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Antioxidant Activity of Selected Indian Medicinal Plants

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Abstract: The objective of the current study was to examine the antioxidant capacity of the *Madhuca longifolia*, *Curcuma caesia*, *Adhatoda vasica*, *Carica papaya* and *Piper longum*. These plants are present in many nations and continents, including Asia, Mauritius, South Africa, Mexico, China, the West Indies, East Africa, and Brazil. It is also used in traditional medicine for a number of indications. The hydro alcoholic extracts were used for the main phytochemical study and *in vitro* antioxidant testing. The plant contains phenolic chemicals, fatty acids, flavonoids, tannins, and glycosides, according to phytochemical analysis. By using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, the antiradical activity of hydro alcoholic extract was evaluated and compared to ascorbic acid. The extract's ability to reduce ferric iron was also assessed using the Oyaizu method. According to the findings, hydro alcoholic fruit extracts have a significant level of antioxidant property. These *in vitro* tests showed that the plant extract is a significant natural source of antioxidants.

Keywords: Antioxidant activity, Ferric reducing antioxidant power, DPPH, Hydroalcoholic extract, Reactive oxygen species

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Introduction

Any agent that prevents or reduces oxidative damage to a target molecule is an antioxidant, according to a broad definition. An antioxidant's primary property is its capacity to snare free radicals (Lobo *et al*., 2010). By scavenging free radicals like peroxide, hydroperoxide, and lipid peroxyl, antioxidant substances including phenolic acids, polyphenols, and flavonoids suppress the oxidative pathways that cause degenerative illnesses. Since ancient times, herbal plants have

been valued as good antioxidants (Aruoma, 2003). An important risk factor in the development of many chronic diseases is oxidative stress (Mohammed and Ibrahim, 2004). Free radicals and other reactive oxygen species (ROS) are now understood to play a role in the pathophysiology of diseases like atherosclerosis, Parkinson's and Alzheimer's disease, diabetes, and asthma. Reactive oxygen species are sometimes cited as the cause of human aging (Bagchi and Puri, 1998).

The goal of the current study was to assess the phytochemical content and ROS scavenging inhibitory activity of 5 medicinal plants from various regions of India, including *Madhuca longifolia, Curcuma caesia, Adhatoda vasica, Carica papaya, and Piper longum*. For many years, these plants have been used historically to treat conditions like bronchitis, diabetes, heart problems, and asthma. *In vitro* tests were used to measure the antioxidant activities and compare the antioxidant effects. Among these is the suppression of FRAP (Ferric reducing antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazyl).

Materials and Methods

Collection and authentication of plant material:

Selected parts of *Madhuca longifolia* (fruits), *Curcuma caesia* (rhizomes), *Adhatoda vasica* (leaves), *Carica papaya* (leaves) and *Piper longum* (fruits) were collected from National Botanical Research Institute, Lucknow, India during February 2021 and authenticated by Dr. Shazia Usmani and Dr. Muhammad Arif, Integral University, Lucknow (India) (Ref No. IU/PHAR/HRB/21/03, IU/PHAR/HRB/21/02, IU/PHAR/HRB/21/04, IU/PHAR/HRB/21/05 and IU/PHAR/HRB/21/06).

Drugs and chemicals:

Methanol, toluene, formic acid, ethyl acetate, and DPPH were purchased from SDFCL, Mumbai. The supplier of the ethanol was Changshu Yangyuan Chemical in China. From Mumbai's Thomas Chemical Laboratory, ascorbic acid and qurecetin was purchased. Sigma Aldrich, a German company, supplied the ursolic acid and lupeol. The experiment only utilized analytical-grade reagents.

Extraction of plant material:

The plant parts were air-dried at room temperature for 10 days and pulverized by grinder. Five hundred grams of the powered plant material was defatted with petroleum ether then extracted with ethanol and water for 24 to 36 h by Soxhlet extraction method. Then, ethanol was separated under reduced pressure to obtain solid mass, after that the powdered material was again treated with ethanol and water using Soxhlet extraction method. Then extracts were dried and stored in air tight amber-colored bottle in refrigerator until further use (Verma and Gupta, 2014).

Identification of Primary and Secondary metabolites by phytochemical screening:

Different leaf extracts were treated with different reagent for the presence and absence of various primary and secondary metabolites (Mayank and Manjul, 2018; Mayank *et al*., 2018a).

Determination of antioxidant activity using DPPH (1, 1-diphenyl-2- picryl - hydrazyl) method:

The 0.1 mM solution of DPPH in methanol was made. To 3 ml of extract solution in water at various concentrations (25, 50, 75, and 100 g/ml), 1.0 ml of solution from this stock was added. After 45 min of incubation at room temperature, the mixture's absorbance at 517 nm was determined. Ascorbic acid was used as a benchmark. Using the control reading, which consisted of DPPH and distilled water without extracts, the percentage inhibition of the DPPH free radical was computed (Mayank *et al*., 2018b).

DPPH scavenged (%) = Absorbance of control – Absorbance of test / Absorbance of control x 100

Determination of antioxidant activity using Reducing power assay:

The standard and test sample extracts were diluted to different quantities (ranging from 25 to 100 g/ml) in 1 ml of deionized water, and then 2.5

Table 1: Phytochemical analysis of different extracts

Phytoconstituents	HAML	HACC	HAAV	HACP	HAPL
Alkaloids	+	++	+++	+++	+++
Glycosides	+	+	++	+++	+++
Tannins	++	+++	+++	++	+++
Flavonoids	+++	+++	+++	+++	++
Fats and oil	+	+	+	+++	+
Carbohydrates	++	++	++	+++	+++
Reducing sugar	+	+	+	++	++
Proteins	+++	++	+++	+++	+++
Saponin	-	-	+	+++	+
Terpenoids	++	++	+++	+++	+++

HAML- Hydroalcoholic extract of *Madhuca longifolia*, HACC- Hydroalcoholic extract of *Curcuma caesia*, HAAV- Hydroalcoholic extract of *Adhatoda vasica*, HACP- Hydroalcoholic extract of *Carica papaya,* HAPL- Hydroalcoholic extract of *Piper longum*

ml of phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%) were added. After cooling, the mixture was incubated for 20 min at 50°C in a water bath. The mixture was mixed with aliquots of 2.5 ml (10%) trichloroacetic acid, which was then centrifuged for 10 min at 3000 rpm. 2.5 ml of the upper layer of the solution was combined with 0.5 ml of freshly made (0.1%) ferric chloride solution. In a UV spectrometer, the absorbance was measured at 700 nm. Extract was not added when creating a blank. Quercetin was used as standard (Tundis *et al*., 2013; Mayank *et al*., 2018a; Mayank and Manjul, 2019).

Statistical analysis:

All the reported data are the mean values of three replicates. Two-way analysis of variance (ANOVA) was done to determine any significant difference in measuring antioxidant activity.

Results and Discussion

The presence of antioxidants in plants may provide protection against lots of diseases. By this way *in vitro* antioxidant activity of different herbal extracts had designed which showed good antioxidant profile of selected plants by using various models. The DPPH radical is widely used in assessing free radical scavenging activity because of the ease of the reaction. Best antioxidant activity was found in HAPL (IC_{50} 3.8)> HAML(IC_{50} 4.6)> HAAV (IC_{50} 5.6)> HACP (IC_{50} 6.2)>HACC (IC_{50} 8.9) by using DPPH (1, 1-diphenyl-2- picryl - hydrazyl) method (Table 2) whereas in case of Ferric-Reducing antioxidant power (FRAP) assay, best antioxidant activity was found in HAPL (IC_{50} 3.8)> HAML (IC_{50} 4.4)> HAAV (IC_{50} 5.3)> HACP (IC_{50} 6.1)>HACC (IC_{50} 8.5) due to presence of antioxidants such as phenolics, flavonoids, tannins etc. (Table 3).

Different plant extracts contain a range of primary and secondary metabolites, including alkaloids, glycosides, saponins, tannins, flavonoids, carbohydrate, and protein. For humans, the discovery of various metabolites such as alkaloids, glycosides, saponins, tannins, flavonoids, carbohydrate, protein, etc. is particularly advantageous because they can be used to treat a variety of ailments (Mayank and Manjul, 2019). Biologically dangerous free radicals are scavenged from our bodies by natural antioxidants found in plants. Any species capable of independent existence that has one or more unpaired electrons reacts with other molecules by taking or giving electrons, and is involved in many pathological conditions (Madhavi *et al*., 1996). These free radicals are extremely unstable, and when their levels in the body are too high, they can harm cells and tissues and contribute to a number of disorders (Upadhye *et al*., 2009). Antioxidants of

Table 2: Antioxidant activity of different extracts using DPPH method

Extracts	Concentration (µg/ml)	Absorbance	IC_{50}
HAML	25 µg/ml	0.0745	4.6
	50 µg/ml	0.0752	
	75 µg/ml	0.0762	
	100 µg/ml	0.0785	
HACC	25 µg/ml	0.0545	8.9
	50 µg/ml	0.0633	
	75 µg/ml	0.0639	
	100 µg/ml	0.0645	
HAAV	25 µg/ml	0.0548	5.6
	50 µg/ml	0.0639	
	75 µg/ml	0.0645	
	100 µg/ml	0.0652	
HACP	25 µg/ml	0.0547	6.2
	50 µg/ml	0.0631	
	75 µg/ml	0.0640	
	100 µg/ml	0.0646	
HAPL	25 µg/ml	0.0758	3.8
	50 µg/ml	0.0762	
	75 µg/ml	0.0772	
	100 µg/ml	0.0779	
Ascorbic acid	25 µg/ml	0.841	1.4
	50 µg/ml	0.845	
	75 µg/ml	0.852	
	100 µg/ml	0.862	

HAML- Hydroalcoholic extract of *Madhuca longifolia*, HACC- Hydroalcoholic extract of *Curcuma caesia*, HAAV- Hydroalcoholic extract of *Adhatoda vasica*, HACP- Hydroalcoholic extract of *Carica papaya,* HAPL- Hydroalcoholic extract of *Piper longum*

Table 3: Determination of antioxidant activity using Reducing power assay

Extracts	Concentration (µg/ml)	Absorbance	IC_{50}
HAML	25 µg/ml	0.0589	4.4
	50 µg/ml	0.0621	
	75 µg/ml	0.0689	
	100 µg/ml	0.0710	
HACC	25 µg/ml	0.0651	8.5
	50 µg/ml	0.0657	
	75 µg/ml	0.0662	
	100 µg/ml	0.0689	
HAAV	25 µg/ml	0.0745	5.3
	50 µg/ml	0.0749	
	75 µg/ml	0.0762	
	100 µg/ml	0.0772	
HACP	25 µg/ml	0.0659	6.1
	50 µg/ml	0.0662	
	75 µg/ml	0.0669	
	100 µg/ml	0.0712	
HAPL	25 µg/ml	0.0541	3.8
	50 µg/ml	0.0549	
	75 µg/ml	0.0552	
	100 µg/ml	0.0562	
Qurecetin	25 µg/ml	0.548	1.5
	50 µg/ml	0.532	
	75 µg/ml	0.552	
	100 µg/ml	0.565	

HAML- Hydroalcoholic extract of *Madhuca longifolia*, HACC- Hydroalcoholic extract of *Curcuma caesia*, HAAV- Hydroalcoholic extract of *Adhatoda vasica*, HACP- Hydroalcoholic extract of *Carica papaya,* HAPL- Hydroalcoholic extract of *Piper longum*

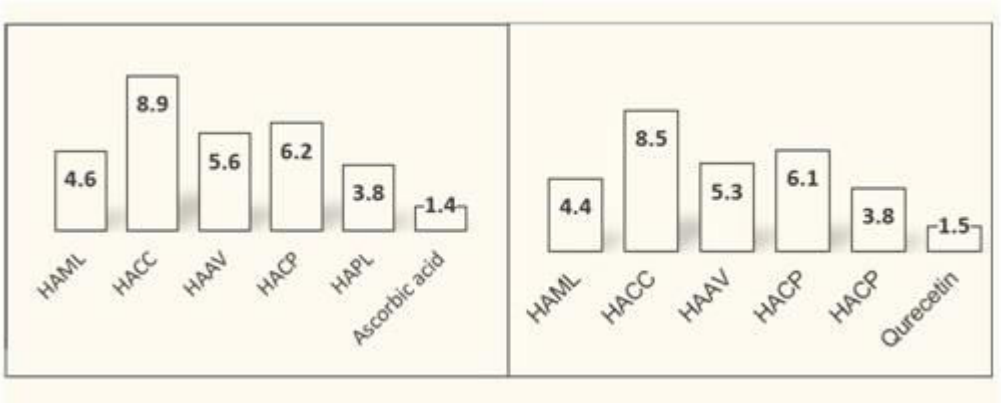

Fig. 1: IC50 of different extracts. HAML- Hydroalcoholic extract of *Madhuca longifolia*, HACC- Hydroalcoholic extract of *Curcuma caesia*, HAAV- Hydroalcoholic extract of *Adhatoda vasica*, HACP- Hydroalcoholic extract of *Carica papaya,* HAPL- Hydroalcoholic extract of *Piper longum.*

natural origin are so necessary since they can shield the body from diseases brought on by free radicals (Mishra *et al.*, 2007). The results of the DPPH radical scavenging activity and reducing power assay are compared to known antioxidant Vitamin C and quercetin are shown in Tables 2 and 3. Here different medicinal plants like *Madhuca longifolia*, *Curcuma caesia*, *Adhatoda vasica*, *Carica papaya* and *Piper longum* from different regions of India were used. Tables 2 and 3 and Figure 1 clearly revealed that fruits part of *Madhuca longifolia* and *Piper longum* have very good antioxidant activity where leaves part of *Adhatoda vasica* and Carica *papaya* have moderate antioxidant activity whereas rhizome part of *Curcuma caesia* has very low antioxidant activity.

Conclusion

Biologically dangerous free radicals are scavenged from our bodies by natural antioxidants found in plants. Any species capable of independent existence that has one or more unpaired electrons reacts with other molecules by taking or giving electrons, and is involved in many pathological conditions. Free radicals play a crucial role in human health and are helpful in preventing and treating a number of diseases, including cardiovascular disorders, lung damage, inflammation, and others. These free radicals are extremely unstable, and when their levels in the body are too high, they can harm cells and tissues and contribute to a number of disorders. Antioxidants of natural origin are so necessary since they can shield the body from diseases brought on by free radicals. The mentioned plants have good antioxidant property except rhizome.

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