

FREE RADICAL SCAVENGING AND ANTIBACTERIAL ACTIVITY OF *MIRABILIS JALAPA* LINN USING IN VITRO MODELS

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ABSTRACT

Medicinal plants form a major source of raw materials for drugs for the prevention and treatment of ailments. *Mirabilis jalapa* linn is used as antinociceptive, anti-inflammatory agent, laxative in folk medicine. Antioxidants play an important role in protecting against damage by reactive oxygen species. The present study was designed to evaluate the plant potential as an antioxidant lead by using various in vitro models like Hydrogen peroxide scavenging method and reducing power assay method and also to determine its antibacterial potential. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, polyphenols like phenolic compounds and tannins. The total flavanoid content of the extract was found to be 4.41 ± 0.02 mg /gram. The plant exhibited significant antioxidant properties and could serve as a free radical inhibitor or scavenger. The antibacterial activity of the extracts was determined by the agar well diffusion method. The greatest zone of inhibition was displayed by methanolic extract against pseudomonas at 3mg/ml. With a wide spectrum of inhibition against Gram-positive and Gram-negative bacteria, the methanolic extract of *Mirabilis jalapa* are worthy of further investigation as a natural wide spectrum antibacterial agent. These results indicates the potential of *Mirabilis jalapa* as a source of antioxidant and antibacterial compound.

Keywords: *Mirabilis jalapa* Linn, Antioxidant, Hydrogen peroxide scavenging activity, Reducing power assay, Antibacterial

INTRODUCTION

Herbal drugs are the potential source of therapeutic aid for the treatment and prevention of a number of ailments. There has always been a continuing emphasis on the herbal medicines as a potential pipeline for novel bioactive molecules that encompass a varied field of application from cancer treatment and Alzheimer's to autoimmune diseases¹⁻³. *Mirabilis jalapa* Linn of family Nyctaginaceae has been called by various vernacular names around the world like 'Four o' clock' in English, 'Gulambasa' in Ayurveda, and 'Gul-abbas' in Hindi⁴. *Mirabilis jalapa* has been extensively used in almost all folklore remedies around the world for treating a variety of conditions. It has been reported that indigenous Mexican population uses various decoctions and preparations of *Mirabilis jalapa* for muscular pain, diarrhoea, dysentery, and abdominal colic⁵. The plant has been extensively studied for a variety of bioactive principles and screened for different pharmacological activities. The ethanolic extract of the leaves and the stem was found to have potent antinociceptive activity in experimental mice⁶. The plant has also proved to possess antibacterial, antiviral, and antioxidant activity⁷⁻⁹. Studies on isolated jejunum muscles indicated that methanolic flower extracts possessed potent contractile activity¹⁰. Efforts towards the identification and isolation of active principles from the plant has resulted in the isolation of eleven compounds including ginglycolipid, 4'-hydroxy-2,3-dihydroflavone, astragaloside VI etc¹¹. Moreover numerous components like β -sitosterol, stigmasterol, ursolic acid, oleanolic acid, brassicasterol, and *Mirabilis* antiviral protein, rotenoids (mirabilalone A-D, boeravinones C and F) have been successfully isolated and characterised¹²⁻¹⁴. The flowers possess antispasmodic activity and investigation of the mechanism of action indicated a role of serotonergic receptor¹⁵. The aqueous extract of the leaves possess potential anti inflammatory activity¹⁶. *Mirabilis jalapa* has also been evaluated for its anti histaminic activity and it has been found that in concordance with the folkloric use of the plant for allergy and asthma it has significant inhibitory action on the release of histamine and subsequent typical allergic responses¹⁷. It has also been evaluated for the anthelmintic activity using in-vitro models and was found to possess vermifugal activity¹⁸. The present study aims to evaluate the methanolic extracts of the aerial parts of the plant for potential antioxidant activity using conventional in vitro models like the reducing power assay, hydrogen peroxide scavenging assay and to determine its antibacterial potential.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh plants 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 of SH College, Kochi. The aerial parts 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 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 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 methods¹⁹⁻²⁰. The extract was dried in a vacuum desiccator to obtain constant weight. The methanolic extract of the aerial parts yielded a dark brown residue (2.5 %). 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 the concentration in mg/ml. The extract was used directly for the reducing power assay, hydrogen peroxide radical scavenging assay, the total flavanoid content estimation and for the determination of antibacterial activity.

Phytochemical evaluation

The dried methanolic extract was used to analyze qualitatively various phytoconstituents such as alkaloids, glycosides, steroids, saponins, phenolic compounds, tannins, flavanoids, carbohydrates and proteins using standard procedures²¹⁻²².

Estimation of flavanoid content using Swain and Hillis method (1959)²³

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. 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 phosphoglucinol. 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 turnings gave pink colour indicated the presence of flavanoids²⁴. The extract with dragendroff reagent gave reddish brown precipitate showed the presence of alkaloid.

Reducing power assay method²⁵

Reducing power of extract of *Mirabilis jalapa* Linn was determined by taking different concentrations of the plant extract. Ascorbic acid was used as reference standard. 50mg of methanol extract was dissolved in 50ml of methanol. From the above different concentrations (1, 2, 3, 4, 5ml) were pipetted out and made up to 10ml with methanol. Added 2.5ml of phosphate buffer 6.6 and 2.5ml of potassium ferricyanide to each of the test tubes and was incubated at 40°C for 20min. After incubation added 2.5ml of Trichloroacetic acid and centrifuged the reaction mixture for 5min. To 2.5ml of this reaction mixture added 0.5ml ferric chloride and 2.5ml water. Measured the absorbance using UV spectrophotometer at 700nm.

Hydrogen peroxide scavenging activity

The scavenging activity of extract towards Hydrogen peroxide radicals was determined by the method described by dehpour²⁶. Solution of hydrogen peroxide (40mM) was prepared in phosphate buffer pH 7.4. Concentration of hydrogen peroxide was determined by measuring the absorption at 230nm using a spectrophotometer. Extract 0.1mg/ml in distilled water were added to hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula

Percentage scavenged $[H_2O_2]$ = $1 - \text{Abs (standard)}/\text{Abs (control)} \times 100$.

Where, Abs control was the absorbance of the control (without extract) at 560 nm; Abs sample was the absorbance in the presence of the extract at 560 nm. The experiment was repeated in triplicate.

DETERMINATION OF ANTIBACTERIAL ACTIVITY - AGAR WELL DIFFUSION METHOD

Test Microorganisms used

Pathogenic microorganism selected for the study includes *Staphylococcus aureus*, *Pseudomonas* sp., *Bacillus* sp.

Agar well diffusion assay²⁷⁻²⁸

The antibacterial activity of the extracts was determined by the agar well diffusion method. Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 100 μ l of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. 5 mm well was cut using a cork borer on the surface of the inoculated agar. The plant extracts were filter sterilized using 25mm syringe filter, loaded into wells and were allowed to dry completely. The antibacterial activity was assessed by measuring the inhibition zone.

Determination of minimum inhibitory concentration²⁹

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to dilution to determine their minimum inhibitory concentration. 1mg, 2 mg and 3 mg (per ml) of the extracts were loaded into the wells and were allowed to dry completely. A well with standard (Chloramphenicol, 1mg/ml) was also prepared. Plates were incubated at 37°C for 24 h. MIC was determined by visual observation and the size of the zone was recorded.

Calculation of IC₅₀

Various concentrations of (100-500 μ g) of methanolic extracts of *M.jalapa* were taken for the study and IC₅₀ values which shows 50% inhibition was calculated using regression analysis in MS excel.

Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as average of three analyses \pm standard deviation. Statistical analyzes was performed by One Sample t-test. P values were done by One way ANOVA employing Graph Pad Prism Software 5 for Windows. The pvalue < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

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 tannins and phenolic compounds. The extract with 5ml 95% ethanol, few drops of concentrated HCl and 0.5g magnesium turnings gave pink colour indicated the presence of flavanoids. The extract with dragendroff reagent gave reddish brown precipitate showed the presence of alkaloids.

Total flavanoid content

The Flavonoid content was found to be 4.41 ± 0.02 mg /gram of dried extract equivalent to phloroglucinol (Figure1). The total flavanoid content shows good linear relation in both standard as well as sample extract.

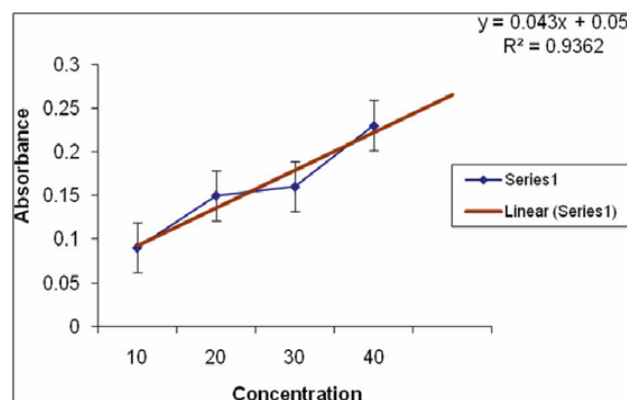


Fig 1: Quantitative estimation of flavanoids

Reducing power assay

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The reducing ability was found to increase with rising concentration in all the samples tested. The reducing power was determined from distinct colour change at 700nm, depending on the reducing power of the sample concentration. The methanolic extract of *Mirabilis jalapa* was found to show significant reductive property. The results are represented in Table 1 and figure 2 and 3. One sample t test were done to find out the Mean, SD and SEM. P values were done and found to be statistically significant ($p < 0.05$). Good correlation was shown between concentration and absorbance of standard and methanolic extracts (0.9378 and 0.9811) respectively.

Table 1. Results of Reducing power Assay

CONCENTRATION (µg/ml)	WAVELENGTH (nm)	ABSORBANCE(nm)	
		Methanol Extract±SD	Ascorbic Acid±SD
1	700	0.0626±0.001732	0.1020±0.000577
2	700	0.1260±0.0005774	0.1983±0.0008819
3	700	0.2600±0.002000	0.5240±0.01155
4	700	0.3023±0.002082	0.6817±0.0008819
5	700	0.4013±0.001528	0.7267±0.008819

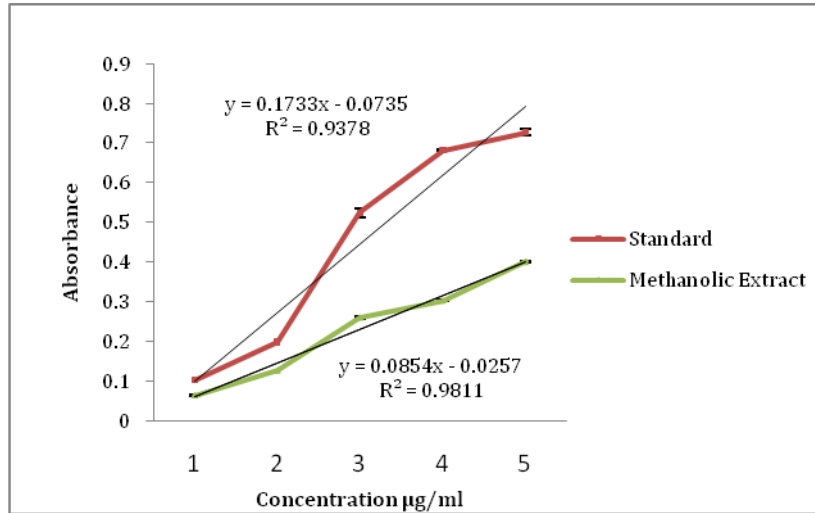


Fig 2: Reducing power of the methanolic extract of *Mirabilis jalapa* Linn.

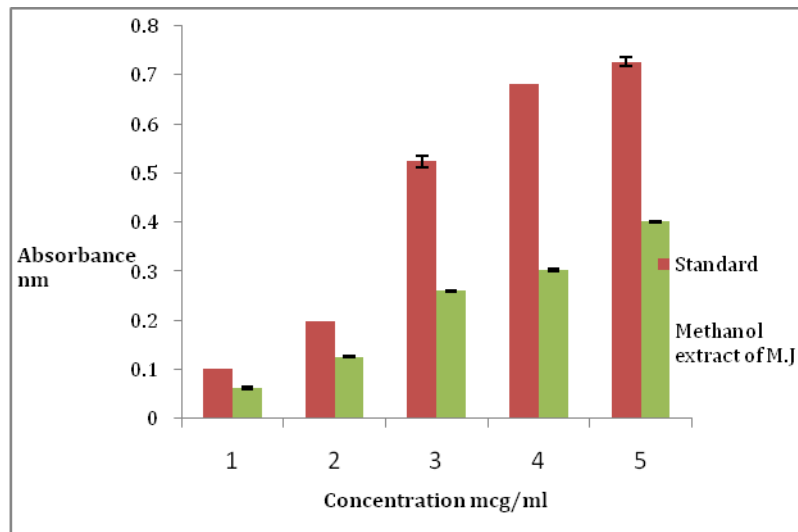


Fig 3: Reducing power assay

Hydrogen peroxide scavenging activity

Mirabilis jalapa extract also caused a moderate dose-dependent inhibition of hydrogen peroxide. The results are indicated in Table 2 and figure 3, 4 and 5. The IC₅₀ values for the extract was found to be

406 µg/ml. P values were found to be significant ($p < 0.05$) both for the standard and extracts. Regression coefficient (R^2 was found to be 0.966 and 0.9497) for the standard and methanolic extract respectively.

Table 2: Hydrogen peroxide scavenging activity

Concentration µG/ML	Percentage Inhibition Of Standard±SD	Percentage Inhibition Of Methanolic Extract±Sd
100	21±0.1	9.967±0.05774
200	25.13±0.1528	16.7±0.0000
300	39.97±0.05773	25±0.1
400	56±0.1	49±0.0000
500	62±0.0000	57.03±0.05773
IC ₅₀	347 µg/ml	406 µg/ml

Values are Mean ± SD (n=3) SD-Standard deviation. P

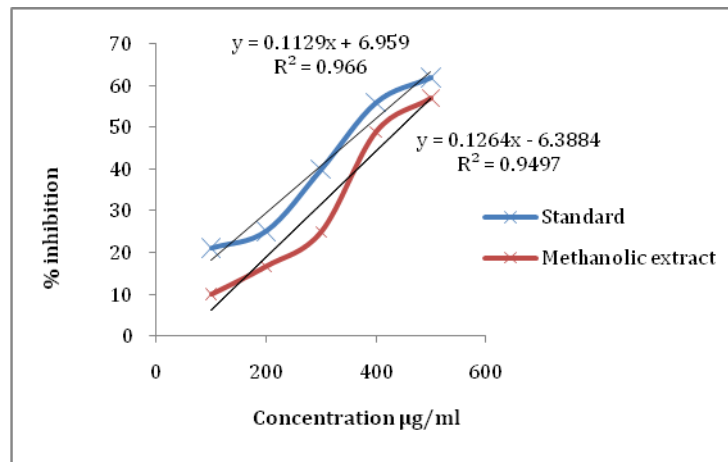


Fig 4: Hydrogen peroxide scavenging activity of the methanolic extract of *Mirabilis jalapa* Linn.

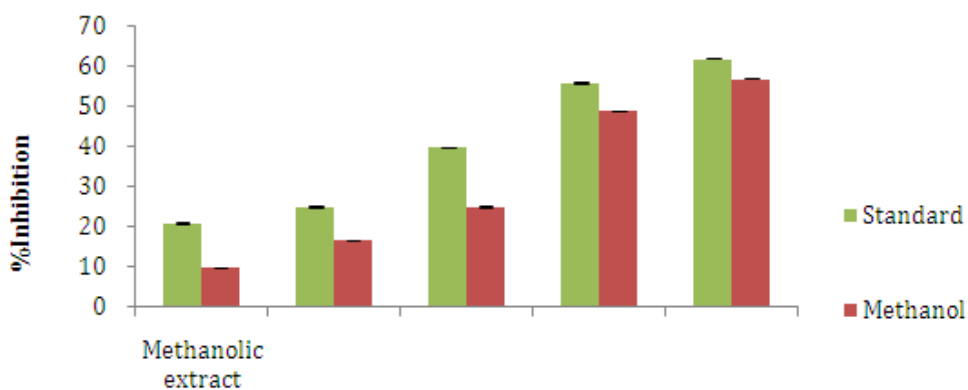
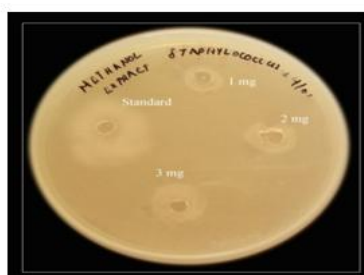


Fig 5: Hydrogen peroxide scavenging activity

Antibacterial activity

Antibacterial activity of *Mirabilis jalapa* was determined against gram positive and gram negative organisms. Chloramphenicol 1mg/ml were used as the reference standard and the activities were found to be concentration dependent for all different samples tested.

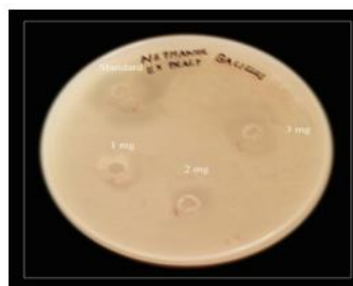
The methanolic extract of *Mirabilis jalapa* displayed significant activity against all organisms tested with minimum inhibitory concentration being 1mg/ml. The results of antibacterial activity are presented in Table 3. Zone of inhibition of the methanolic extract of *Mirabilis jalapa* Linn against organisms are shown in figure 6.



Staphylococcus aureus spp (17mm)



Pseudomonas spp (18mm)



Bacillus spp (16 mm)

Fig 6: Inhibition zone of methanolic extract of *Mirabilis jalapa* Linn against organisms

CONCLUSION

The present study reports for the first time the free radical scavenging activity of methanolic extracts of aerial parts by hydrogen peroxide radical scavenging method. The results of the present study established potent free radical scavenging activity through reducing power assay method and hydrogen peroxide scavenging assay method. This is due to presence of flavonoids, polyphenols like phenolic compounds and tannins. which may act in similar fashion as reductones by donating electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction. The separation and identification of these constituents present in the plant can help researchers find new molecules which can be used as natural antioxidants. In conclusion, the traditional claim of *Mirabilis jalapa* linn. as an anti bacterial have been confirmed as the methanolic extract displayed activity against the micro organism used in the study. Screening and proper evaluation of this plant could offer possible alternatives that may be both sustainable and environmentally acceptable.

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