Isolation of Chemical Constituents and *In vitro* Antidiabetic Activity of Mirabilis Jalapa Flower Extracts

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

**Objectives:** The present work was designed to investigate the phytochemical and *in vitro* screening of the flower extracts of *Mirabilis jalapa*.

**Materials and Methods:** Phytochemical constituents were isolated using column chromatography and characterized of the compounds were carried out using IR, $^1$H NMR, $^{13}$C NMR and mass spectroscopy data. *In vitro* anti-diabetic activity was evaluated by alpha-amylase inhibition assay and Alpha-glucosidase inhibition assay.

**Results:** Phytochemical investigation of the ethanol extract revealed the presence of a flavonoid Kaempferol and a triterpenoid Lupeol. *In vitro* investigation indicated that the *Mirabilis jalapa* flowers extracts has considerable anti-diabetic activity

**Conclusion:** The anti-diabetic activity may due to the presence of phytoconstituents like flavonoids, triterpenoids, steroids.

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1. INTRODUCTION

Utilization of plants as medicines is the earliest remedies known to human. Phytotherapy is the utilization of any of the plant parts to either cure or prevent diseases. Ayurveda and Chinese traditional medicine are the two main systems which utilize herbal medicines as remedies. Ayurveda has historical significance in India. Ayurveda is the medical system involving extensive use of herbs. Herbal medical system is considered as fundamental framework for the modern pharmaceutical drug regimen. In olden days medicines derived from plants were used for treatment, there was no allopathic system of medications. Once the allopathic system was developed the popularity of herbal medicines decreased due to the rapid action of drugs. Demand for plant based medicines have increased in last two decades due to limitations of allopathic medicines [1]. About 80% people in developing countries still depend upon the traditional herbs. According to WHO more than 65% of world’s population have incorporated herbal medicines in health care. More than 25% of prescribed drugs contain plant based APIs. This includes its analogues also. Almost 70% of medicines used in India are derived from plants [2].

Herbal drugs mainly include whole part of the plant. Herbal drugs have many advantages like minimum side effects, high approachability, high potency etc. Medicinal plant resources act as the sources of new lead compound. It has long been recognized that natural product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favorable as lead structures of drug discovery. Each plant act as a substances based on which new chemical moieties are designed [3]. Natural products have the ability to cross the biological barriers hence they can easily penetrate the cells. The pharmacokinetic properties are also favorable which makes them a good leads. Even though the modern medicines have improved a lot in recent decades herbal medicines are also contributing in its own way.

**Mirabilis jalapa** (Family: Nyctaginaceae) commonly known as four "o" clock plant, as introduced as an ornamental plant [4]. It acts as emetic and purgative”. It can also be used to treat viral infection, microbial infections, malaria, helminthiasis and it can also be used as antioxidant agent [5]. For centuries it was used as antidiabetic in China. Root and leaf infusions were used in the treatment of inflammation of skin as topically. In vitro antioxidant property was found in extract [6]. The research carried out on the medicinal herbs in last few decades has revealed the use of herbal anti-diabetic agents in the traditional medicinal system. The study on the natural product may act as a driving force to achieve this goal. The present study was designed to evaluate the phytoconstituents and in vitro anti-diabetic activity of *Mirabilis jalapa* flower extracts.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The flora of Mirabilis jalapa were gathered from in and around Mangalore, Dakshina Kannada district, in the month of June - August 2019. The plant was authenticated by Dr. Krishna Kumar G, Professor, Department of Applied Botany, Mangalore University.

2.2 Preparation of Extracts

The flowers were washed, cleaned; dried under shade and using mechanical grinder dried flowers were powdered. Such coarse powdered flower was used for extraction. Cold maceration and infusion were performed for extraction [7,8]. Ethanol and water are used as solvents. Cold maceration method was done by using ethanol as a solvent. The powder (100g) was kept for maceration with ethanol for 7 days. Filtration was carried out using muslin cloth. The filtrate was then distilled to concentrate the extract and also recover the solvent. The solvent recovered was utilized for further extraction. The obtained concentrated extract was further concentrated by evaporating in water bath. Infusion method was carried out by boiling the solvent and placing the floral powder in the hot solvent for definite time. Distilled water was used as solvent in infusion. With the help of muslin cloth extracts were filtered. The marc was dried and filtrate was evaporated in the water bath. Both the crude extracts were then kept in the desiccator for further use. Chemical tests were employed for preliminary phytochemical analysis by standard procedures [3,10].

**Keywords:** *Mirabilis jalapa*; *Nyctaginaceae*; *Kaempferol*; *Lupeol*; anti-diabetic; alpha-amylase; alpha-glucosidase; acarbose.
2.3 Fractionation and Isolation

The ethanolic extract obtained by the maceration process was suspended in distilled water (10g in 10ml) and fractionated using different solvents like diethyl ether, chloroform, Petroleum ether, and methanol. The yields obtained in each solvent were in trace amount hence couldn’t process further.

2.4 Isolation of Compound 1

Using solvent petroleum ether the powdered flower material was defatted. Further this was extracted using ethanol. The dried extract (5g) was dissolved in the small volume of methanol to prepare slurry and was loaded into a silica gel column (150g) saturated by ethyl acetate. The column was eluted with 100% ethyl acetate followed by graded mixture of 10%, 20%, methanol in ethyl acetate, finally up to 100% methanol. Elutes of the different fraction were continuously watched by TLC (Silica Gel G; Ethyl acetate: Methanol and visualized by UV/NH₃ 20:80, 30:70 and 10:90 (ethyl acetate: methanol) fractions showed similar spots. On concentration of these elutes yellow coloured mass was obtained which was recrystallized using methanol the residue obtained was termed as MJ1 (1.02g). MJ1 gave orange colour with shinoda’s test. The elutes obtained from other fractions produced a brown resinous material that was not further processed.

2.4.1 Hydrolysis of compound I

To the solution of the MJ1 (15mg) an equal volume of 2N HCl was added. The mixture was refluxed at 100°C for 2hrs and then evaporated to dryness under reduced pressure. After the addition of the distilled water (6 ml) it was extracted with ether and concentrated. The concentrated product was then dried over anhydrous Na₂SO₄ to deposit a yellow solid with melting point of 175-179°C.

2.5 Isolation of Compound II

The ethanolic extract (10g) of the powdered flower was saponified with 20% Alc.KOH for 2hrs. The unsaponified portion was further extracted using diethyl ether. The ethereal portion was evaporated to obtain the yellow residue (2.5g). This yellow residue was dissolved in chloroform and loaded into the chromatographic column containing neutral alumina (150g) initially saturated with petroleum ether. The column was eluted with petroleum ether 100% initially and gradual decreases 90%, 80% etc. with the increase in the concentration of benzene. After that the chloroform, benzene mixture was also examined in similar manner. The elutions obtained were further examined using TLC (chloroform: benzene, 70:30, silica gel G visualization: vanillin/sulphuric acid heated at 110°C). The elutes obtained from the 70:30 of chloroform: benzene fraction gave a good spot. These elutes were further concentrated to yield a residue. The residue was further recrystallized using benzene. Light green coloured (0.72g) product was obtained which was labeled as MJ2 and it has showed green colour for Liebermann burchard test.

2.6 Screening of Anti-diabetic Activity

2.6.1 Alpha Amylase inhibition assay [11]

Alpha-amyrase solution (0.5µL/ml) prepared using sodium phosphate buffer (pH-6.9) and plant extracts of different concentrations (6.25µg — 100µg) was mixed together in test tubes and incubated at 25°C for 10 minutes. To this solution 250µL of 1% starch solution was added and incubated at 25°C for 10 minutes.500µL of dinitro salicylic acid was added to stop the reaction. The above mixture was incubated in the boiling water bath for 5 minutes. The test tubes were cooled to room temperature. All the test tubes were diluted with 5ml of distilled water. The absorbance of the solution was measured at 540nm. The % inhibition was calculated using following formula,

$$\%\text{Inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

Where Abscontrol is the absorbance of control and Absrest is the absorbance of test extract.

2.6.2. Alpha glucosidase Inhibition assay [12]

50µL of phosphate buffer(100mM, pH = 6.8), 10µL of alpha-glucosidase solution(1U/ml) and 20µL of various concentrations of plant extracts were taken in a 96 well plate and incubated at 37°C for 15minutes. To the above solution 50µL of p-nitrophenyl α-glucopyranoside solution (5mM) was added and further incubated at 37°C for 20 minutes. The reaction was stopped by the addition of sodium carbonate (0.1M). The absorbance was absorbed at 405nm % Inhibition was calculated using the following formula.
%Inhibition = (Abs_{control} - Abs_{test}) / Abs_{control} \times 100

Where Abs_{control} is the absorbance of control and Abs_{test} is the absorbance of test extract.

3. RESULTS AND DISCUSSION

**Compound I:**

IR cm⁻¹: 3314.23 cm⁻¹ (O-H), 2967.46 cm⁻¹ (C-H Str), 1725.41 cm⁻¹ (C=O Str), 1653.09 cm⁻¹ (C=C), 1202.32 cm⁻¹ (C-O-C). ¹H NMR (DMSO): δ 12.44 (s, 1H, 5-OH), δ 10.66 (s, 1H, 3-OH), δ 10.042 (s, 1H, 7-OH), δ 9.24 (s, 1H, 4″-OH), δ 8.042 (m, 1H, 2″-H), δ 6.93 (m, 1H, 6-H), δ 6.90 (s, 1H, 3″-H), δ 5.89 (s, 1H, 8-H), δ 6.40 (d, 1H, 6″-H), δ 6.18 (d, 1H, 5″-H). ¹³C NMR: (DMSO) δ 177.75 (C-4), δ 165.97 (C-7), δ 162.30 (C-9), δ 161.27 (C-3″), δ 157.43 (C-5), δ 148.36 (C-2), δ 136.30 (C-3), δ 124.28 (C-2″), δ 124.18 (C-6″), δ 130.98 (C-1″), δ 123.54 (C-3″), δ 123.45 (C-5″), δ 105.78 (C-10), δ 98.42 (C-8). Mass spectra (EI-MS): 286 (M⁺, C₁₅H₁₀O₆, 34%), 281 (76%), 158 (87%), 130 (15%), 86.87 (100%), 72 (69%), 42 (35%).

Compound 1 gave an orange colour with shinoda’s test which is the characteristic colour given by flavonoids. Melting point of the compound was found to be 277°C. In the IR spectra the absorption band for OH group was observed at 3314.23 cm⁻¹, a peak at 2967.46 cm⁻¹ indicated C-H Str. C=O Str was observed at 1725.41 cm⁻¹, C=C at 1653.09 cm⁻¹ and C-O-C at 1202.32 cm⁻¹. In the ¹H NMR spectra the protons of the hydroxyl groups were indicated at δ 9.24 ppm - δ 12.44 ppm. ²H appeared at δ 8.042 ppm, ₆¹H at δ 6.93 ppm, ₆″H at δ 6.90 ppm, ₇H at δ 6.89 ppm, ₈H at δ 6.90 ppm and ₅″H at δ 6.18 ppm. In ¹³C NMR C=O was observed at δ 177.75 ppm. The carbon atoms of the side aromatic ring were observed in the range δ 161.27 ppm - δ 123.45 ppm. From the mass spectrum the molecular mass of the compound was found to be 286. The compound was identified as Kaempferol which was confirmed by IR, ¹H NMR, ¹³C NMR and Mass spectral characters and by chromatography with an authentic sample of Kaempferol [13].

I-a (IR spectral data)  
I-b (¹H-NMR spectral data)  
I-c (¹³C-NMR spectral data)  
I-d (Mass spectral data)

Fig. 1. Spectral data of compound 1
Compound II: IR (cm\(^{-1}\)): 3585.1 cm\(^{-1}\) (O-H), 2919.06 cm\(^{-1}\) (C-H stretch in alkene), 2919.06 cm\(^{-1}\) (C-H stretch in alkanes), 1171.25 cm\(^{-1}\) (C=O stretch), 887 cm\(^{-1}\) (C-H unsaturated vibrations). \(^1^H\)NMR (DMSO): \(\delta\) 5.18 (s, 1H, vinylic proton), \(\delta\) 1.21 - \(\delta\) 1.48 (m, 20H, 10×CH\(_2\)), \(\delta\) 3.56 (s, 1H, 2-OH), \(\delta\) 0.82 - \(\delta\) 1.23 (m, 5×CH\(_3\)), \(\delta\) 2.07 (q, 4H, CH\(_2\)), \(\delta\) 1.91 (s, 3H, 3×CH\(_3\)). \(^1^3^C\)NMR (DMSO): \(\delta\) 38.75 (C-1), \(\delta\) 151.01 (C-20), \(\delta\) 109.34 (C-29), \(\delta\) 79.02 (C-3), \(\delta\) 55.29 (C-5), \(\delta\) 50.43 (C-9), \(\delta\) 48.29 (C-18), \(\delta\) 48.00 (C-19), \(\delta\) 543.01 (C-17), \(\delta\) 42.83 (C-14), \(\delta\) 40.83 (C-8), \(\delta\) 40.01 (C-22), \(\delta\) 38.87 (C-4), \(\delta\) 38.04 (C-13), \(\delta\) 37.17 (C-10), \(\delta\) 35.58 (C-16), \(\delta\) 34.28 (C-7), \(\delta\) 27.99 (C-23), \(\delta\) 27.74 (C-15), \(\delta\) 25.13 (C-12), \(\delta\) 20.93 (C-11), \(\delta\) 19.31 (C-30), \(\delta\) 18.32 (C-6), \(\delta\) 16.13 (C-25), \(\delta\) 15.98 (C-26), \(\delta\) 15.38 (C-24), \(\delta\) 14.55 (C-27).

Compound II showed a positive result to the Liebermann burchard test. The melting point of the compound was found to be 216°C. In IR spectral data peak at 3585.1 cm\(^{-1}\) indicated hydroxyl group. C-H Str was observed at 2919.06 cm\(^{-1}\), C-H bend for alkenes was observed at 1461.99 cm\(^{-1}\) and C-H unsaturated vibrations was observed at 887 cm\(^{-1}\). In the \(^1^H\)NMR spectra the vinylic proton was indicated at \(\delta\) 5.18 ppm. The proton of hydroxyl group was indicated at \(\delta\) 3.56 ppm. Methyl protons appeared in the region \(\delta\) 0.82 - \(\delta\) 1.23 ppm. The sp\(^2\) hybridized carbon gave signal at \(\delta\) 151.01 ppm for C-20 and \(\delta\) 109.34 ppm for C-29. The carbon attached to the hydroxyl group at C-3 gave signal at \(\delta\) 79.02 ppm. The protons of methyl group attached to the skeleton gave peaks at \(\delta\) 14.55 - 16.13 ppm. \(\delta\) 27.99 ppm is for C-23. From the mass spectrum the molecular weight of the compound was found to be 426. Its identity as Lupeol was further confirmed by IR, \(^1^H\)NMR, \(^1^3^C\)NMR and Mass spectral characters and by chromatography with an authentic sample [14, 15].

### 3.1 In vitro Anti-diabetic Activity

#### 3.1.1 Alpha-amylase Inhibition assay

This assay is based on the inhibition of the enzyme alpha-amylase which results in the reduction of the metabolism of carbohydrates which ultimately decreases the postprandial blood glucose levels. The ethanol extract showed the maximum% inhibition of alpha-amylase at the concentration of 100 µg/mg, value was found to be 38.77%. The aqueous extract showed maximum% inhibition at the concentration of 50 µg/ml and the value was found to be 38.36%. The maximum percentage inhibition of standard at 100 µg/ml was 62.83%. The IC\(_{50}\) values of aqueous and ethanol extracts were found to be 518.93 and 208.17 respectively. The IC\(_{50}\) Value of standard drug acarbose was found to be 42.23.

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition of Alpha-amylase ±SEM</th>
<th>IC(_{50}) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarbose</td>
<td>6.25</td>
<td>29.60±0.050</td>
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</tr>
<tr>
<td></td>
<td>12.5</td>
<td>37.81±0.163</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>47.97±0.26</td>
<td>42.23</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>56.17±0.052</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>62.83±0.231</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract of Mirabilis jalapa</td>
<td>6.25</td>
<td>29.64±0.101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>34.21±0.101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>36.31±0.101</td>
<td>518.93</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>38.36±0.154</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>34.50±0.058</td>
<td></td>
</tr>
<tr>
<td>Ethanolic extract of Mirabilis jalapa</td>
<td>6.25</td>
<td>29.76±0.058</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>31.22±0.101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>33.39±0.058</td>
<td>208.17</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>36.08±0.233</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>38.77±0.202</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed in terms of ± SEM and are found to be significant when compared to control (P=0.05).
3.1.2 Alpha-glucosidase Inhibition assay

Alpha-glucosidase inhibition assay also approved the results obtained in the alpha amylase method. The maximum percentage inhibition was exerted by the ethanol extract but at the concentration of 50µg/ml (26.82%). The percentage inhibition of standard was found to be 50.79% at 50µg/ml. The IC$\textsubscript{50}$ values of aqueous and ethanol extracts were 299.84 and 221.45 respectively. Concentration required for 50% inhibition of standard acarbose was found to be 57.10µg/ml.

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition of Alpha-glucosidase ± SEM</th>
<th>IC$\textsubscript{50}$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarbose</td>
<td>6.25</td>
<td>23.36±0.127</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>34.48±0.095</td>
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</tr>
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<td>57.10</td>
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<td></td>
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<td>50.79±0.199</td>
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<td></td>
<td>100</td>
<td>59.31±0.192</td>
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<tr>
<td>Aqueous extract of</td>
<td>6.25</td>
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</tr>
<tr>
<td>Mirabilis jalapa</td>
<td>12.5</td>
<td>11.56±0.123</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>17.74±0.046</td>
<td>299.84</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>21.48±0.081</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>21.16±0.046</td>
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<tr>
<td>Ethanol extract of</td>
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<tr>
<td>Mirabilis jalapa</td>
<td>12.5</td>
<td>11.70±0.046</td>
<td></td>
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<tr>
<td></td>
<td>25</td>
<td>19.85±0.093</td>
<td>221.45</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>26.82±0.081</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>24.85±0.243</td>
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</table>

All values are expressed in terms of ± SEM and are found to be significant when compared to control (P=0.05)
4. CONCLUSION

The preliminary phytochemical analysis of the ethanolic extract of the flower showed the presence of flavonoids, triterpenoids, steroids, resins, tannins and reducing sugars. Further, thorough analysis of the flower extract by the separation of the chemical constituents and characterization by various methods like IR, $^1$H NMR, $^{13}$C NMR and mass spectroscopy revealed the presence of a flavonoid Kaempferol(compound I) and a triterpenoid Lupeol(compound II). Further the anti-diabetic activity was analyzed using methods like alpha-amylase and alpha-glucosidase inhibition assay. It was observed that the inhibition of enzymes by the two extracts (aqueous and ethanolic) of the flower of Mirabilis jalapa were not as prominent as the standard drug acarbose. It only showed the slight activity when compared with the standard. Even the IC$_{50}$ values of the standard and extracts suggested the same. The presence of flavonoids, triterpenoids, steroids and tannins might be responsible for these activities. Even though the anti-diabetic activity was moderate it may be useful in decreasing the postprandial blood glucose level and in the treatment of type 2 diabetes [16].

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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