Identifying and Study of some of Phytochemical compounds and Anti-Jaundice Activity for powder of leaves and seeds of *Moringa oleifera* in male albino rats.

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**Abstract**

The present study aimed to revealing and identifying some of phytochemical compounds, bioactive fatty acids and antioxidants for powder of leaves and seeds of *Moringa oleifera* that cultivated in Iraq which have been many identified by using modern techniques such as gas chromatography mass spectrometry (GC-MS) technique. And then study and test the preventive and Therapy effects for powder of leaves and seeds in present study against hepatotoxicity that induced by carbon tetrachloride (*CCl₄*) and against jaundice. These effects were tested by measuring total bilirubin concentrations of total bilirubin and γ-glutamyl transferase (GGT) in serum of normal male rats, exposed to *CCl₄* and that treated with powder of leaves and seeds of *Moringa oleifera* along the period of experiment (30) days, used (20) albino male rats divide to (4) groups, that is meaning (5) rats for each group. The rats group that exposed to *CCl₄* caused a significant increase (P<0.05) in concentrations of total bilirubin and GGT enzyme in comparison with normal control group. Whereas the animals which exposed to *CCl₄* then treated with powder of leaves and seeds of *Moringa oleifera* lead to significant decrease (P<0.05) in total bilirubin and GGT enzyme concentration in comparison with the group that exposed to *CCl₄* only. It could be concluded of this study that using powder of leaves and seeds of *Moringa oleifera* may be have Preventive and therapeutic effects against damage and hepatotoxicity, subsequently, anti-jaundice activity through its nutritional and antioxidant influences for remove of free radicals and repairing cellular and tissues from toxic damage.

**Introduction**

In recent times, focus on plant research has increased all over the world because the plants contain numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities [1], as well as, throughout history, plants have been used by human beings for medicinal purposes and even in modern times have formed the basis of many pharmaceuticals in use. Under traditional purposes, herbal medicines and medical plants like *Moringa oleifera* Lam [2], is one of the best known and most widely distributed and naturalized species of a monogeneric family *Moringaceae* [3]. *M. oleifera*, native of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia [4]. *M. oleifera* is an important food commodity which has had enormous attention as the ‘natural nutrition of the tropics’. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries [5]. In Thailand, it is locally known as ‘Marum’. Seed of this plant is used as human food, medicine, in oil production, and also for efficient treatment of hard water [6]. Also, *M. oleifera* can be used to treat diabetes and intestinal worms [7].

**Keywords:** *Moringa oleifera*, *CCl₄*, GC-MS, Total bilirubin, GGT enzyme.

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Jaundice is the most common of liver disorders. It is a condition in which yellow discoloration of the skin and mucous membranes occurs due to an increase in the bile pigments (bilirubin) in the blood [9]. Excess bilirubin in blood gives rise to jaundice. The common causes are increase destruction of red cells with rapid release of bilirubin into blood. Obstruction of bile duct cause damage to liver cells. There are many different causes of jaundice such as hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E, liver cirrhosis, obstruction of bile ducts, gallstones, pancreatic cancer, alcoholic liver disease, inflammation of the liver, haemolytic anaemia, typhoid, malaria, yellow fever, tuberculosis, certain medication and pregnancy.

The aim of the present study was to identified the presence of some phytochemicals and antioxidants in seeds and leaves samples of *M. oleifera* by GC-MS analysis and study of biologically active to some of phytochemicals on bilirubin level and GGT enzyme as special markers for jaundice.

**Materials and methods**

This study was conducted on uses the plant samples (seeds and leaves) of *Moringa oleifera* were identified and collected from Herbarium in Baghdad, Iraq. Seeds and leaves were screened to remove bad ones and dried then pulverized by using grinder transform to powder form and sieved and put in an approximate 1 ml of blood from each animal, put in EDTA tubes containing anticoagulant for measuring the total bilirubin and GGT enzyme concentration.

**GC-MS Analysis:**

The phytochemical properties and fatty acid composition of leaves and seeds of *M. oleifera* was quantified using Gas Chromatography–Mass spectrometry (GC-MS) analysis. This analysis was performed by Instrument of Gas Chromato-graphy–Mass spectrometry (QP-2010Japan, in laboratories of al - Mustansiriyah university \ college of Science\ department of chemistry then were evaluated with Postrun system and searched in national institute of standard and technology center (NIST).

**The biochemical tests:**

Diagnostic kit were employed in the analysis of serum total bilirubin concentrations [13]. In addition to the concentration of GGT measured by modified method [14].

**Statistical analysis:**

Finally, the statistical analysis was carried out by using statistical program (SAS,2001) and Comparison between groups were made by using one-way analysis of variance (ANOVA), and tried out the arithmetic means for parameters by using test of duncun multiple range to delimitating significantly differentes especially between groups. The level of statistical significance was taken at (P<0.05). All data are expressed as mean± standard error (M±S.D) and put above it duncun value (letters).

**Results and Discussion:**

The results of preliminary qualitative phytochemical for powder of seeds and leaves of *Moringa oleifera* showed the presence of phenols, glycosides,
flavonoids, steroids, alkaloids, terpenoids, tannins, volatile oils, vitamin C, resins, proteins and amino acids in all of samples in the iraqi \textit{Moringa oleifera}, as well as, presence of coumarin and saponins only in leaves sample.

\begin{table}[h]
\begin{tabular}{|c|c|c|c|}
\hline
No & Active substances & \textit{Moringa oleifera} leaves & \textit{Moringa oleifera} seeds \\
\hline
1 & Phenols & + & + \\
2 & Glycosides & + & + \\
3 & Flavonoids & + & + \\
4 & Coumarin & + & - \\
5 & Steroids & + & + \\
6 & Alkaloids & + & + \\
7 & Terpenoids & + & + \\
8 & Tannins & + & + \\
9 & Volatile oils & + & + \\
10 & Saponins & + & - \\
11 & Resins & + & + \\
12 & Vitamin C (ascorbic acid) & + & + \\
13 & Proteins & + & + \\
14 & Amino acids & + & + \\
\hline
\end{tabular}
\caption{Phytochemical screening of Extract from leaves and seeds of \textit{Moringa oleifera}.}
\end{table}

+, - represent presence and absence of phyto-constituents respectively.

\textbf{Determination of Components by Gas Chromatography – Mass spectrometry technique:}

Analyzing the results of mass spectrum of GC-MS was done using the database of NIST. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and mass fragmentation of some of the components of the test materials were ascertained as follows:

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{GC-MS-Chromatogram.png}
\caption{GC-MS Chromatogram for leaves powder of \textit{M. oleifera}.}
\end{figure}

The results concerning to GC-MS technique led to the determination of number of compounds in leaves and seeds powder of \textit{M. oleifera}. GC-MS chromatogram revealed many climaxes that indicating to numerous of compounds (figure 2), (figure 3), (Table. 2).
Figure (3): GC-MS Chromatogram for seeds powder of *M. oleifera*.

Table (2): Phyto-components identified in the leaves and seeds of *M. oleifera*.

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<th>Height</th>
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Total bilirubin concentration in serum of male albino rats:
Results showed in figure (4) that there was a high significant increase (p<0.05) of total bilirubin concentration in serum of male albino rat group which administrated carbon tetrachloride CCl₄ in comparison with normal control group.

Figure(4): Effect of treatment by leaves powder (300 mg/kg of b.w) and seeds powder (200 mg/Kg of b.w) of Moringa oleifera on total bilirubin concentration in male albino rats that exposed to liver toxicity Induced by CCl₄.

CCl₄ is one of the most commonly used hepatotoxins in induced the liver injury by associated with oxidative stress and free radicals. The hepatotoxicity of CCl₄ is due to reductive dehalogenation products, such as trichloromethyl (CCl₃) and trichloro-methyl peroxyl (CCl₃O₂.) radicals, these radicals can bind to organic compounds such as lipids, proteins and nucleic acids, thus occurrence lipid peroxidation and damage in hepatocytes [15], and subsequently, elevate concentration of serum bilirubin.

While the groups that administrated CCl₄ then treated by leaves and seeds powder of Moringa oleifera observed significant decrease (p<0.05) in total bilirubin concentration in comparison with group which administrated CCl₄ only. This may be due to that treatment with parts of M. oleifera which contain a rich source of proteins, β-carotene, vitamins A, B, C, E, riboflavin, nicotinic acid, folic acid and pyridoxine, amino acids, unsaturated fatty acids, minerals and various phenolic compounds [16], this effect may lead to repairing damage in tissue and cells of liver, and respectively, decrease of serum bilirubin concentration.

Whereas the results showed no significant variations in TB concentration in all groups that administrated CCl₄ then treated by leaves and seeds powder of Moringa oleifera in comparison with normal control group.

γ-glutamyl transferase (GGT) concentration in serum of male albino rats:
The results in figure (5) indicates that there was a significant increase (p<0.05) in concentration of GGT in serum of male albino rats group which administrated CCl₄ only and the groups which administrated CCl₄ then treated by leaves and seeds powder of Moringa oleifera successively, in comparison with normal control group. The causes in increasing of GGT may be due to; when cell membrane of hepatocyte is damaged, a variety of enzymes normally that located in the cytosol are released into the blood stream [17]. The activity of serum GGT is generally elevated as a result of liver disease [18]. Also, elevated of GGT in serum may be result from biliary epithelial necrosis, intra-hepatic cholestasis or hepatic infiltration [19], that harm induced by CCl₄. Moreover, if the liver is damaged or the normal flow of blood or bile is obstructed, the cellular content of GGT enzyme leak or secreted into the blood and thus may result in increase of GGT concentration.

While the groups that administrated CCl₄ then treated by leaves and seeds powder of M. oleifera showed significant decrease (p<0.05) in GGT concentration in comparison with group which administrated CCl₄ only. This effect may be refer that treatment with M. leaves can be cause increase of antioxidant activity and remove of free radicals, subsequently, reduced oxidative stress and damage for major biomolecules and prevent tissue damage by free radicals (oxidative stress) [20], therefore, indicating on promising hepatoprotective activity of Moringa leaves that may conserve the structural integrity of hepatocytes membranes and consequently preventing GGT enzyme leakage into blood stream.
**Figure (5):** Effect of treatment by leaves powder (300 mg/kg of b.w) and seeds powder (200 mg/Kg of b.w) of *Moringa oleifera* on GGT concentration in male albino rats that exposed to liver toxicity Induced by CCl₄

### Conclusion
The results of the present study indicated that under the present experimental conditions, administration of leaves and seeds powder of *Moringa oleifera* showed hepatoprotective abilities by improved values of total bilirubin and GGT enzyme against carbon tetrachloride which induced liver damage in male albino rats.

### References


تشخيص ودراسة بعض المركبات الكيميائية النباتية والنشاط المضاد لليرقان لمسحوق أوراق وبذور نبات مورنجا إوليفيرا Moringa oleifera

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قسم علوم الحياة ، كلية التربية ، جامعة سامراء ، سامراء ، العراق

الملخص

استهدفت هذه الدراسة الكشف وتشخيص بعض المواد والمركبات الكيميائية النباتية والاحماض الدهنية الفعالة بيولوجياً ومضادات الأكسدة في مسحوق اوراق وبذور نبات مورنجا اوغريفا Moringa oleifera المزروع في العراق والتي تم تحديد العديد منها باستخدام التقنيات الحديثة مثل تقنية كروماتوكرافيا الغاز GC-MS وتقنية كروماتوكرافيا الغاز GC-MS. ومن ثم دراسة واختبار التأثيرات الوقائية والعلاجية لمسحوق الورق والبذور للذكور في فحص قيد الدراسة ضد السمية الكبدية المقدمة بواسطة رابع كلوريد الكاربون CCl4، وضد اليرقان. وتم اختبار هذه التأثيرات من خلال قياس تراكيز البيليروبين الكلي وانزيم GGT في مصل دم ذكور الجرذان السليم والموضوع بـ CCl4 وتم اختبار مجموعة مجمعة من ذكور الجرذان الموضوعة بـ CCl4 بمسحوق اوراق وبذور Moringa oleifera وتم استخدام كُلوتاميل كتيميناهي GGT في مصل من ذكور الجرذان السليم والموضوع بـ CCl4.

التجربة البالغة 30 يومًا، إذ استخدم 20 من ذكور الجرذان البيض قسمت إلى 4 مجموعات، أي 5 جرذان في كل مجموعة. إذ أدى تعرض الحيوانات إلى CCl4 إلى ارتفاع معنوي (P<0.05) في تراكيز البيليروبين الكلي والانزيم GGT. ومع ذلك، فإن معالمة مجموعات الحيوانات المعوضة بـ Moringa oleifera أدى إلى انخفاض معنوي (P<0.05) في تراكيز البيليروبين الكلي والانزيم GGT. وقد استنتجت الدراسة أن اتباع مسحوق الورق والبذور من Moringa oleifera قد يكون له تأثيرات وقائية وعلاجية ضد اليرقان والسيمي الكبدية وبالتالي ضد اليرقان من خلال فعالية التغيير المضادة للأكسدة لازالة الجذور الحرة وإصلاح النسيج الخلوي والنسيجي.

الكلمات المفتاحية: Moringa oleifera، GGT، CCl4، GC-MS، الكيمياء، التغذية، النباتات.