In-vitro Antioxidant Potential of Methanolic Extracts of *Mirabilis jalapa* Linn

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ABSTRACT

Introduction: Over the past decade, herbal and ayurvedic drugs have become a subject of world importance, with both medicinal and economical implications. The relatively lower incidence of adverse reactions to plant preparations compared to modern pharmaceuticals is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs [1, 2, 3, 4] . Various crude extracts of *Mirabilis jalapa* Linn of family Nyctaginaceae has been widely used in traditional medicine. The infusion or decoction of *Mirabilis jalapa* Linn leaves is being used to treat inflammatory and painful diseases [5, 6]. Of date there has not been any report on any kind of antioxidant screening of the areal extracts of *Mirabilis jalapa* Linn including the kernel and the leaves. **Methods:** This is one of the first studies reporting the antioxidant potential of aerial (bark and leaves) and the root extracts of *Mirabilis jalapa* separately using ABTS and DPPH assay methods The present study was aimed to extract, identify the various phytochemical constituents and to study the antioxidant activities of the plant by various chemical and instrumental methods. **Results:** The extracts showed the presence of alkaloids, tannins, phytosterols, triterpenoids and flavonoids in significantly detectable amounts. In the present study we examined the antioxidant effects of methanolic extract of the aerial parts and root extracts of *Mirabilis jalapa* Linn at various concentrations in the ABTS+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay. **Conclusions:** The results of the study reveal the immense potential of the plant for further research that aims at identifying the bioactive components responsible for the anti-oxidant activity and elucidating their tentative mechanisms of action.

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Keywords: ABTS radical, DPPH, Free radical scavenging, *Mirabilis jalapa*, Reductive capacity, total flavonoids.

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INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves.

"Free radicals" are dangerous substances, chemical compounds, and toxins found within the body. Herbal antioxidants help rid the body of these toxins and help people live healthier and longer lives as free radical damage have been proposed as one major factor for natural aging.

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to neutralize free radicals. Thus, antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells[7].Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke, neurodegenerative diseases, cancer and coronary heart disease. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline [8].

Mirabilis jalapa (also known as Four O'clock flower or marvel of Peru), of the family Nyctaginaceae, is a perennial herb. It can also be grown as an annual, tall herbaceous climbing plant with opposite leaves, large showy flowers, coriaceous obovoid fruits and prominent tuberous roots, planted as an ornamental plant throughout the country. Phytochemical investigations revealed that the plant contains alanine, alphaamyrins, arabinose, beta-amyrins, campesterol, daucosterol and dopamine[9]. Previously, antimicrobial, antinociceptive[4] and antigonorrhoeal properties of this plant have been reported. *Mirabilis jalapa* is rich in many active compounds of which, particular interest to researchers is a group of amino acid-based proteins, called mirabilis antiviral proteins (MAPs) [10], [11].

The plant has shown specific antiviral actions. It is also found to possess antispasmodic and antinociceptive properties. In traditional medicine Mirabilis jalapa is widely used as antidysenteric, antiparasitic, carminative, detoxifier, digestive stimulant, diuretic, purgative, tonic, vermifuge, wound healer, for skin problems. *Mirabilis jalapa* is rich in many active compounds including triterpenes, proteins, flavonoids, alkaloids, and steroids.

MATERIAL AND METHODS

Collection and Identification of Plant material

The fresh plant of *Mirabilis jalapa Linn* were collected in the months of July-August from the local areas of Kochi and authenticated by the authority of the botany department. A voucher specimen was submitted at Institute's herbarium department for future reference. The bark, leaves and root were washed with water, shade dried powdered in a mechanical grinder and kept in air tight container till use.

Preparation of the Plant Extract

The extraction of the *Mirabilis jalapa* aerial parts and root were carried out by known standard procedures. The plant materials were dried in shade and powdered in a mechanical grinder. The powder(100gm) of the root and aerial parts each were initially de-fatted with petroleum ether (60-80°C), followed by 500ml methanol by Soxhlet extraction method for 72 hrs separately .Solvent elimination under reduced pressure afforded the petroleum ether and methanol extract of which methanol extract was further used for antioxidant assay methods[12],[13],[14]. The extract was dried in a vacuum desiccator to obtained constant weight. The phytochemical screening was carried out as described by Norman. The methanolic extract root and aerial parts yield a reddish brownish solid residue (2.33 %) and dark drown residue (2.5 %) respectively. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts were obtained by the solvent evaporation and used to determine concentration in mg/ml. the extract was used directly for DPPH assay, ABTS assay and total flavanoid content and also for assessment of antioxidant capacity through various chemical assays.

PHYTOCHEMICAL EVALUATION

Determination Of Total Antioxidant Activity

In-vitro antioxidant activity

Protocol for ABTS+ radical scavenging assay

The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation has been used to screen the relative radical-scavenging capacity.This is a measure of antioxidant activity as opposed to antioxidant concentration which includes a proportion of biologically inactive antioxidants. It also permits the measurement of antioxidant activity of mixtures of substances and hence helps to distinguish between additive and synergistic effects [15].

Protocol for preparing Standard Solution

ABTS+ radical was freshly prepared by adding 4.9 mM ammonium persulfate solution to 14 mM ABTS solution and kept for 16 hrs in dark. This solution was diluted with ethanol (99.5%) to yield an absorbance of 0.70±0.02 at 734 nm and the same was used for the assay. To 950 µl of ABTS radical solution, added 50 µl of extract solutions (100-500 µg/ml) and the reaction mixture was vortexed for 10 sec. After 6 minutes the absorbance was recorded at 734 nm and compared with the control ABTS solution. Percentage inhibition was calculated from the formula

$$\textit{Percentage inhibition} = 1 - \frac{\text{absorbance of test}}{\text{absorbance of control}} \times 100$$

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Statistical analysis

Values were represented as mean ± SD of three parallel measurements and data were analyzed using the *t*-test.

Free radical scavenging activity (DPPH Assay)

Protocol for estimation of DPPH scavenging activity [16], [17], [18], [19]

This is the most widely reported method for screening of antioxidant activity of many plant drugs. DPPH assay method is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger [20], [21]. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC_{50}.

Preparation of the Sample Solution

Quantitative measurement of radical scavenging property was carried out in a universal bottle. Sample stock solutions [22]1.0 mg/ml) were diluted to final concentrations of 50, 40, 30, 20, 10 μg/ml, in ethanol. One ml of a 0.3 mM DPPH ethanolic solution was added to 2.5 ml of sample solutions of different concentrations, and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (AA) using the following formula:

$$AA\ \% = 100 - \frac{ABS_{SAMPLE} - ABS_{BLANK}}{ABS_{CONTROL}} \times 100$$

Average percent of antioxidant activity from three separate tests were calculated [23].

Estimation of flavonoid content using Swain and Hillis method (1959): [24]

Preparation of test and standard solutions

The plant extract (50 mg) were dissolved separately in 50 ml of methanol. These solutions were serially diluted with methanol to obtain lower dilutions. Phloroglucinol (50 mg) was dissolved in 50 ml of distilled water. It was serially diluted with water to obtain lower dilutions.

Protocol for total Flavanoid content

0.2 ml of the extract was taken in a test tube and the final volume was made up to 2 ml with distilled water and to this 4 ml of vanillin reagent was added rapidly. Exactly after 15 min. absorbance was recorded at 500 nm against blank. The unknown was read from a standard curve prepared using different concentration of phosphoglycinol.

In the phytochemical identification, the aqueous extract of Mirabilis jalapa with 5% ferric chloride solution gave deep blue colour and with lead acetate solution gave white precipitate indicated the presence of tannin and phenolic compounds. The extract with 5ml 95% ethanol, few drops of concentrated HCl and 0.5g magnesium turnigs gave pink colour indicated the presence of flavanoids. The extract with dragendroff reagent gave reddish brown precipitate showed the presence of alkaloid.

RESULTS AND DISCUSSION

Total Flavanoid Content

The Flavonoid content was found to be 4.41 ± 0.02 mg / gram of dried extract equivalent to Phloroglycinol (Fig. 3). The total flavanoid content shows good linear relation in both standard as well as sample extract.

ABTS+ radical scavenging assay

It is evident from Fig. 1 that the *Mirabilis jalapa* extracts exhibited ABTS free radical scavenging activity compared to that of quercetin. The EC50 values were found to be 1249 μg/ml,974 μg/ml, 70.28 μg/ml respectively for methanol root and aereal extracts and Quercetin respectively.

In DPPH radical scavenging assay, MJM root extract at the concentration of 500 μg/ml exhibited 17.53605 ± 0.1071422, MJM aerial at the concentration of 500 μg/ml extract exhibited 17.20884 ± 0.08971401% inhibition, Nevertheless it was 81.29971 ± 0.27 % in the presence of 100 μg/ml quercetin on the DPPH radical (Fig. 2) .The EC-50 values of MJM root and MJM areal was found to be 1679μg/ml and 3723 μg/ml respectively and for quercetin it is 17.84 μg/ml.

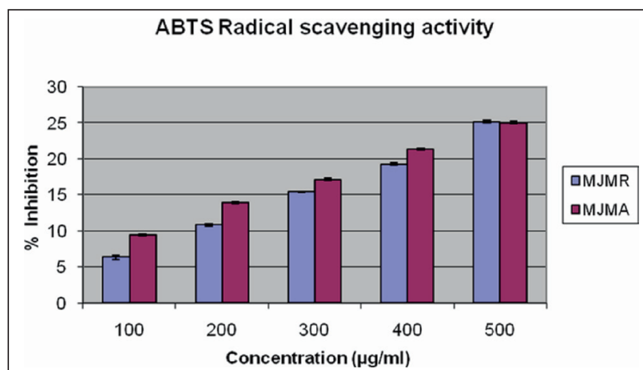

Figure 1. ABTS Radical scavenging activity.

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Table 1. ABTS Radical Scavenging Assay

Concentration (µg/ml)	Percentage Inhibition		
	MJMR	MJMA	Quercetin
100	6.32 ± 0.29	9.45 ± 0.15	72.30 ± 0.14
200	10.81 ± 0.14	13.94 ± 0.13	76.95 ± 0.09
300	15.42 ± 0.10	17.16 ± 0.14	82.12 ± 0.24
400	19.29 ± 0.14	21.33 ± 0.13	86.73 ± 0.11
500	25.15 ± 0.17	25.05 ± 0.21	91.02 ± 0.12
EC50	974.1 µg/ml	1249 µg/ml	70.28 µg/ml

Values are mean ± SD of three parallel measurements
MJMR - Methanolic root extract of Mirabilis jalapa
MJMA- Methanolic areal extract of Mirabilis jalapa.

Table 2. DPPH Free Radical Scavenging Assay

Concn (µg/ml)	Percentage Inhibition			
	Quercetin	Conc (µg/ml)	MJMR	MJMA
20	53.12 ± 0.14	100	3.56 ± 0.11	5.93 ± 0.12
40	64.47 ± 0.07	200	6.05 ± 0.16	8.43 ± 0.09
60	71.02 ± 0.08	300	8.56 ± 0.13	10.57 ± 0.16
80	76.94 ± 0.06	400	13.76 ± 0.06	13.74 ± 0.24
100	81.29 ± 0.27	500	17.53 ± 0.10	17.20 ± 0.08 1
EC-50	17.84 µg/ml		1679 µg/ml	3723 µg/ml

Values are mean ± SD of three parallel measurements
MJMR - Methanolic root extract of Mirabilis jalapa
MJMA- Methanolic areal extract of Mirabilis jalapa

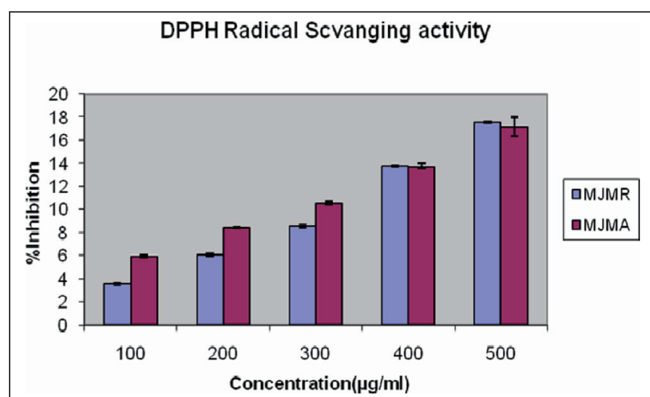

Figure 2. DPPH Radical scavenging activity.

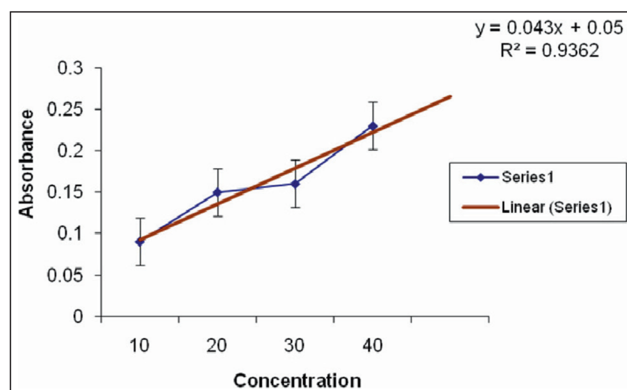

Figure 3. Quantitative Estimation of Flavanoids.

CONCLUSION

In this present study extract of *Mirabilis jalapa* shows anti oxidant property which might be helpful in preventing the progress of various oxidative stress related diseases followed by the DPPH and *ABTS*$^+$ *free radical scavenging assay*. Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of reactive oxygen species attack [25]. The biological properties, including cytotoxic and antioxidant properties, of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds. The results of the present study shows

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that the methanolic extract of the aerial parts and root of *Mirabilis jalapa* Linn possess antioxidant activity through the DPPH free radical scavenging activity at 517nm and *ABTS$^+$ radical scavenging assay*. As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant quercetin, the reference antioxidant. The preliminary phytochemical investigation indicates the presence of flavonoids, tannic acid and phenolics in the plant. In addition, the methanolic extract of *Mirabilis jalapa* found to contain a noticeable amount of total flavanoids, which plays a major role in controlling antioxidants .The separation and identification of flavonoids present in the plant can help researchers find new molecules which can be used as natural antioxidants. Further studies are currently in fact underway to isolate and characterize the active constituents responsible for its antioxidant activity.

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