test compound, allowing for the quantitative measurement of the test compound's antioxidant capacity. Antioxidants donate electrons to H_2O_2, stabilizing it and preventing it from forming more harmful radicals like the hydroxyl radical (•OH), which can damage DNA. This assay provides a way to evaluate a substance's ability to protect against oxidative stress by removing reactive oxygen species (ROS).

In the current study, Ethanolic extract of *Mangifera indica Linn.* exhibited the antioxidant activity by inhibiting the Total antioxidant activity (phosphomolybdate assay), hydrogen peroxide radical scavenging assay breakdown at different concentrations when it is compared with standard.

CONCLUSION

The study concludes that the ethanolic extract of *Mangifera indica Linn*. leaves exhibits antioxidant activity. Using the Phosphomolybdenum method and Hydrogen Peroxide significant Radical Scavenging assay, the extract showed strong free radical neutralization and protective are recommended. Overall, mango leaves represent a promising natural source of antioxidants with effects. The presence of bioactive compounds like polyphenols, flavonoids, and tannins contributes to its antioxidant potential. These findings indicate its therapeutic value in combating oxidative stress-related disorders. Further, studies on specific active compounds and mechanisms potential applications in nutraceuticals and traditional medicine.

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