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RESEARCH ARTICLE

FREE RADICAL SCAVENGING ABILITY OF A POTENT THERAPEUTIC PLANT *MUSSAENDA LUTEOLA* DELILE (RUBIACEAE)

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ABSTRACT

The aim of present study was to investigate the *in vitro* antioxidant potential and total extractive yield of *Mussaenda luteola* Delile leaves. Antioxidant activity was assessed by using 2,2- diphenyl-1-picryl-hydrazyl (DPPH•) assay, reducing power activity and [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)] ABTS•+ assay. Here ascorbic acid (ASA) and rutin were used as standard antioxidants. The results of the study indicates that the chloroform extracts of the leaf of *Mussaenda luteola* possesses significant scavenging activity against DPPH• (17.56) and reducing power activity (0.759) at 700nm absorbance. The ethanolic leaf extracts holds high free radical scavenging activity (ABTS•+) at 735nm (94.59). The free radical scavenging and antioxidant activities may be attributed to the presence of adequate phenolic and flavonoid compounds. The ethanolic leaf extract of *M. luteola* yields maximum extractive yield percentage (37.08%). This study revealed that the leaf extracts of *Mussaenda luteola* has demonstrated significant antioxidant activity.

Keywords: *Mussaenda luteola*, total phenolic, flavonoids, DPPH• assay, ABTS•+ assay

1. INTRODUCTION

Natures are sources of medicinal agents and produces enormous number of new drugs based on their use in traditional medicine. Medicinal plants typically contain combinations of different chemical substances that may act individually, additively or in synergy to improve the quality of health. Traditional medicines are found to be fundamentally preventive, protective, nutritive and curative. They are safe and harmless and could treat patients without side effects. In spite of the phenomenal progress in the area of development of new drugs from synthetic sources and appearance of antibiotics as major therapeutic agents, plants continue to provide basic raw material for some of the most important drugs [1]. Phytoconstituents are becoming a great source of interest in the present because of its wide applications in pharmaceutical industry. While finding the prior plants with high potent medicinal values, it can be used to treat various ailments. In such case we could prevent and save the present and future generations from health hazards, new novel diseases and various unknown health deteriorating ailments. The main aim of this study is to find plants with strong antioxidant assay which could serve as good candidate for the development of quality phytomedicine. The *Mussaenda* genus has been instrumental in the discovery of medicinal natural products. The plants are members of the Rubiaceae (madder or coffee family) and are native to the Old World tropics, from West Africa through the Indian sub-continent, South-East Asia and to Southern China [2]. There are more than 200 species of *Mussaenda* known. Some species of *Mussaenda* have been used in Chinese and Fijian traditional medicine.

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2. MATERIALS AND METHODS

Extractive yield

The extract yield contains different phytoconstituents. Extractive value is the measure of the chemical constituents in a plant material. The powdered plant material (crude drug) contains active chemical constituents which are responsible for its biological activity. Total soluble quantity of the drug in any particular solvent or mixture is referred to, as its extractive value. Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by other means.

The percentage yield (recovery) of evaporated plant extracts were calculated as follows:

$$\text{Yield (\%)} = \frac{[\text{Extract + container (g)}] - [\text{Empty container (g)}]}{\text{Sample weight (g)}} \times 100$$

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Determination of in vitro antioxidant activity

DPPH radical scavenging activity

Free radical scavenging activity of the plant extracts was assessed accordance with Blois [3], the stable DPPH▪ method. A solution of radical was prepared by dissolving 2.4mg of 0.1mM DPPH▪ in 100mL methanol. The test samples (5µg/mL) were mixed with 3.9 mL of methanolic DPPH▪ solution. The reaction mixture was shaken vigorously, incubated in dark at room temperature for 30 min and the absorbance of the reaction mixture was measured at 517 nm spectrophotometrically. The radical scavenging capability were compared with the activity of rutin, quercetin, BHA and BHT. Per cent DPPH▪ discoloration of the samples was calculated using the formula:

DPPH radical scavenging activity (%) = [(Control OD – Sample OD)/Control OD] ×100.

Antioxidant capacity of the extracts to decrease the initial concentration of DPPH were expressed as inhibitory concentration (IC_{50}), these values were calculated from the linear regression of the % of DPPH scavenged versus concentration of the extracts [4].

ABTS▪+ antioxidant assay

$ABTS^{▪+}$ radical scavenging activity was performed according to the method suggested by Siddhuraju and Manian [5]. The radical cation ($ABTS^{▪+}$) was pregenerated by adding 5 mL of 14mM $ABTS^{▪+}$ solution to 5mL of 4.9 mM potassium persulphatesolutionand was incubated in dark for 12-16 h at room temperature. Before initiating the reaction, the solution was suitably diluted with ethyl alcohol (about 1:89 v/v) to obtain an absorbance of 0.700 ± 0.02 at 734 nm and then used for initiating the antioxidant assay. 50 µL/mL of sample was added to 950µL of diluted $ABTS^{▪+}$ solution and vortexed for 10 seconds. After 30 min of incubation, the reduction in absorbance was recorded at 734 nm. Trolox (50 µg/mL) was used as a reference compound.

Determination of Reducing power [6]

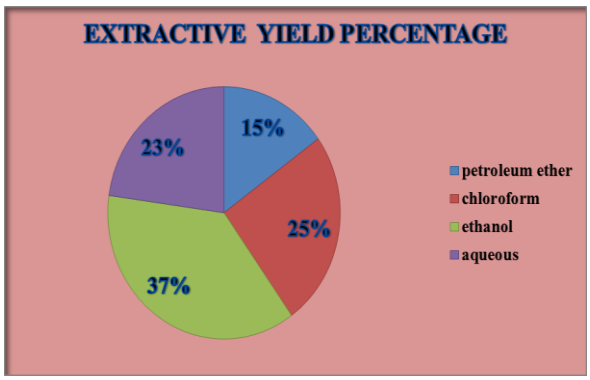
Different concentrations of *Mussaenda luteola* extract (100– 1000 µg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was taken and is expressed as mean ± standard deviation.

3. RESULTS

For the present study *M. luteola* leaves were collected for total extractive yield and antioxidant activities.

Extractive yield

The total extractive yield of *M. luteola* leaves was estimated in all the four selective solvents through standard procedures and it results in the separation of medicinally active portions of plant leaves. The results of the extractive yield from *M. luteola* was measured and calculated and it ranged between 15.03% - 37.08% respectively and their results were depicted in the Figure 1. The ethanolic leaf extract of *M. luteola* yields maximum yield percentage (37.08%) followed by chloroform (25.09) and aqueous (22.80%). The minimum extractive yield percentage was found in the low polarity solvent petroleum ether (15.03%). This result shows that the ethanolic leaf extract under the observed study proves that most of the phytoconstituents are found active in the leaves of *M. luteola.*



In vitro antioxidant analysis

DPPH radical scavenging activity in different solvent extracts of M. luteola leaves

The antioxidant activity of *M. luteola* was evaluated using various solvent extracts such as petroleum ether, chloroform, ethanol and aqueous. The radical scavenging activity based on the DPPH assay was determined and found the percentage of inhibition with the increase in concentration.

Ascorbic acid is used as a control. Among the solvent extracts chloroform (17.56) holds the highest IC_{50}valuefollowed by petroleum ether and ethanol. The minimum IC_{50} was recorded in aqueous (2.67) (Table 1).

ABTS radical scavenging activity

The total antioxidant activity of *M. luteola* was assessed by ABTS cation as the percentage of inhibition at 743 nm. In the present investigation ethanol extract registered the highest amount 94.59µmol/ml and followed by aqueous and chloroform. The lowest value was occurred in the petroleum ether 55.17µmol/ml (Table 2).

Determination of Reducing power [6]

Different concentrations of *M. luteola* leaf extract (100– 1000 µg/g) were taken to estimate the radical scavenging activity based on the reducing power and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates the increased reducing power. In this case the ascorbic acid was used as the standard. Phosphate buffer was used as blank solution. The absorbance of the final reaction mixture is calculated and revealed that chloroform (0.759 $\mu l/ml$) in different concentration has the higher absorption and the minimum is calculated in ethanol (0.440 $\mu l/ml$) (Table 3).

Table 1. DPPH antioxidant activity in different solvent leaf extracts of *Mussaenda luteola*

S. No.	Solvents	IC_{50} Value (µg/ml)
1.	Petroleum Ether	7.77
2.	Chloroform	17.56
3.	Ethanol	4.72
4.	Aqueous	2.67
5.	Standard	1.8

Values are mean ± SD of three independent experiments. Values not sharing a common letter in a column are significantly different (P<0.05).
*Values expressed as TEAC (Trolox equivalent antioxidant capacity) in µmol/ml extract.

Table 2. ABTS radical scavenging activity in different solvent leaf extracts of *Mussaenda luteola*

S. No.	Solvents	ABTS* RADIAL SCAVENGING ACTIVITY (µmol/ml)
1.	Petroleum Ether	55.17
2.	Chloroform	90.46
3.	Ethanol	94.59
4.	Aqueous	91.10

Table 3. Reducing power assay in leaf extracts of *Mussaenda luteola*

S. No	Solvents	Concentration (g/ml)	OD value	Mean value $(\mu l/ml)$
1	Petroleum ether	10	0.354	0.606
		20	0.661	
		30	0.687	
		40	0.722	
2	Chloroform	10	0.656	0.759
		20	0.793	
		30	0.772	
		40	0.815	
3	Ethanol	10	0.426	0.440
		20	0.483	
		30	0.425	
		40	0.427	
4	Aqueous	10	0.761	0.748
		20	0.734	
		30	0.725	
		40	0.772	

4. DISCUSSION

Medicinal plants are a crucial source of natural antioxidants they produce a diverse range of secondary metabolites with antioxidative properties that have therapeutic potential. Antioxidants can be effective in preventing free radical formation by scavenging them or increasing their decomposition rate and suppressing disorders [7]. Currently, there is a growing interest towards natural antioxidants of herbal resources. In the present study the free radical scavenging ability of various solvent extracts of *M. luteola* leaf were analyzed using DPPH, ABTs and reducing power assay and was depicted in Table

1-3. It was noted that ethanol leaf extract exhibited remarkable scavenging ability than other solvent. Whereas, in reducing power assay chloroform extract exhibited antioxidant property. The reducing ability is generally associated with the presence of reductants which employ antioxidant potential through breaking down the free radical chain by donating a hydrogen atom or preventing peroxide formation [8]. These results are in accord with high levels of phytochemical contents in *M. luteola* leaf. The presence of bioactive compounds in the ethanol extracts of the studied species positively correlated with their antioxidant potential, confirming their major role in antioxidant activity.

5. CONCLUSION

Plants are being widely used in the production of new natural products in various fields such as pharmaceuticals, nutraceuticals and food production. Antioxidant studies pave the way to claim the traditional medicinal property of plants. Hence it is also proved in the current investigated plant *M. luteola.* The plant that is taken under studies shows that it could serve as a potent therapeutic plant. To find the action mechanism of antioxidants further studies can be done in *M. luteola* by way of isolation and characterization of antioxidant compounds, and also by *in vivo* study models.

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