Study of Some *Mirabilis jalapa* L. Leaves Components and Effect of Their Extracts on Growth of Pathogenic bacteria

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الخلاصة

شملت الدراسة معرفة المكونات الكيميائية الفعّالة الموجودة في أوراق نبات لالة عباس *Mirabilis jalapa*، إذ أظهرت الدراسة أن محلولي المستخلصين المائي والكحولي يحتويان على مجموعة من المركبات الكلايكوسيدية والعفصية والفينولية والراتنجات والقلويدات والبروتينات بينما لا تحتوي على الصابونيات والفلافونويدات .

اثبت التحليل الدقيق للعناصر المعدنية لأوراق النبات احتواءها على تراكيز من K و Na وFe وهي 161.2 19 18.7 µg/ml, على التوالي ، وكميات من Zn و Ca وهي 12, 14.2µg/ml وكميات على Cd، Cu و Pb وهي 0.8 ,0.3,0.1 µg/ml . وعلى التوالي، وعدم احتوائها على Cr.

كما درس تأثير المستخلصات المائية والكحولية على أنواع مختلفة من البكتريا أذ لوحظ أن للتركيز 0.5 ملغم /مل تأثيرا فعّالا تجاه تثبيط نمو بكتريا *Proteus mirabilis* و *E.coli* و *Staphylocoous aureus* خاصة تجاه المستخلص الكحولي .

ABSTRACT

The chemical components of the *Mirabilis jalapa L.* leaves in the watery and alcoholic extracts were identified .The results showed that the extract contain : glycosides ,tannins ,phenolic compounds , resins ,alkaloids and proteins ,while the saponins and flavonoids were not found.

The results also showed that there were concentrations of the following trace elements in the leaves K , Na , Fe with 161.2 ,19 ,18.7 µg/ml , respectively and concentrations of Zn ,Ca with 14.2 , 12 µg/ml respectively , concentrations Cd ,Cu, Pb with 0.8 ,0.3,0.1 µg/ml respectively ,and Cr were not founds.

The effects of these extracts on growth of different bacteria were studied, has been found that 0.5 mg/ml concentration was effective inhibitor of growth of *Staphylocoous aureus* , *E.coli* and *Proteus mirabilis* .

INTRODUCTION

Mirabilis jalapa Linn (Nyctaginaceae), known as four o'clock, maravilla, belle de nuit, buenas tardes, Dondiego de noche, jalap, noche buena,[1,2] *Mirabilis jalapa* L. is widely used to treat dysentery, diarrhea, muscular pain, and abdominal colics in different countries [3-5] and its extract has antibacterial, antiviral, and antifungal functions [6-8]. In China, *Mirabilis jalapa* L. is widely distributed and commonly used with its root, and has been used as traditional Chinese medicine and ethnic drug to treat diabetes [9,10], constipation [11], genitourinary system disorders and injuries [12].Several constituents of the *Mirabilis jalapa* plant have been isolated from the root and aerial part, including some rotenoids (mirabijalones A-D, boeravinones C and F), an isoquinoline derivate [13], as well as terpenoids, steroids, phenolic compounds, stigmasterol, **p**-sitosterol, **p**-sitosterol- **p**-D-glucoside, ursolic acid, mirabalisoic acid, mirabalisol, trigonellin and an antiviral

protein [14]. Furthermore, *Mirabilis jalapa* extracts have also been reported to have biological activities (antibacterial, antiviral, antifungal, protein synthesis inhibition) by many research groups [15], though few studies have been performed on muscular activity [16]. It is difficult to correlate the biological effects with the traditional use of this plant, because common people employ this medicinal plant for many different illnesses, as spasmogenic or spas- molitic drug between others, with apparently opposite effects as purgative or for treating dysentery. That contradictory use given at *Mirabilis jalapa* could be due to a lot of bioactive elements this plant contains (as trigonellin, a purgative and **p**-sitosterol- **p**-D-glucoside, some rotenoids and other compounds reported as antispasmodics), their irregular distribution in the enter plant and to differences in the traditional extraction processes.

The aim of this study the watery and alcoholic extracts *for Mirabilis jalapa* leaves posses *in vitro* antibacterial activity because of its content (glycosides , tannins , ,proteins ,various phenolic compounds ,alkaloids , trace elements) , however if plant leaves extracts are to be used for food preservation or medical purposes .

MATERIALS AND METHODS

Collection and treatment of samples:

The leaves of *Mirabilis jalapa* were collected from the north of Baghdad, Iraq. The leaves were transported to the laboratory, washed, cleaned with filter paper or soft clothes to remove all traces of dust and insects, then dried in shade 25-30ºC for one week, with continuous overturn to prevent mould .weighed, ground in a mortar and pestle, placed in airtight bottles and stored in dissicator to be used for extraction [17].

Preparation of extracts:

a) Watery extract:

Air dried leaves 50 g were suspended in one liter of distilled water and left for 24 hrs at 35ºC with continuous stirring in shaking incubator .Then the mixture was filtered by filter paper ,the filtrate was centrifuged for 10 min. at 2500 rpm ,and the extract evaporated to dryness at 40ºC in the incubator for 24 hrs .

b) Alcoholic extract:

Prepared as in watery extract described above ,but with using 70% ethanol alcohol instead of water to give alcoholic extract powder [18-21].

Determination of Ash content:

Dried leaves 2 g were taken and heated at 900ºC for 20 min. in muffle furnace until the material converted to white powder, after its cooling the percentage of ash content was determined [22].

Chemical detection of the plant components:

The chemical components of the prepared watery and alcoholic extract were detected: glycosides, alkaloids, saponins, phenolic compounds, tannins, resins, flavonoids (20-22) and proteins [23].

Determination of trace element levels:

Dried leaves 3 g were taken and mixed with 8 ml of concentrated nitric acid and 2 ml of 60% prechloric acid in a conical flask, the mixture was kept for 24hrs covered with watch glass .After that it was left for 6hrs at sand bath at 80ºC, until the digestive material converted to white powder. Deionized water 8 ml were added to this powder, and the trace elements were determined [22] by (Shimadzu AA-670, Flame Atomic Absorption Spectrophotometer).

The biological activity:

The biological activity against various bacterial species was determined by using wells-diffusion method. From gram negative bacteria, *E.coli* and *Proteus mirabilis* was chosen, while *Staphylococcus aureus* was used as gram positive bacteria. These isolates were obtained from department of Biology /College of Science /Al-Mustansiryih University .The concentrations for both extracts were 0.1 , 0.5, 1 mg/ml [20,21].

Inhibition of hyaluronidase:

Hyaluronidase inhibition activity was determined turbidimetrically by the method of Kass *et al.* [24] by using 0.01 mg/ml enzyme mixed with 250 µg/ml from the extracts with inhibition time 45 min. and the percentage of inhibition was calculated according to this equation [25]:

$$\%\text{inhibition} = \frac{\text{Activity of control - Activity in the presence of Extract}}{\text{Activity of control}} \times 100$$

Assay system :

1- 100µl of hyase (serum) was made up to 900 µl with 0.1 M acetate buffer (pH 3.7 containing 0.15 M NaCl), then incubated for 15 min at 37 ^{0}C .
2- After preincubation .the assay was commenced by added HA (100µl) to each tube and incubated for 45 min. all incubations were carried out in triplicate.
3- Other tube was prepared for the initial HA in sample , contained (100 µl) of substrate and the volume was made up to 900 µl with acetate buffer pH 3.7 and a 100µl of serum sample was then added after 45 min of incubations time with a total volume of 1ml.

4- The blank which was contained 100µl of enzymes were prepared and the volume was made up to 1ml with acetate buffer , but in the case of serum sample the contained only 1ml acetate buffer pH 3.7 .
5- Reaction were terminated by the addition of 2ml of Cetrimide (CTAB) (25% w/v) in 2% (w/v) NaOH solution (stop reaction)and produced the turbidity.

RESULTS AND DISCUSSION

The results showed that Ash content for the *Mirabilis jalapa* leaves is 34 %. The qualitative chemical analyses of the watery and alcoholic extracts are represented in Table.1,Which shown that leaves contents are (glycosides, proteins, tannins, resins and various phenolic compounds) Our results similar with other studies (26)**,** alkaloids are obtained in alcoholic extract only, while the saponins and flavonoids are not found.

Table-1: Chemical components analysis for watery and alcoholic extracts of *Mirabilis jalapa* leaves.

components	Reagents	Note	Result Watery extract	Result Alcoholic extract
Glycosides	Iodine test Molish test Benedict test	Blue ppt. Violet ring Orange ppt.	Ve+ Ve+ Ve+	Ve- Ve + +Ve
Proteins	Folin-Ciocalteau reagent	Blue color	Ve+	Ve+
Saponins	Fast stirring Mercuric Chloride	No Dense foam for long time No White ppt.	Ve - Ve -	Ve- Ve-
Phenolic compounds	Aqueous%1 Ferric chloride	Green ppt.	Ve+	Ve+
Tannins	Aqueous%1 Ferric chloride Lead acetate%1	Green ppt. Preface yellow ppt.	Ve+ Ve+	Ve+ Ve+
Resins	Ethanol + Boiling + D.w.	turbidity	Ve+	Ve+
Flavonoids	aqueous%1 Ferric chloride Ethanol hydroxide alcohol	No Green ppt. No Yellow ppt.	Ve- Ve-	Ve- Ve-
Alkaloids	Mayer's reagent Wagner reagent Picric acid	No white ppt. Brown ppt. Yellow ppt.	Ve- Ve- Ve-	Ve+ +Ve Ve+

The concentrations of trace elements in *Mirabilis jalapa* leaves are represented in Table.2 .which shows , concentrations of (K , Na , Fe) with (161.2 , 19 , 18.7) µg/ml , respectively and concentrations of (Zn ,Ca) with (14.2 ,12) µg/ml , respectively , concentrations (Cd , Cu , Pb) with (0.8 , 0.3, 0.1) µg/ml and (Cr) were not founds .

Table-2: The concentration of trace elements content of *Mirabilis jalapa* leaves.

Trace elements	symbol	Concentration(µg/ml)
Potassium	K	161.2
Sodium	Na	19
Iron	Fe	18.7
Calcium	Ca	12
Zinc	Zn	14.2
Cadmium	Cd	0.8
Chromium	Cr	Nil
Lead	Pb	0.1
Cupper	Cu	0.3

The effect of these extracts on different microorganisms were studied and compared between them .In addition to that the results seen in Table .3 showd that the concentrations 0.5 , 1 mg/ml exhibit very effective inhibition towards tested bacteria , *P. mirabilis* and *E.coli* and *S. aureus* ,specially for the alcoholic extract , while less inhibition effects were seen for the same concentrations when the watery extract was used .In general , when the both extracts were tested against the intended bacteria they were no activity at concentration of 0.1 mg/ml .

Table-3: The effect of watery and alcoholic extracts of Mirabilis jalapa represented by inhibition zone (mm) on different bacteria species.

Bacterial species	*mg/ml(Alcoholic extract)*			*mg/ml (Watery extract)*		
	0.1	0.5	1	0.1	0.5	1
S. aureus	-	+	+	-	+	+
E.coli	-	+	++	-	+	+
P. mirabilis	-	+ +	++	-	++	++

(-) No inhibition zone
(++) Inhibition zone between (4-10) mm .
(+++) Inhibition zone more than (10) mm .

This work shows , the two extracts were examined for their effects on hyaluronidase .The percentage of inhibition for watery extract was

7.5% , and 6.25% for Alcoholic extract with respect to control assays run simultaneously .

CONCLUSION

the present study confirm that the watery and alcoholic extracts *for Mirabilis jalapa* leaves posses *in vitro* antibacterial activity because of its content (glycosides , tannins , ,proteins ,various phenolic compounds ,alkaloids , trace elements) , however if plant leaves extracts are to be used for food preservation or medical purposes ,issues of safety and toxicity will need to be addressed ,and this results will serve as a precursor for further research.

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