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SCREENING OF BIOACTIVE COMPOUNDS AND STRUCTURAL ELUCIDATION FROM LEAVES OF *MIRABILIS JALAPA* L.

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ABSTRACT

The present investigation has been carried out to find the phytochemicals present in the leaves of *M.jalapa*. A preliminary phytochemical testing of the leaves extract where than to identify the phytoconstuents, which reveals that present of tannin, saponin, flovonoids, steroids, terepenoids, triterpenoid, carbohydrate, anthroquinone polyphenol, glycoside bioactive compound was isolated by column chromatography technique. The collected flovonoid fractions was purified by thin layer chromatography. FTIR and NMR studies were carried out to find the structure of bioactive compound 3,3',4',5,7-pentahydroxy flavones-3-rutinoside(Rutin).^{1}H- NMR and ^{13}C – NMR that reveals the structure of flavonoids. The compound was identified as 3,3',4',5,7- pentahydroxy flavones-3- rutinoside(Rutin) by ^{1}H- NMR and ^{13}C – NMR.

KEYWORDS: Phytochemicals, *Mirabilis jalapa* L, FTIR and NMR studies.

INTRODUCTION

Research regarding medicinal plant is a highlighted issue today. Medicinal plants are the nature's gift of human being to make disease free healthy life. It plays a vital role to preserve our health. Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Dahanukar, 2000).

Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants. It is estimated that there are more than 250,000 plant species. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Purification and isolation of bioactive compounds from plants is a technique that has undergone new development in recent years. This modern technique offers the ability to parallel the development and availability of many advanced bioassays on the one hand, and provided precise techniques of isolation, separation, and purification on the other. The goal when searching for bioactive compounds is to find an appropriate method that can screen the source material for bioactivity such as keeping in view, the present study was understand to isolation, identification and characterization of active compounds from *M. jalapa* leaves.

MATERIALS AND METHODS

Collection of Plant materials

The leaves of *Mirabilis jalapa* L. were collected in January 2018 from, Thanjavur, Tamil Nadu, India.

Preparation of alcoholic extract

The leaves of *M. jalapa* were first washed and dust was removed. The leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with aqueous and 70% methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Quantitative assay

Determination of total phenols by spectrophotometric method. Flavonoid determination by the method of Bohm and Kocipai-Abyazan (1994).

Column Chromatography

Separation of flavonoid compound using Column Chromatography adopted by the method (Javed Intekhab and Mohammad Aslam, 2009). The column of about 15cm long and 4cm in diameter was thoroughly washed with detergent, rinsed with distilled water and then allowed to dry. When the column has been fully dried, small piece of glass wool was inserted into the lower part of the column and the column was supported using a clap and retort stand. A funnel is attached to the open end and little clean white sand was poured on top of the glass wool already inserted, after this some quantity of the solvent was poured down the column. The silica gel (40micron) used was activated in the oven at 120 ^{0}C for 2hours. A well-stirred suspension of silica gel (100–150 g in petroleum ether at 60°C–80°C was poured into a column (150 cm long and 50 mm in diameter). When the absorbent was well settled, the excess of petrol-ether was allowed to pass through the column. The slurry was passed through the silica gel in petrol-ether and was digested to well stirred column. Gradual setting was arranged by maintaining a gentle agitation while there was solvent flow through the column in order to obtain a homogenous packing. The ethyl acetate (EtOAc) extract sample was mixed with silica gel slurry and applied to the top of the column as evenly as possible and distortion of the column packing avoided as this would lead to distorted bands. The column was successively eluted with EtOAc, hexane, chloroform, methanol and their mixtures of increasing polarity. Elution with $CHCl_{3:}$ MeOH (7:1) afforded a yellow colour. The eluted solution further tested in Shinoda test to give a pink or orange or red to purple colour.

Thin layer chromatography

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic Fig.5 Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces (Harborne, 1984, 1973). A thin-layered Fig.5 is prepared by spreading aqueous slurry of Silica gel G on the clean surface of a glass or rigid plastic. Calcium carbonate or starch is also added to the adsorbent to increase adhesion. The Fig.5 is then heated in an oven for about 30 mins at 105°C to activate the Fig.5 It is then cooled inside the oven itself. Test samples were applied in the form of spots using capillary tube. The choice of solvents depends upon the nature of compound to be separated and also on the adsorbent used. The solvent is poured

into the chamber and closed tightly and the chamber is saturated for a few hours before running the chromatogram.

The fraction was drawn with capillary tubes and applied as spots on a stationary phase (silica-gel coated) about 1 cm from the base. The plate was then dipped into a suitable solvent system (n-Butanol, acetic acid and water (4:1:5). The solvent migrates up to the top. As the solvent rising through thin layer separates different components of the mixture at different rates which appear as spots in the thin layer. After the solvent has reached almost the top edge of the Fig.5 nearly 3/4th of the top is removed from the tank and dried briefly at moderate temperatures 60-120°C. The presences of secondary metabolites in the extracts were detected by TLC using suitable spraying reagents. The presence of flavonoid was detected by the formation of yellow colour spot in the Fig.5 a positive reaction by exposure of ammonia (Adam *et al*., 2002).

Rf Value

It is a ratio of distance travelled by the sample and distance travelled by the solvent.

$$R_f = \frac{\text{Distance of the sample (solute) from the origin}}{\text{Distance of the solvent from origin}}$$

Fourier Transform Infrared (FTIR) spectroscopic analysis

FTIR spectrophotometer (Perkin Elmer Spectrophotometer system, USA) used to investigation of spectrum. A small amount of liquid of plant sample was respectively placed directly on sample holder of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 cm^{-1} to 400 cm^{-1} and computerized for analyses by using the 21 CFR part 11 software. The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. The peak values of FTIR were recorded.

NMR Spectroscopy

After the separation of plant extract to fractions using Column chromatography, Thin Layer chromatography was used for further purification of collected fraction. The NMR experiment was carried out in BRUKER-AMX400 MHz instrument with 5mg of purified compound in DMSO were used for ^{1}H NMR and ^{13}C NMR spectra recorded. Tetra Methyl Silane is used as the internal standard and chemical shifts are expressed in ppm.

RESULTS AND DISCUSSION

The present study was carried out on the revealed the presence of medicinally active constituents. The phytochemical characters of the *Mirabilis jalapa* leaves investigated and summarized in Table-1 and fig-2 The phytochemical screening of methanol extract of *M. jalapa* leaves showed that the presence of flavonoids, tannin, terepenoids, steroids, saponins, triterpenoids, phenolics, carbohydrate, anthriquinone and glycosides while phlobatannins, alkaloids and protein were absent.

Table 1: Phytochemical screening leaves of *Mirabilis jalapa* L.

S. No.	Phytochemical analysis	Methanol extract	Quantitative analysis (mg/gm)
1	Tannin	+	
2	Phlobatannins	-	
3	Saponin	+	
4	Flavonoids	+	140
5	Steroids	+	
6	Terepenoids	+	
7	Triterpenoids	+	
8	Alkaloids	-	
9	Carbohydrate	+	
10	Protein	-	
11	Anthroquinone	+	
12	Polyphenol	+	230
13	Glycoside	+	

(+) Presence, (++) highly presence and (-) Absence

Chromatographic Separation

Column chromatography of *M. jalapa* leaves extract afforded 5 fractions. The result of the chromatographic separation is given in table 2.

Table 2: Separation of fractions from column chromatography.

S. No.	Eluents	Number of fraction(s)	Nature of fractions
1.	Hexane	01	White
2.	Chloroform	01	White
3.	Ethyl acetate	01	White
4.	Methanol	01	Light green
5.	$CHCl_3$:MeOH (7:1)	01	Greenish Yellow

Table 3: Analysis of flavonoid by TLC.

Phytoconstituents	Rf Value	Results	Literature (Gordana, 2003)
Flavonoid	8.2/9.4	0.85	Flavonoid derivatives (Rf = 0.90)

Fig. 3: Column chromatography Setup.

Fig. 4: Collection of fractions.

Fourier Transform Infra-Red Spectroscopy analysis of *Mirabilis jalapa* leaves extract

The FTIR spectrum of the *M. jalapa* leaf extract was pronounced absorbance was recorded in the region between 4000 and 400 cm^{-1}. The peak 3404.72 indicates alcoholic and phenolic groups, 2977.22 indicates alkenes (C-H strech stretch), 2901.97 indictes alkenes (C-H stretch) and 2541.22 indicates carboxylic acids (O-H strech), 2131.55 indictes alkenes (–C=C– stretch) and 1649.61 indicates alkynes (C=C– stretch), 1452.26 indictes aromatics and alkenes (C-C stretch (in-ring) and C-H bend) 1407.35 indicates aromatics (C-C stretch (in-ring), 1254.26 indicates aromatic amines (C-N stretch), 1331.97 indicates nitro compounds (N-O symmetric stretch), 1080.25 indicates alkynes C-O stretch and C-N stretch), 1049.33 indicates aliphatic amines C-N stretch) 880.89 indicates $1,^{o}$ 2^{o} amines (N-H wag).

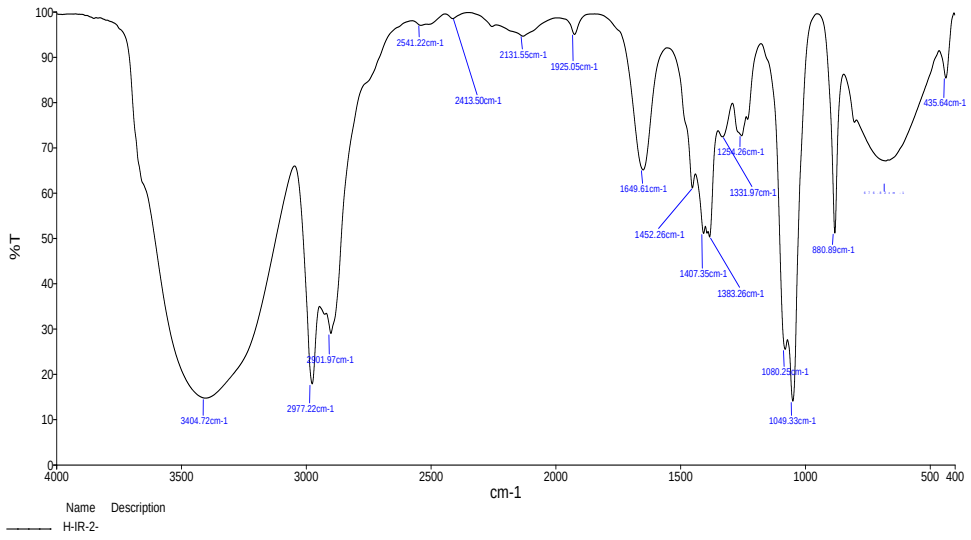


Table 6: FTIR Peak Values of Extract of leaves of *Mirabilis jalapa.*

Peak Value	Bond	Functional group
3404.72	O-H stretch, free hydroxyl	Alcohols, phenols
2977.22	C-H strech	Alkenes
2901.97	C-H strech	Alkenes
2541.22	O-H strech	Carboxylic acids
2131.55	-C=C- strech	Alkynes
1649.61	-C=C- strech	Alkenes
1452.26	C-C stretch (in-ring) C-H bend	Aromatics Alkanes
1407.35	C-C stretch (in-ring)	Aromatics
1254.26	C-N stretch	Aromatic amines
1331.97	N-O symmetric stretch	Nitro compounds
1080.25	C-O stretch C-N stretch	Alcohols, Carboxylic acids, esters, ethers Aliphatic amines
1049.33	C-N stretch	Aliphatic amines

NMR spectrum of *Mirabilis jalapa* leaves extract

^{1}H-NMR spectrum

^{1}H-NMR spectrum of rutin was compared to^{1}H-NMR spectrum of rutin-standard. In ^{1}H-NMR spectrum (400MHz, DMSO) the aromatic protons produced signals at C-6 and C-8 appear as δ 6.13 and δ 6.40ppm respectively. Thus, in the rutin spectrum could be seen some supplementary signals in the areas: 3.38-3.56 (m,12H of ramnose moieties), 3.81(d, J=1.15Hz, 1H-glucose), 1.12(3H, d, J=6Hz, CH3-Rham), 4.52(4H, d, J=7.8Hz, H-1 Ramnose), 5.13 (1H, d, J=2Hz H-6-glucose) (Fig 4).

^{13}C -NMR spectrum

Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C-NMR data and a complete assignment is given in Table 1. ^{13}CNMR (400MHz, DMSO) δppm=158.5 (C-2), 135.7 (C-3), 179.4 (C-4), 163.069 (C-5,) 100 (C-6), 166.09 (C-7),.09 (C-8), 158.50 (C-9,) 105.6 (C-10,) 123.7 (C-1’), 117.8 (C-2’), 145.8 (C-3’), 149.9 (C-4’) 116.1 (C-5’), 123.2 (C-6’), 104.7 (C-1’’) 75.7 (C-2’’), 77.1 (C-3’’), 71.5 (C-4’’), 78.2 (C-5’’) 68.7 (C-6’’), 102.4 (C-1’’’), 72.3 (C-2’’’), 72.2 (C-3’’’) 74.10 (C-4’’’), 69.7 (C-5’’’), 18.10 (C-6’’’) (Şerban Georgeta *et al*., 2016) (Table 5 and Fig 5).

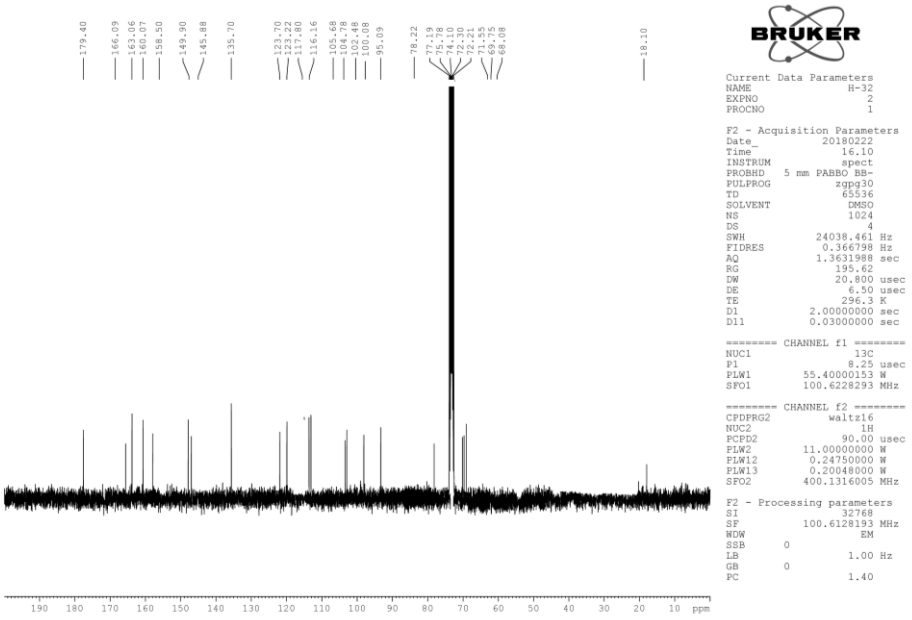


Fig. 7: ^{13}C -NMR spectrum *Mirabilis jalapa* leaves extract.

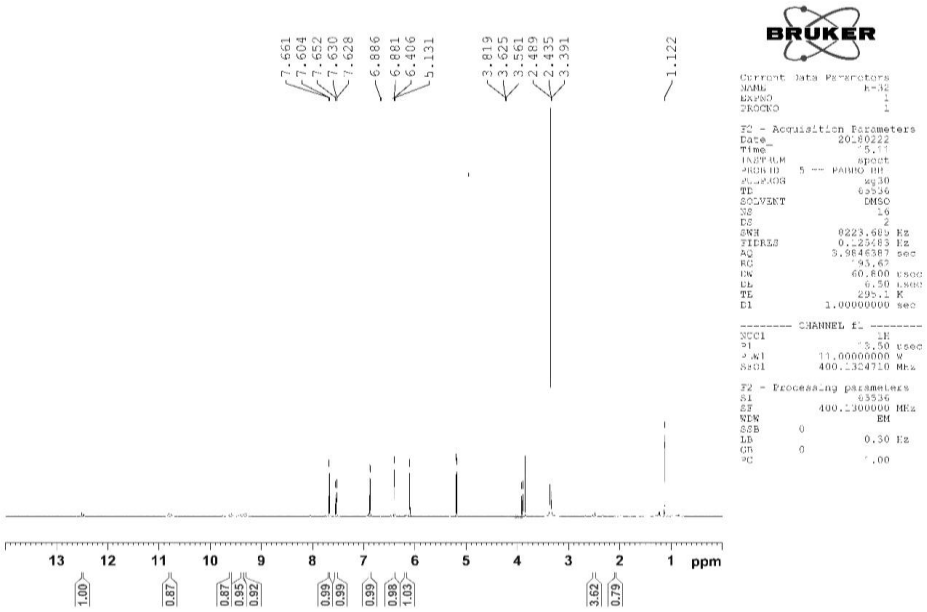


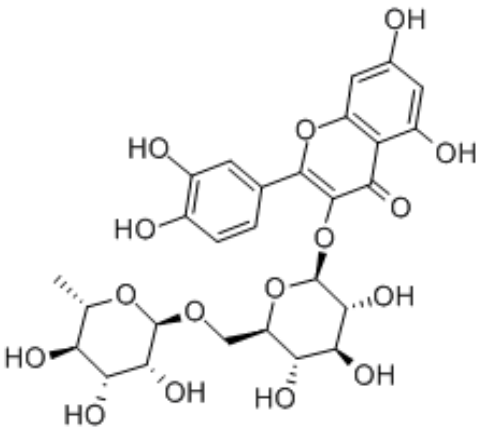
Fig 8: ^{1}H -NMR spectrum *Mirabilis jalapa* leaves extract.

Table 5: ^{13}C-NMR Data and Their Assignment for Flavonoid Derivative.

Carbon	Literature*	Reference Rutin Standard*	*Mirabilis jalapa* extract
2	158.50	158.50	158.50
3	135.70	135.60	135. 70
4	179.40	179.40	179.40
5	163.00	162.50	163.06
6	100.00	99.90	100.00
7	166.0	166.0	166.09
8	95.0	94.8	95.09
9	159.40	159.30	158.50
10	105.70	105.6	105.68
1'	123.70	123.10	123.70
2'	117.80	117.6	117.80
3'	145.90	145.8	145.88
4'	149.90	149.7	149.90
5'	116.10	116.1	116.16
6'	123.20	123.5	123.22
Glucose 1"	104.748	104.7	104.78
2"	75.780	75.7	75.78
3"	77.20	77.2	77.19
4"	71.50	71.4	71.55
5"	78.20	78.1	78.22
6"	68.60	68.6	68.75
Rhamnose 1"'	102.50	102.4	102.48
2"'	72.30	72.0	72.30
3"'	72.20	72.20	72.21
4"'	74.00	73.9	74.10
5"'	69.80	69.7	69.75
6"'	18.00	17.9	18.10

Finding compound

Based on this data has been characterized as It was characterized as 3, 3',4', 5, 7-pentahydroxy flavones-3-rutinoside (Rutin).



3, 3',4', 5, 7-pentahydroxy flavones-3-rutinoside (Rutin) (Molecular Formula: $C_{27}H_{30}O_{16}$).

CONCLUSION

The present investigation has been carried out to find the phytochemicals present in the leaves of *M.jalapa*. A preliminary phytochemical testing of the leaves extract where than to identify the phytoconstuents, which reveals that present of tannin, saponin, flovonoids, steroids, terepenoids, triterpenoid, carbohydrate, anthroquinone polyphenol, glycoside bioactive compound was isolated by column chromatography technique. The collected flovonoid fractions was purified by thin layer chromatography. FTIR and NMR studies were carried out to find the structure of bioactive compound 3,3',4',5,7-pentahydroxy flavones-3-rutinoside(Rutin).^{1}H- NMR and ^{13}C – NMR that reveals the structure of flavonoids. The compound was identified as 3,3',4',5,7- pentahydroxy flavones-3- rutinoside(Rutin) by ^{1}H-NMR and ^{13}C – NMR.

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