

Study of Some Fenugreek (*Trigonella foenum-graecum* L.) Components and Effect of Their Extracts on Liver Enzymes and Growth of Some Microorganisms

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ABSTRACT

The chemical components of the *Trigonella* leaves in the watery and alcoholic extracts were identified .The results showed that the extract contain : glycosides , saponins ,tannins ,phenolic compounds , resins ,alkaloids and flavonoids .

The results also showed that there were high concentrations of K , Na , Ca with (171.2 , 19 , 18.3) ppm, respectively and low concentrations of Fe, Zn ,Cu, Mg with (5.1, 3.2 ,2.3 ,1.9) ppm, respectively ,very low concentrations Cr ,Cd , Pb with (0.9 ,0.7, 0.2) ppm

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

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an yearly plant from legume family. This native crop is extending from Iran to northern India [1]. Fenugreek leaves and seeds have been used extensively for preparing extracts and powders in medicinal performance [2]. In some state in Asia, the young plants are performed as potherbs and the seeds for herbal medicine usages. The species name "*foenum-graecum*" means"Greek hay" indicating its use as a forage harvest in the past[1]. Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses [2]. Fenugreek is a rich source of steroidal saponin. Previous studies reported the anti-diabetic [3], Antibacterial [4], Anti-oxidant [5] hypocholesterolaemic [6], anti-cancer [7], thyroxin-induced hyperglycaemia [8], anti-inflammatory [9],

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antiaflatoxic [10] and protective properties against ethanol toxicity [11] of fenugreek. It also plays an important role in the control of cholesterol metabolism [12].

Trigonella foenum-graecum is produced diosgenin as a steroidal sapogenin, belonging to triterpene group and has great significance for pharmaceutical industry because of its oestrogenic effect on the mammary gland [13]. It also plays an important role in the control of cholesterol metabolism, variation in the lipoxygenase activity of human erythroleukaemia cells and responsible for morphological and biochemical variations in megakaryocyte cells. Diosgenin is performed mainly as basic material for partial synthesis of the oral contraceptives, sex hormones and the other steroids [14]. Methyl protodioscin, as the potent agent by anti-tumor properties, has been synthesized from diosgenin [15]. as well the role of ethylene on diosgenin production in *T. foenum-graecum* seedlings reported before [16]. concerned about the expression of sapogenin in this plant material contained the roots and hairy roots cultures and its expression in their seeds [17]. The present study confirm that the watery and alcoholic extracts for *Trigonella* leaves posses *in vitro* antibacterial activity and liver enzymes.

MATERIALS AND METHODS

Plant material:

Trigonella foenum-graecum L. (Fenugreek) leaves were collected from the north of Baghdad, Iraq. Cleaned, dried and finely powdered [18].

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 35°C with continuous stirring in shaking incubator. Then the mixture was filtered by filter paper and centrifuged at 2500 rpm for 10 min. the supernatant was collected [18].

b) Alcoholic extract:

Prepared as in watery extract described above, but with using 70% ethanol alcohol instead of water to give alcoholic extract powder [19-22].

Determination of Ash content:

Ash content was determined by using method [22].

Chemical detection of the plant components:

The chemical components of the prepared watery and alcoholic extract were detected. They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, resins, flavonoids [23].

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Determination of trace element heavy:

For trace elements and heavy metals the plant material was digested with nitric acid and perchloric acid and the trace elements were determined [23] estimated was used 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 [21,22] .

Biochemical analyses and Inhibition

Plasma was used to measure AST, ALT as indicative parameters of hepatic function. The plasma activities of AST, ALT were estimated by commercially available kits using an autoanalyser . inhibition activity was determined turbidimetrically by the method of kits 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 %I was calculated according to this equation [26]:

RESULTS AND DISCUSSION

The results showed that Ash content for the *Trigonella* leaves is 28 %. The qualitative chemical analyses of the watery and alcoholic extracts are represented in Table.1, Which shown that leaves contents are (glycosides, saponins, tannins, resins, various phenolic compounds, alkaloids, and flavonoids) our results similar with other studies.

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Table.1: Chemical components analysis for watery and alcoholic extracts of *Trigonella* leaves.

components	Reagents	Note	Result Watery extract	Result Alcoholic extract
Glycosides	Iodine test	Blue ppt.	+Ve	-Ve
	Molish test	Violet ring	+Ve	+Ve
	Benedict test	Orange ppt.	+Ve	+Ve
Saponins	Fast stirring	Dense foam for long time	+Ve	+Ve
	Mercuric Chloride	White ppt.	+Ve	+Ve
Phenolic compounds	1% Aqueous Ferric chloride	Green ppt.	+Ve	+Ve
Tannins	1% Aqueous Ferric chloride	Green ppt. Preface yellow ppt.	+Ve	+Ve
	1% Lead acetate		+Ve	+Ve
	Ethanol + Boiling + D.w.	turbidity	+Ve	+Ve
Flavonoids	1% Aqueous Ferric chloride	Green ppt.	+Ve	+Ve
	Ethanol hydroxide alcohol	Yellow ppt.	+Ve	+Ve
Alkaloids	Mayer's reagent	white ppt.	+Ve	+Ve
	Wagner reagent	Brown ppt.	+Ve	+Ve
	Picric acid	Yellow ppt.	+Ve	+Ve

The concentrations of trace elements and heavy metal in *Trigonella* leaves are represented in Table.2 .which shows ,high concentrations of (K , Na , Ca) with (171.2 ,19 , 18.3) ppm, respectively and low concentrations of (Fe, Zn ,Cu, Mg) with (5.1, 3.2 ,2.3 ,1.9) ppm, respectively ,very low concentrations (Cr ,Cd , Pb) with (0.9 ,0.7,0.2) ppm .

Table .2: The concentration of trace elements content of *Trigonella* leaves.

Elements	Concentration(ppm)
Potassium K	171.2
Sodium Na	19
Calcium Ca	18.3
Iron Fe	5.1
Zinc Zn	3.2
Copper Cu	2.3
Magnisium Mg	1.9
Chrom Cr	0.9
Cadmium Cd	0.7
Lead Pb	0.2

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The effect of these extracts on different microorganisms were studied and compared between them .In addition to that the results seen in Table .3 show 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 *Trigonella* represented by inhibition zone (mm) on different bacteria species.

Bacterial species	Alcoholic extract(mg/ml)			Watery extract (mg/ml)		
	0.1	0.5	1	0.1	0.5	1
<i>S. aureus</i>	-	1-3mm	2-4mm	-	1-3mm	2-5mm
<i>E.coli</i>	-	1-3mm	4-7mm	-	1-3mm	2-4mm
<i>P. mirabilis</i>	-	4-6mm	5-8mm	-	4-7mm	5-10mm

- (-) 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 aspartate aminotransferase (AST) .The percentage of inhibition for watery extract was 9.2% , and 7.5% for Alcoholic extract with respect to control assays run simultaneously . while for their effects on alanine aminotransferase (ALT), .The percentage of inhibition for watery extract was 8.5% , and 8.2% for Alcoholic extract with respect to control assays run simultaneously . The present study confirm that the watery and alcoholic extracts for *Trigonella* leaves posses *in vitro* antibacterial activity because of its content (glycosides , tannins , saponins ,various phenolic compounds ,alkaloids and flavonoids , 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 and improvement strategies of this important plant .

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دراسة بعض مكونات أوراق نبات الحلبة (*Trigonella foenum-graecum* L.) وتأثير مستخلصاتها على انزيمات الكبد و نمو بعض الأحياء الدقيقة

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الخلاصة

شملت الدراسة معرفة المكونات الكيميائية الفعالة الموجودة في أوراق نبات الحلبة (*Trigonella foenum-graecum* L.) إذ أظهرت الدراسة أن المستخلص المائي والكحولي يحتويان على مجموعة من المركبات الكلايكوسيدية والتانينات والصابونيات والفينولية والراتنجات والقلويدات والفلافونيدات .

اثبت التحليل الدقيق للعناصر المعدنية لأوراق النبات احتواءها على تراكيز عالية من K , Ca , Na وهي 171.2 , 19 , 18.3 جزء بالمليون على التوالي ، وكميات أقل Fe, Zn , Cu وهي 5.1 , 3.2 , 2.3 , 1.9 وكميات قليلة جدا على Cr, Cd, Pb وهي 0.7, 0.9 , 0.2 على التوالي.

كما درس تأثير المستخلصات المائية والكحولية على أنزيمات الكبد ووجد أن لها تأثير تثبيطي على أنزيمات الناقلّة للامين AST, ALT ودرس كذلك تأثير المستخلصات على أنواع مختلفة من البكتريا أذ لوحظ أن للتركيز 0.5 ملغم /مل تأثيرا فعالا تجاه تثبيط نمو بكتريا *Staphylococcus aureus* , *E.coli* , *Proteus mirabilis* لاسيما تجاه المستخلص الكحولي .