Int J Pharm Bio Sci 2012 Oct; 3(4): (P) 440 - 446

Research Article **Natural Chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF RED FLOWERED *MIRABILIS JALAPA* LEAF IN DIFFERENT SOLVENTS

P. SELVAKUMAR[*1], DEVI KANIAKUMARI[2] AND V. LOGANATHAN[1]

[1]Department of Chemistry, Periyar University, Salem, Tamilnadu, India
[2]Department of Chemistry, Quaid-E-Millath Government College for Women, Chennai, Tamilnadu, India

ABSTRACT

The present phytochemical study investigates about red flowered *Mirabilis jalapa* leaf in different extracts using acetone, ethyl acetate, ethanol and petroleum ether solvents. From the crude extracts various parameters of pharmacognostical standards such as ash and extractive values, microscopical characters of leaf powder were determined. The village people of India use this plant to cure many infirmities including dysentery, diarrhea and muscular pain.

Further, the qualitative phytochemical screening of ethanolic extract of red flowered plant leaf reveals the presence of tannins, alkaloids, carbohydrates, terpenes and saponins. Moderate amount of glycosides were also observed. Acetone extract of plant leaf showed the presence of alkaloids, carbohydrates. Ethyl acetate extract of plant leaf showed the presence of alkaloids, carbohydrates and glycosides. Petroleum ether extract of plant leaf showed the presence of alkaloids. The research indicated clearly that red flowered *Mirabilis jalapa* plant has strong antibacterial potential and is active against a wide range of microorganisms. The observed antibacterial activities were believed to be due to the presence of phytochemical actives. We have further characterized some pharmacological properties of ethanolic extract of leaf. Therefore, we intend to contribute to understand the pharmacological effects and clarify the complex use of this medicinal plant. The results of the present study led us to the inference that the plant extract possesses modest medicinal and antioxidant properties. Further studies are necessary to identify the exact active compounds within the extract and to elucidate the mechanism of action.

KEYWORDS: ***Mirabilis jalapa*, Phytochemical screening, Ash value, Extractive value, Antibacterial activity, Antioxidant activity.**

P. SELVAKUMAR
Department of Chemistry, Periyar University, Salem, Tamilnadu, India

Corresponding author

INTRODUCTION

Mirabilis jalapa[1] belongs to the family Nyctaginaceae. It is known as Anthi-Mandhaari in Tamil, Naalumanipoovu in Malayalam, Gulabakshi in Marathi, GodhuliGopal in Assamese, Vieruurtjie in Afrikaans, Zi Mo Li in Chinese, Belle-De-Nuit in French, Belle Di Notte in Italian, Punkkot in Korean, Hoja De Xalapa in Spanish, Beauty of night, Four O' clock, Marvel of Peru in English. Four O'clock are leafy, shrub like, multibranched perennials that produce flowers all summer long. The plant is a tall herbaceous climbing plant with opposite leaves, large showy flowers, curvaceous obovoid fruits and prominent tuberous roots, and is planted as ornamental plant throughout the country. The flowers are used in food coloring. The leaves may be eaten cooked as well, but only as an emergency food. An edible crimson dye is obtained from the flowers to colour cakes and jellies. In herbal medicine, parts of the plant may be used as a diuretic, purgative, and for vulnerary (wound healing) purposes[2]. The root is believed to be an aphrodisiac as well as diuretic and purgative. It is used in the treatment of dropsy.

Mirabilis jalapa is used in traditional medicine by the people from different countries for the treatment of diarrhea, dysentery, conjunctivitis, edema, inflammation, swellings and muscular pain[3]. *Mirabilis jalapa* is widely used to treat dysentery, diarrhea, muscular pain, and abdominal colics in many countries[4] and its extract has antibacterial, antiviral, and antifungal functions[5].

Figure 1.0
Red flowered Mirabilis Jalapa

MATERIALS AND METHODS

Plant collection

Mirabilis jalapa red flower plant was collected at the peak of its flowering cycle in February from Coimbatore, South India and identified by personal interviews with the village people in Kovilpalayam. The flowers were removed, washed with water and dried under the shadow. The dried leaves were fine powdered and stored in polythene bags at room temperature (30±2ºC) until use.

Sample preparation and extraction procedure

Fine powder (250 g) of the sample was weighed into 500 mL of ethanol (95%) in a conical flask. This was covered, shaken every

30 min. for 6 h and then allowed to stand for about 48 h. The solution was subsequently shaken and filtered using Whatman filter paper. The extract was concentrated gently in a water bath at 60°C and stored in a desiccator until analysis. It was stored in refrigerator in amber colored bottle to avoid degradation. Acetone, ethyl acetate, petroleum ether solvent extracts were also prepared and stored in the same ways.

PHYTOCHEMICAL SCREENING

Phytochemical screenings were performed using standard procedures for acetone extract, ethyl acetate extract, ethanol extract and petroleum ether extracts[6].

Tests for alkaloids

To 0.5 g of each extract, was added to 10% tannic acid solution in a test tube. The solution was shaken vigorously and observed for the formation of a turbidity or precipitation.

Test for carbohydrates-Molisch test

Small portion of each extract was dissolved in 5 mL of distilled water and filtered. To this solution, three drops of *α*-naphthol was added and 1mLof con. sulphuric acid was added along the sides of inclined test tube so as to form two layers and observed for formation of violet coloured ring at the interface to detect the presence of carbohydrates.

Test for tannins

Small portion of each extract (0.5 g) was taken in different boiling tubes and boiled with 20 mL of distilled water. To the filtrate few drops of 0.1% ferric chloride was added, mixed well and allowed to stand for few seconds. The solution was observed for brownish green or a blure-black color.

Test for terpenes

Small portion of each of the decolorized extract residue (0.5 g) was taken in different tubes. To this 10 mL of chloroform, 0.5 mL of acetic anhydride and few drops conc. H_2SO_4 were added, mixed well and allowed to stand for few seconds. The solution was observed for brown precipitate.

Tests for saponins

To 0.5 g of extract, was added 5 mL of distilled water in different test tubes. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Tests for glycosides-keller-killani test

Small portion of each 0.5 g of extract was taken and subjected to the following test. Glacial acetic acid (1 mL) containing traces of ferric chloride and 1 mL of con. sulphuric acid were added to extract and observed for the formation of reddish brown color at the junction of two layers. The upper layer would turn bluish green if glycosides are present.

The results are summarized in Table 1.

PHYSICO-CHEMICAL ANALYSIS

Ethanolic extract alone was taken and subjected to the following tests. Ash values such as total ash, acid insoluble ash and water soluble ash; extractive values such as water soluble, alcohol soluble and ether soluble extractive values were determined using the powdered leaf of ethanolic extract according to Indian Pharmacopoeia and the results are shown in Table 2.

ANTIBACTERIAL ACTIVITY

Agar well diffusion method

Antibacterial activity of the acetone, ethyl acetate, petroleum ether and ethanol extracts of leaves of were tested using agar well diffusion method[7]. A loop full of culture was inoculated into peptone broth and incubated for 2 to 5 h at 35°C until it achieved the turbidity of 0.5 McFarland's standard. The test cultures were swabbed on nutrient agar plates, within 15 min after adjusting the turbidity of the inoculum suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removed the excess inoculum from the swab.

The dried surface of a nutrient agar plate was inoculated by streaking the swab and the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed and wells were made using the sterile well puncture.

Different concentrations (200 to 1000 µg) of the sterile acetone, ethyl acetate, petroleum ether and ethanol extracts were added to each well. The plates were incubated in an upright position at 37°C for 24 h. The diameters of inhibition zones were measured in mm and the results are recorded in Table 3.

ANTIOXIDANT ACTIVITY

FRAP assay

Total antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) assay [8-12]. The principle of this method is based on the reduction of a ferrictripyridyltriazine complex to its ferrous complex in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 mL of 10 mmol/L TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3.6H_2O$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.7) and was prepared freshly and warmed at 37°C. Working FRAP reagent (2900 µL) is mixed with 100 µL of each extract and the mixtures were allowed to react for 30 min in dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm using spectrophotometer. The final result was expressed as concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$. Results are expressed in µM Trolox equivalents (TE)/g fresh mass and are tabulated in Table 4.

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical active compounds of leaf were qualitatively analyzed and the results are presented in Table 1. The data indicate that the ethanolic extract of leaf showed the presence of phytochemical active[13] compounds such as tannins, glycosides, saponins, carbohydrates, alkaloids and terpenes. Acetone extract tested positive for the presence of only alkaloids and carbohydrates. Petroleum ether extract contained only alkaloids. Alkaloids, carbohydrates, tannins[14], saponins, glycosides were present in the ethyl acetate extract.

Table 1

Preliminary phytochemical screening of various extracts of leaf of M. jalapa

S.No	Phytoconstituents	Ethanol	Acetone	Petroleum ether	Ethyl Acetate
1	Alkaloids	+++	+++	++	+++
2	carbohydrates	+++	+++	-	+++
3	Tannins	+++	-	-	+
4	Terpenes	+++	-	-	-
5	Saponins	+++	-	-	+
6	Glycosides	++	+	-	+++

"+++" = Highly present; "++" = moderately present; "+" =Present; "-" = absent.

Physico-Chemical Analysis

Ash values such as total ash, acid insoluble ash and water soluble ash; extractive values such as water soluble, alcohol soluble and ether soluble extractive values were determined using the powdered leaf ethanolic extract according to Indian Pharmacopoeia. The results are shown in Table 2. Ash value is useful in determining authenticity and purity of drug and also these values are important

quantitative standards. Total ash value of plant material indicated the amount of minerals, and earthy materials present in the plant material. Analytical results showed the total ash value as 15.15% w/w. The water-soluble extractive value indicated the presence of sugar, acids, and inorganic compounds. The alcohol soluble extractive values indicated the presence of polar constituents.

Table 2
Microscopical characters of ethanolic extracts of leaf of M. jalapa

S.No	Physico-Chemical parameters	Percentage (w/w)
1	Total ash value	15.15
2	Acid insoluble ash value	4.57
3	Water soluble ash value	3.75
4	Water soluble extractive value	26.22
5	Alcohol soluble extractive value	21.81
6	Ether soluble extractive value	24.94

Antibacterial activity

In vitro antibacterial activities[15] of leaves of *M. jalapa* have been investigated against biofilm producing Uropathogenic *E. coli* (UPEC) and the results are shown in Table 3. The biofilm producing strains employed for the antimicrobial activity include UPEC 1, UPEC 17, UPEC 57 and UPEC 82. The organisms are selected based on the resistance pattern exhibited against the antibiotics used to treat urinary tract infection caused by *E. coli.* The acetone extract exhibited a zone of inhibition of 22, 20 and 17 mm respectively against biofilm producing strains UPEC 1, UPEC 17 and UPEC 82. The petroleum ether extract exhibited a zone of inhibition of 18 and 15 mm respectively against biofilm producing strains UPEC 1 and UPEC 17. The ethyl acetate extract exhibited a zone of inhibition of 20, 19 and 21 mm respectively against biofilm producing strains UPEC 1, UPEC 17 and UPEC 82.

Table 3
Antimicrobial activity of different extracts of leaves by well diffusion method against biofilm producing uropathogenic E. coli

S.No	Solvents	UPEC 1	UPEC 17	UPEC 57	UPEC 82
1	Ethanol	23	20	21	21
2	Acetone	22	20	-	17
3	Petroleum ether	18	15	-	-
4	Ethyl Acetate	20	19	-	21

UPEC= Uropathogenic E. coli, '-' indicates no significant zone of inhibition

Antioxidant activity

Antioxidant activities of different solvent extracts as determined by the FRAP assays are furnished in Table 4. Ethanolic extract showed more antioxidant potential compared to all other extracts used in this study. The antioxidant activity of each extract using FRAP assay was measured three times to test the reproducibility of the assays.

Table 4
Evaluation of antioxidant activity of crude solvent extract of the leaf of M. jalapa

S.No	Solvent used for extraction	Antioxidant activity (µM (TE)/g fresh mass)
1	Ethanol	11.5
2	Acetone	1.1
3	Petroleum ether	0.12
4	Ethyl Acetate	5.2

CONCLUSION

The results of the present study led us to the inference that the plant extract possess modest medicinal and antioxidant properties. Although the extract is reported to contain a range of phytochemical components, it is difficult to ascribe these observed activities to any specific group of compounds. The observed antibacterial activities were believed to be due to the presence of phytochemical actives like alkaloids, carbohydrates, tannins, terpenes, saponins and glycosides identified in different solvent extracts in the present investigation. The results of this study showed that the leaves exhibited varied antimicrobial activities against the biofilm producing Uropathogenic *E. coli*. Total ash value indicated the amount of minerals and earthy materials present in the plant material. Analytical results showed the total ash value to be 15.15% w/w. The water-soluble extractive value indicated the presence of sugar, acids, and inorganic compounds. The alcohol soluble extractive value indicated the presence of polar constituents. Further study is necessary for isolation and characterization of the active antioxidants, which can be used to treat various oxidative stress-related diseases.

REFERENCES

1. Kayser O., Quax W.J. Eds., Medicinal plant biotechnology: from basic research to industrial applications. Weinheim, Wiley-VCH, (2007)
2. Yoganarasimhan S.N., Medicinal Plants of India, Bangalore, Tamilnadu Regional Research Institute,; 2: 357, (2000)
3. Daniel M., Medicinal plants chemistry and properties. Science Publishers, Enfield, NH, U.S.A. pp.107, (2006)
4. Holdsworth D,K., A preliminary study of medicinal plants of Easter Island, South Pacific. Int J Pharmacognosy, 30: 27-32, (1992)
5. Oladunmoye M,K., Comparative Evaluation of Antimicrobial Activities of Leaf Extract of Mirabilis jalapa. Trends Med Res, 2 (2):108-112, (2007)
6. Soforowa E,A., Medicinal plants and traditional medicine in Africa. John Wiley and Sons, Chichester, pp198, (1982)
7. Oskay M., Sari D., Antimicrobial screening of some Turkish medicinal plants. Pharmaceut Biol, 45:176-181, (2007)
8. Rumzhum N.N., Rahman M.M., Islam M.S., Chowdhury S.A., Sultana R., Parvin M.N., Cytotoxicity and antioxidant activity of extractives from Mirabilis jalapa, J Pharm Sci, 1(1,2): 85-88, (2008)
9. Benzie, F.F., and Strain, J.J., Ferric reducing/ antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified versionfor simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 299:15-23, (1999)

10. Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., Jiang, Y., Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research 23, 1719–1726, (2003)
11. Jimenez-Escrig, A., Rincon, M., Pulido, R., Saura-Calixto, F., Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. Journal of Agricultural and Food Chemistry 49,5489–5493, (2001)
12. Alagumani vasagam. G., Kottai muthu.A., Manavalan.R., In-vitro antioxidant potential of tuberous root of methanolic extract of *ipomoea digitata (linn.),* Int J Pharm Bio Sci V1(2) 2010
13. Chibale K., Economic drug discovery and rational medicinal chemistry for tropical diseases. J P Appl Chem, 77(11): 1957-1964, (2005)
14. Lü L., Liu S.W., Jiang S.B., Wu S.G., Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacol Sin, 25 (2):213-218, (2004)
15. Akujobi C., Anyanwu B.N., Onyeze C., Ibekwe V.I., Anibacterial activities and preliminary phytochemical screening of four medicinal plants. J Appl Sci 7(3): 4328-4338, (2004)