Chemistry Research Journal, 2022, 7(5):1-7

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Assessment of Antioxidant Activity of Leaf Extract of *Mussaenda erythrophylla*

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Abstract Traditional medicines have deep roots in culture of ancient heritage. Ancient scholars believed that herbs are only solution for many health-related problems. The advances in various scientific fields have proved that plants contain active chemical constituents. *Mussaenda erythrophylla* belongs to Rubaceae family, is cultivated as ornament plant in India and native of Western tropical Africa. The main objective of the study was to determine *in vitro* anti-oxidant evaluation by different methods on ethanolic extract of *Mussaenda erythrophylla.* The scavenging activity of DPPH assay and NO assay is determined. The results of the present study, proves the extract which have an anti-oxidant activity in a dose dependent manner. The ethanolic extract of *Mussaenda erythrophylla* leaves which have high activity. Further studies are needed for compound identification and its action in an *in vivo* model.

Keywords *Mussaenda erythrophylla*, anti-oxidant activity, ascorbic acid, DPPH assay

Introduction

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety. Modernization has made many changes and modifications in the lifestyle of society, resulting in drastic increase of several disorders and diseases. Various studies have reported that consumption of good diet (vegetables, fruits) is necessary in reducing the risk factor of many diseases.

Mussaenda are increasingly popular for the showy color they provide during much of the year in South Florida landscapes. The *Mussaendas* are a group of highly ornamental shrubs suited to tropical and subtropical climates with a bright future, both as landscape plants and as potted floral decorations. The most distinctive feature of *Mussaenda* (and some other genera of the *Rubiaceae*) is that the floral display is primarily derived from the calyx, with some individual flowers within an inflorescence carrying an enlarged petaloid sepal. Some cultivars have all five sepals enlarged. These are called calycophylls or sometimes semaphylls (that is, a structure which signals a pollinator).

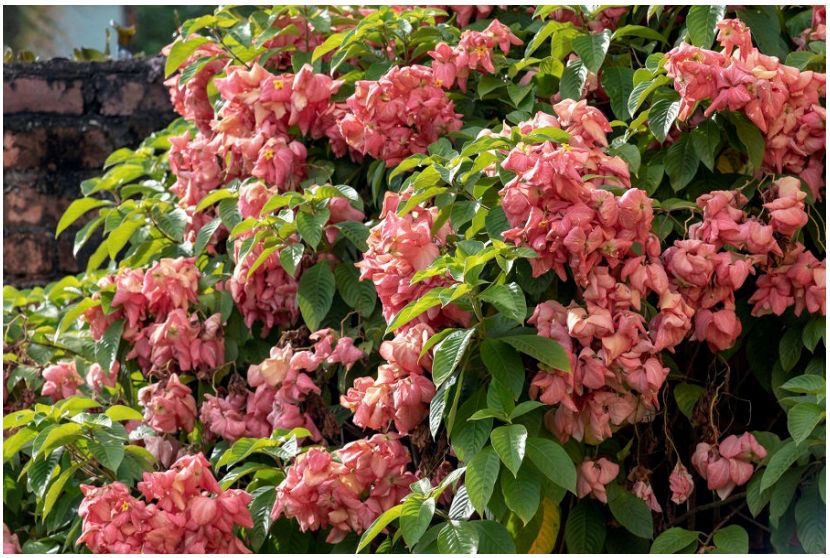

Figure 1: Mussaenda erythrophylla plant

The major attractions of *Mussaendas* in the landscape is their extended flowering period. They will loosen their leaves and go dormant through the cooler and drier winter, but put on a spectacular display throughout the warm, wet months. If conditions are suitable, they can flower year-round. They have poor drought and cold tolerance. The present aim is to screen the anti-oxidant activity on methanolic extract of *Mussaenda erythrophylla* leaves s in an *in vitro* model.

Figure 2: Mussaenda erythrophylla leaves

Materials and Methods

Collection of the plant

The fresh leaves of *Mussaenda erythrophylla* were collected from forest area of Tirupati, Chittoor dist., Andhra Pradesh, India.

Identification and authentication of plant

The plant is identified and authenticated by Prof. K. Madha Shetty, Department of Botany, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen as deposited in School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Medchal, Hyderabad, Telangana.

Preparation of the extract

The shade dried leaves were powdered mechanically and stored in an air tight container. The extraction was carried out by Soxhlet apparatus. The extract was concentrated to dryness under controlled temperature 40-50 ^{0}C. The extract was closed in air tight container and preserved in refrigerator till further use.

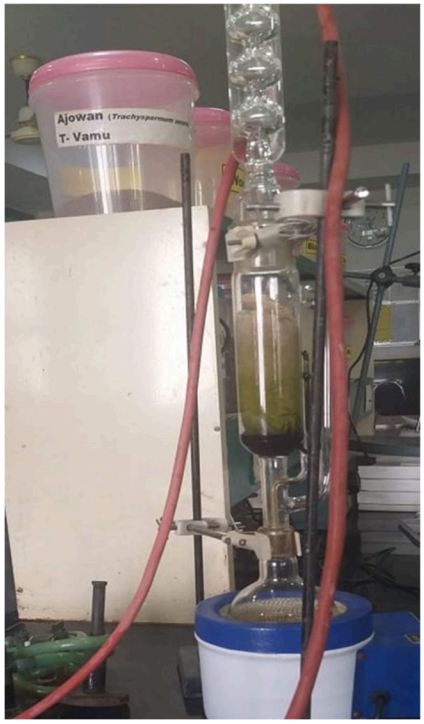

Figure 3: Soxhlet extraction of Mussaenda erythrophylla leaves

***In vitro* anti-oxidant activity**

Oxidation is one of the important biological processes for the production of energy in living organism. Living organisms uses oxidation for the production of energy to fuel biological processes. A variety of physiological and biochemical lesions increasingly deteriorate degenerative diseases such as aging, cancer and coronary artery disease due to free radicals. Despite of anti-oxidant defence and other defence mechanism in human these systems are insufficient to prevent the damage entirely. Anti-oxidants are the substances that can inhibit or restrict oxidative cellular oxidizable substrates.

Nitric oxide scavenging activity

Nitric oxide was generated by sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations of ethanolic extract (50, 100, 200, 400, 800, 1000 µg/ml), vitamin C as reference standard (50, 100, 200, 400, 800, 1000 µg/ml) and dissolved in phosphate buffer saline (0.025 M, pH: 7.4) and the tubes were incubated at 25 ^{0}C for 5 h. Control experiments without the test compounds but equivalent amounts of buffer were conducted in an identical manner. After 5 h, 0.5 ml of incubation solution is removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% *O*-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with

naphthyl ethylene diamine was read at 545 nm. All the determinations were performed in 6 replicates. Percentage inhibition of nitric oxide radical was calculated by using the following formula;

$$\textbf{Percentage inhibition } (\%) = \frac{(\textbf{Abs of control} - \textbf{Abs of test})}{\textbf{Abs of control}} \textbf{X 100}$$

DPPH radical scavenging activity

Preparation of DPPH solution:

About 0.1 mM DPPH crystalline solid was taken in test tube, slowly dissolve the crystalline using organic solvent like methanol to form a solution. The ethanolic extraction, standard (vitamin C) and control (without the test compound but with an equivalent amount of methanol) with a different concentration (50, 100, 200, 400, 800, 1000 µg/ml) of about 3 ml each were taken in test tube. To this ethanolic extract, add 1 ml of DPPH solution slowly. Shake the concentrate solution and enable it to remain at room temperature for about 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC_{50} value (half of inhibitory focus in µg/ml) was compared with standard solution i.e., vitamin C (Ascorbic acid). Free radical scavenging activity is identified by the decrease in the absorbance of the reaction mixture. The percentage inhibition of DPPH radical was calculated using the below formula;

$$\textbf{Percentage inhibition } (\%) = \frac{(\textbf{Abs of control} - \textbf{Abs of test})}{\textbf{Abs of control}} \textbf{X 100}$$

Results and Discussion

Natural compounds or primary as well as secondary metabolites like glycosides, volatile oils, alkaloids, tannins, steroids and other secondary metabolites which exert physiological activity were synthesized in the plant, in addition to the carbohydrates lipids and proteins utilized by man as food particles. A systematic and complete study of crude drugs includes a thorough investigation of both primary and secondary metabolites derived as a result of plant metabolism. Different qualitative chemical tests were performed to investigate the phytochemical profile of given extracts. Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reactions.

The results of radical assay is reported in the below tables.

Table 1: Nitric oxide scavenging activity

S. No.	Concentration (µg/ml)	% Inhibition	
		Standard (Vitamin-C)	**Leaf extract**
1	50	8.31 ± 0.45	38.496 ± 0.0534**
2	100	16.67 ± 0.90	41.748 ± 0.1393**
3	200	27.76 ± 1.08	45.18 ± 0.024**
4	400	52.77 ± 0.60	46.178 ± 0.030**
5	800	61.12 ± 0.51	47.872 ± 0.081**
6	1000	65.88 ± 0.84	49.594 ± 0.048**
7	IC_{50}	380 (µg/ml)	560 (µg/ml)

*The values are expressed as Mean ± SEM, n=6 in each group. If * P<0.05, ** P<0.01 and *** P<0.001 vs. control.*

Some of the studies suggest that it contain of high amount of ethanolic extract substances. Hence the present research emphasis on isolation of ethanolic extract and its evaluation for anti-oxidant activity. The young leaves juice is used to treat the imbalances of the digestive function. It is also said to be a remedy for the toothache. Mammalian cells produce nitric oxide in form of free radicals, which is involved in the regulation of various physiological reactions. Under aerobic conditions nitric oxide is very unstable species.

Nitric oxide when undergoes metabolism, produce nitrate and nitrite as an end product through intermediates. It is estimated by using the Griess reagent and in presence of test compound which was the scavenger. In the present research, sodium nitroprusside in standard phosphate saline buffer at 25 ^{0}C was incubated to produce nitrite. It was

estimated that due to the presence of ethanol, free radical scavenging property can be produced using the above method.

DPPH assay is considered as a valid method to evaluate scavenging activity of antioxidants, since the radical compound is very stable and do not have to generate as in other radical assays. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solutions loses colour stoichiometrically with the number of electrons taken up. such reactivity has been widely used to test the ability of plant extract to act as free radical scavengers. DPPH assay of ethanolic extract showed a dose dependent increase in the percentage of inhibition of free radicals. The ethanolic extract fraction was found to show a good anti-oxidant potential.

Table 2: DPPH radical scavenging activity

S. No.	Concentration (µg/ml)	% Inhibition	
		Standard (Vitamin-C)	**Leaf extract**
1	**50**	56.437 ± 0.7557	14.95 ± 0.2474**
2	**100**	65.55 ± 0.679	20.164 ± 0.4004**
3	**200**	70.255 ± 0.8019	32.316 ± 0.6935**
4	**400**	73.377 ± 0.7377	42.239 ± 0.7685**
5	**800**	76.41 ± 0.7823	48.957 ± 0.5805**
6	**1000**	82.37 ± 0.7078	61.905 ± 0.4995**
7	**IC_{50}**	540 (µg/ml)	630 (µg/ml)

*The values are expressed as Mean ± SEM, n=6 in each group. If * P<0.05, ** P<0.01 and *** P<0.001 vs. control.*

The *Mussaenda erythrophylla* leaves are popular in an indigenous system of folk medicine. It is mainly used for the treatment of various ailments. Some investigations examined anti-microbial and anti-cancer activity. *Mussaenda erythrophylla* leaves contain many major pharmacologically active ingredients, and so many other bioactive compounds. The important active constituents are essential oils, flavonoids, phenolic compounds, and triterpenoids. In view of the huge medicinal importance of this plant, the present study is carried out to evaluate the *in vitro* anti-oxidant activity of the *Mussaenda erythrophylla* leaves of various extracts. DPPH is a free radical, it easily damages the cell membrane. DPPH is easily accepting the electrons or hydrogen radical from anti-oxidant compounds. The DPPH which gains the hydrogen atom from the anti-oxidant compounds and the colour will be changed. In the present study the in density of the colour is directly proportional to the inhibitory activity of the anti-oxidant compound. It shows the inhibitory activity is due to the maximum hydrogen donating ability of *Mussaenda erythrophylla* leaf extract.

Based on this result the maximum inhibitory activity is noticed in the ethanolic extract at 1000 µg/ml. The results obtained in the present study; all the three extracts have an anti-oxidant activity in a dose dependent manner. The reducing power assay is determined by the electron transfer ability of the plant extracts. The Fe^{3+} ions are converted into Fe^{2+} ions, this ability is minimizing the oxidative damages in the tissues. In the present study proves that the inhibitory activity NO formation by ethanolic extract of *Mussaenda erythrophylla* leaves. The ethanolic extract have a maximum inhibitory activity against the NO formation in the concentration of 1000 µg/ml. NO is a free radical. It changes the structure and functions of the cellular membranes. It is formed from sodium nitroprusside and it react with free radicals to form nitrite. The anti-oxidant compound which directly reacts with the free radicals and other nitrogen compounds and it prevents the nitric oxide formation. This may prevent the cellular damages.

Conclusion

Oxidation is an essential process in living organisms to produce energy to biological processes. But uncontrolled oxidation process produces the free radicals and it causes the cell damage and it leads to various diseases such as cancer, atherosclerosis, aging etc. In normal conditions our body produces the natural antioxidants and it prevents the cell from oxidative damages, but in pathological conditions our body needs additional supply of anti-oxidants. Synthetic anti-oxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are used to prevent the cell damages but it causes the adverse effects due to this, continuous searching for alternative drugs. In

now-a-days the researchers are interested to find the drugs from natural sources. In all over the world nearly three-quarters were used plant and plant materials to treat various diseases.

From this, we can infer that ethanolic extract from leaves of *Mussaenda erythrophylla* have huge significant anti-oxidant activity in all *in vitro* models based on free radical searching property. The anti-oxidant activity is most likely due to the presence of ethanolic extract. These findings show that the *Mussaenda erythrophylla* extract possesses anti-oxidant activity. DPPH and NO assay revealed that leaf extract had the highest anti-oxidant activity comparable with Vitamin C. The leaf extract is a promising candidate for use as natural products-based anti-oxidant for the health of human being.

Acknowledgement

The authors wish to thank the management of School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Telangana, India for providing necessary equipment for research, praiseworthy inspiration, constant encouragement, facilities and support.

Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

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