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Anti-Oxidant Activity of *Linderina Madayiparense* Extracts

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Abstract : India has rich biodiversity of medicinal plants which are widely used for the treatment of various human ailments since ancient times. There is no scientific evidence available for a newly identified plant, *Lindernia madayiparense* (Family: Linderniaceae). In the present study petroleum ether, ethyl acetate, ethanol and water extract obtained from soxhlet extraction method and decocted extracts of *L. madayiparense* were assessed for the *in-vitro* antioxidant potential by DPPH free radical scavenging assay and reducing power assay. In DPPH assay, the activity was increased by increasing the concentration of the extracts. Among the tested extracts, ethanol extract exhibited highest percentage inhibition (72.65%) followed by decocted extract (71.59%) and ethyl acetate extract (62.80) whereas the lowest % inhibition was found in pet ether extract (50.19%) at a concentration of 100 µg/ml. In reducing power assay, the absorbance was increased by increasing the concentration of the extracts which indicates their greater reducing power. Also, there is a good the inhibition concentration (IC_{50}) value was found for ethanol extract followed by decocted extract (270 µg/ml) and ethyl acetate extract (410 µg/ml). All the results were well comparable with standard antioxidant, ascorbic acid. The findings revealed that in both assays, the highest antioxidant activity was observed in ethanol extract of the plant L. Madayiparense.

Introduction

Radical scavenging antioxidants are mostly important in antioxidative-defence in protecting cells from the injury of free-radical such as superoxide radical ($O_2^{\cdot}$), Hydroxyl radical (OH) and non-free radical species such as H_2O_2 and singlet oxygen ($^1O_2$)[1]. Free radicals and oxidants play an important role for the development of degenerative diseases which affect the survival of the life for the humans[2]. The oxidation induced by Reactive Oxygen Species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury, diabetes and cardiovascular disease. This might be due to the continuous exposure to chemicals and contaminants through air, water and food by the humans in the part of their daily lives. These activities may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage. Still the human possesses such defense mechanisms, as enzymes and antioxidant nutrients which arrest the damaging properties of ROS[3].

Moreover, organisms possess antioxidant defence systems to counteract oxidative damage not only produced by reactive species due normal biochemical and physiological processes but also to radicals of

environmental origin. But if the antioxidant defence systems fail and/or an overproduction of radicals occur, the damage of biomolecules and consequently life threatening disease will occur. For this reason, an intake of antioxidants may be necessary which can be supplied through diet[4]. Scientific evidence also suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Most of the antioxidants are present in a diet including plant sources which consists various classes of phytochemicals[5].

Antioxidants which are immensely present in Green leafy vegetables significantly slowing or preventing the oxidative or damage from oxygen process caused by free radicals and associated with cellular and metabolic injury, accelerating aging, cancer, liver diseases, cardio-vascular diseases, neurodegenerative diseases and Inflammation[6]. India has abundant species of medicinal plants and showed practiced with the usage of traditional medicines since ancient times to cure various human ailments. *Lindernia madayiparense* (Family: Linderniaceae) is a plant discovered first from the laterite hills, Madayipara, Kannur District, Northern Kerala, India. The Plant is used by the tribal inhabitants and local villagers for therapeutic purposes since long time. But there is no scientific evidence for the pharmacological effects of this plant. Therefore, a detailed phytopharmacological study is warranted to explore the major active compounds and related bioactivities of this plant[7]. Hence the present study was undertaken for first time to explore the *in-vitro* antioxidant activity of the extracts of the plant, *L. madayiparense*.

Materials and Methods

Collection and identification of plant materials

Wild crafted plant, *Linderina madayiparanse* was collected during its flowering season in the month of October to December, 2013 in Kannur District, Kerala, India. The plant material was identified and authenticated by botanist Mr. P. Biju, Assistant Professor, Government College, Kasaragod, Kerala, India.

Preparation of plant extracts

Various extracts of *L. madayiparanse* were prepared by two decoction and continuous hot extraction methods.

In the decoction method, the fresh plant, *L. madayiparanse* (500 gm) were washed with distilled water and chopped into small pieces as size of 0.5 cm to 1.0 cm and allowed to boil in one liter of distilled water for 20 min from the time the water started to boil or until the original volume was reduced to half. After 20 min, the mixture was allowed to cool and filtered using cheesecloth[8]. The collected decoction was concentrated using rotary vacuum evaporator under reduced pressure to remove the excessive solvent and stored in an air tight container and preserved in 8^0C until further use. Lyophilizer was used to get an absolute dry decocted extracts [9] and the percentage yield of the decocted extract was calculated.

In continuous hot extraction method, the whole plant, *L. madayiparense* was washed thoroughly with distilled water and dried under air-shade. The dried plant materials were pulverized mechanically into coarse powder. The dried coarse plant material (1000gm) has been successively extracted with four different solvents by changing the solvent polarity from non polar to polar solvents such as petroleum ether, ethyl acetate, ethanol and distilled water respectively using Soxhlet apparatus. At the end of each successive extraction, the mixture was collected and concentrated using rotary vacuum evaporator under reduced pressure to remove the excessive solvents[10]. To get an absolute extracts, Lyophiliser was used for water extract and others were freeze dried. All the extracts were stored properly and preserved in 8^0C until further use[9] and the percentage yield of all the extracts was calculated.

Preliminary phytochemical studies

All the extracts of *L. madayiparanse* were subjected for preliminary qualitative analysis to identify the presence of phytochemical constituents such as alkaloids, carbohydrates, glycosides, flavonoids, steriods, triterpenoids, phenols, proteins, tannins etc. as per the standard methods[11]. The presence of the secondary metabolites was noted in table 1.

Evaluation of *in-vitro* anti-oxidant activity

All the extracts of *L. madayiparanse* were assessed for their anti-oxidant potential by DPPH Free radical scavenging assay and reducing power assay.

DPPH free radical scavenging assay

The free radical scavenging activity was determined by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH)[12, 13]. A serial dilution of all the extracts of *L. madayiparanse* were prepared using methanol to obtain various concentrations (10, 20, 40, 60, 80 and 100 µg/ml). The reaction mixture (3.0 ml) consisted 2.0 ml of DPPH in methanol (0.004%) and 1.0 ml of respective concentrations of decoction and all the extracts. The reaction mixtures were left in the dark at room temperature for 30 min and then incubated for 10 min. Then the absorbance was measured at 517 nm using UV-visible spectrophotometer against methanol as a blank. A solution containing DPPH instead of test substance in methanol was prepared and used as control. Ascorbic acid was used as positive control in this assay. The reduction capability of DPPH radical was determined by the decrease in its absorbance[14]. The percentage of inhibition was calculated and tabulated (table 2) using the formula:

$$\% \text{ Inhibition} = (A_0 - A_1 / A_0) \times 100$$

Where, A_0 is the absorbance of control and A_1 is the absorbance of test.

Reducing power assay

The reducing power assay was determined using various concentrations (50, 100, 200, 300, 400 and 500 µg/ml) of all the extracts of *L. madayiparanse* were prepared in methanol. 0.5 ml of the respective test solution was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in water bath for 20 min and cooled. After that 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min and the supernatant liquid was separated out. Then 2.5 ml of supernatant was mixed with distilled water (2.5 ml) and a freshly prepared 1% ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm using UV-visible spectrophotometer and tabulated (table 3). Control was prepared in similar manner excluding test samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates greater reducing power[15,16]. The inhibition concentration (IC_{50}) value is the concentration of extract or standard to require absorbance is 0.50. IC_{50} value is the amount of antioxidant necessary to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions which was determined from extrapolating the graph of absorbance versus the concentration of the test substance. Lower the IC_{50} value indicates higher the antioxidative effect[17].

Results

Decocted extract and pet ether, ethyl acetate, ethanol and water extracts of *L. madayiparense* were obtained by decoction and continuous hot extraction method respectively. The extracts were ranged from 4.78% - 36.42%. The percentage yield of decocted extract was highest (36.42%) compared with all the extracts obtained by continuous hot extraction method. Among the extracts obtained by soxhlet extraction method, the ethanol extract had the highest percentage yield (26.40%) followed by water extract (15.87%), ethyl acetate extract (15.46%) whereas pet. ether extract had the lowest percentage yield (4.78%).

Phytochemical tests revealed that the presence of coumarins and the absence of proteins and amino acids in all the extracts. Fats and oils were present in pet ether extract only. The other phytoconstituents such as alkaloids, carbohydrates and glycosides, flavonoids, steroids, terpenoids, tannins, and phytosterols and poly phenols were also found in all the extracts of *L. madayiparense* (Table 1) in various degree of concentration which was identified by the various range of % yield of extracts obtained.

Table 1: Preliminary phytochemical analysis of the various extracts of the plant, *L. madayiparense*

Phytoconstituents	Decocted extract	Pet. ether extract	Ethyl acetate extract	Ethanol extract	Water extract
Alkaloids	+	-	+	+	-
Carbohydrates	+	-	+	+	+
Glycosides	+	-	+	+	-
Flavanoids	+	-	+	+	+
Proteins and Aminoacids	-	-	-	-	-
Steroids	+	-	+	+	+
Coumarins	+	+	+	+	+
Terpenoids	+	-	+	+	-
Tannins	+	-	+	+	+
Phytosterols	+	-	+	+	+
Saponins	+	+	+	+	-
Oils/ Resins	-	+	-	-	-
Poly phenols	+	-	+	+	+
Fats	-	+	-	-	-

The antioxidant potential of the plant, *L. madayiparense* was assessed by *in-vitro* assay techniques such as DPPH free radical scavenging assay and reducing power assay. In both assays, the highest antioxidant activity was observed in ethanol extract of the plant *L. madayiparense*.

In DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of *L. madayiparense*, the activity was increased by increasing the concentration of the extracts. Among the tested extracts, ethanol extract exhibited highest percentage inhibition (72.65%) followed by decocted extract (71.59%) and ethyl acetate extract (62.80) whereas the lowest % inhibition was found in pet ether extract (50.19%) at a concentration of 100 µg/ml. All the results were well comparable with standard antioxidant, ascorbic acid (table 2 and figure 1).

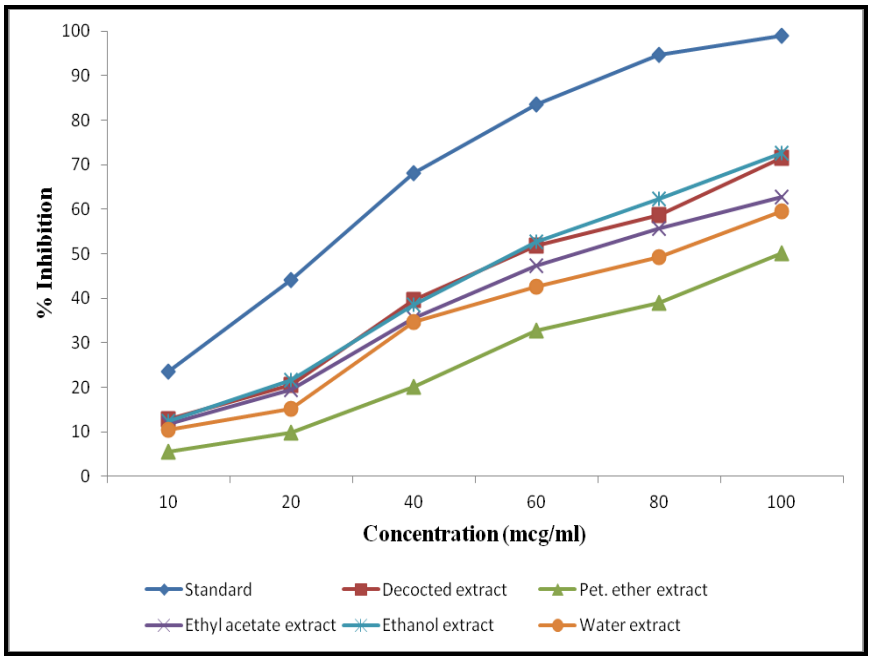

Figure 1: DPPH radical scavenging activity of various extracts of the plant, *L. madayiparense*

Table 2: DPPH free radical scavenging activity of various extracts of the plant, *L. madayiparense*

Tested compounds	%Inhibition of DPPH					
	10 (µg/ml)	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)
Standard	23.58	44.21	68.14	83.58	94.74	98.93
Decocted extract	12.85	20.63	39.53	51.92	58.71	71.59
Pet. ether extract	5.45	9.78	20.13	32.65	38.98	50.19
Ethyl acetate extract	11.85	19.53	35.62	47.37	55.68	62.80
Ethanol extract	12.49	21.56	38.44	52.70	62.31	72.65
Water extract	10.47	15.27	34.62	42.66	49.30	59.45

In reducing power assay, the absorbance was increased by increasing the concentration of the extracts which indicates their greater reducing power of the extracts of *L. madayiparense*. The inhibition concentration (IC_{50}) which required the absorbance of 0.5 was also determined from the graph plotting between the concentration of extract and the absorbance. Among the tested extracts at various concentrations, ethanol extract was found to be effective IC_{50} value (268 µg/ml) followed by decocted extract (270 µg/ml) and ethyl acetate extract (410 µg/ml) whereas no effective IC_{50} value was found in water extract followed by pet ether extract. All the results were well comparable with standard antioxidant, ascorbic acid (table 2 and figure 2).

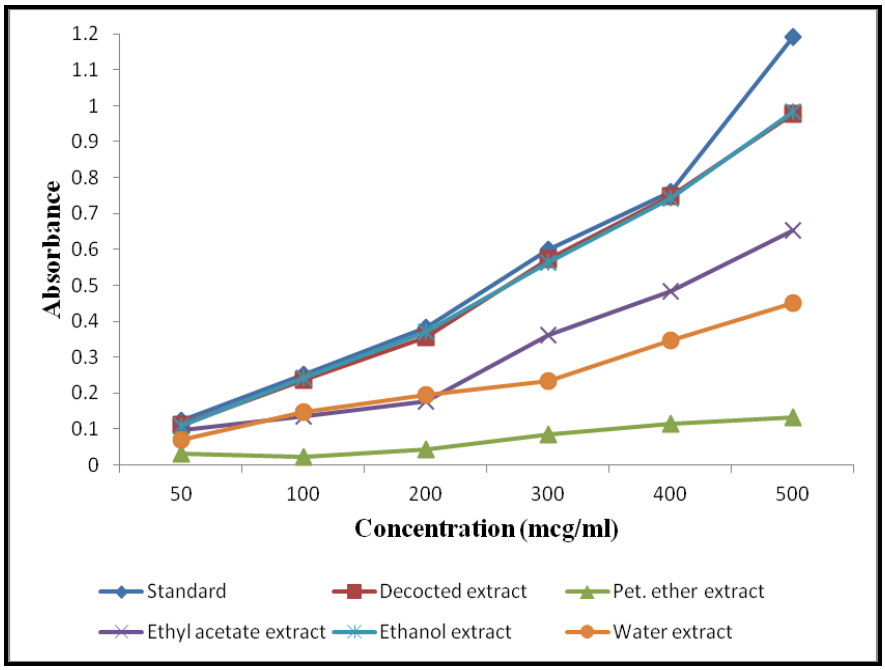

Figure 2: Reducing power ability of various extracts of the plant, *L. madayiparense*

Table 3: Reducing power ability of various extracts of the plant, *L. madayiparense*

Tested compounds	Absorbance						IC_{50} value
	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)	
Standard	0.124	0.252	0.382	0.599	0.761	1.192	252
Decocted extract	0.112	0.238	0.355	0.574	0.748	0.978	270
Pet. ether extract	0.03	0.023	0.042	0.086	0.114	0.132	>500
Ethyl acetate extract	0.098	0.134	0.178	0.362	0.484	0.652	410
Ethanol extract	0.108	0.243	0.369	0.564	0.743	0.984	268
Water extract	0.071	0.148	0.196	0.233	0.346	0.452	>500

Discussion

DPPH radical scavenging assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the formation of visible deep purple color and read the absorbance at 517 nm. The decolorization of DPPH is possible when it accepts an electron donated by an antioxidant compound and can be quantitatively measured from the changes in absorbance[3]. The reduction capability of DPPH radical was determined by the decrease in its absorbance which might be the reason for the increased antioxidant activity of the plant, *L. madayiparense* in DPPH assay.

An earlier study by Tanaka *et al*., 1988 have reported a direct correlation between antioxidant activity and reducing power of certain plant extracts[18]. Reducing power ability assay is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) which was observed by the increased absorbance read by spectrophotometrically at 700 nm. The absorbance was increased by increasing the concentration of the extracts which indicates their greater reducing power. A previous report by Oyaizu (1986) supported this transformation occurred which might be due to the presence of phytochemicals in the plant extracts[19]. Moreover, the reducing property of the plant extracts are normally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[20]. The reducing power of the plant, *L. madayiparense* extracts was found to gradually increase as the increased concentration of the extracts.

Conclusion

The present study scientifically validates the antioxidant potential of the plant, *L. madayiparense* for the first time. As plant extract is a mixture of phytocompounds, it is necessary to identify the active phytoconstituent and to test this effect in animal models. Further investigations on the various effects of this plant and isolation of the phytoconstituent(s) responsible for the antioxidant activity are in process.

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