BIOLOGICAL AND CHEMICAL EVALUATION OF THE USE OF ACACIA NILOTICA (L.) IN THE EGYPTIAN TRADITIONAL MEDICINE

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SUMMARY

Acacia nilotica (L.) pods are commonly used in the Egyptian traditional medicine since pharaonic ages. In vitro and In vivo screening of biological activity of Acacia nilotica (L.) pods emphasized its use in the Egyptian traditional medicine. Further chemical investigation on fractions of the hot water extract of Acacia nilotica (L.) pods revealed that the water extract is more potent than any of its fractions. Some compounds are isolated and identified from fractions of the hot water extract of Acacia nilotica (L.) pods.

KEYWORDS

Phytochemistry, traditional medicine, Acacia nilotica (L.), water extract, In vitro, In vivo.

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INTRODUCTION

In modern medicine, there is no satisfactory effective therapy to cure the diabetes mellitus. Though insulin therapy is also used for the management of diabetes mellitus but there are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver. In recent years, there has been renewed interest in plant medicine. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential. Several such herbs proved to have anti-diabetic activity when assessed using presently available experimental techniques. Thus, plants are potential source for anti-diabetic drugs but this fact has not gained enough momentum in the scientific community. The reasons may be many including the lack of belief among the practitioners of conventional medicine over alternative medicine, alternative forms of medicine are not very well-defined, possibility of quacks practicing such medicine providing alluring and magical cures and natural drugs may vary tremendously in content, quality and safety.

Acacia nilotica L. (gum arabic, babul, Egyptian thorn, thorn mimosa or scented thorn in South Africa) is a species of Acacia native to Africa and the Indian subcontinent. The ethanolic extract of Acacia nilotica (L.) stem exhibit a remarkable hypoglycemic activity. Screening of the different organs of Acacia nilotica (L.) revealed the presence of tannins, saponins, coumarins, carbohydrates and/or glycosides, unsatd. Sterol, triterpenes, alkaloids and nitrogenous bases, flavonoids and cyanogenic compounds.

MATERIAL AND METHODS

Plant Material

Dried Acacia nilotica (L.) pods were purchased from the Egyptian markets and were grinded by electric grinder.

Extraction of Plant Material

One kilogram of dried powder of Acacia nilotica (L.) pods were macerated using boiling water then filtrated using folded muslin. This process was repeated several times until complete exhaustion of the plant materials. The extracts were evaporated under vacuum and weighed.

Preparation of the Mucilage Fraction

The mucilage of the hot water extract of Acacia nilotica (L.) pods has been isolated. The yield was 7% w/w of dry extract. The isolated mucilage was odorless, soluble in water, insoluble in ethanol, ether and chloroform. It gave positive Molish’s test and did not reduce Fehling’s and Barfoed’s solutions. In addition, it gave negative test for proteins and left no ash on ignition.

Fractionation of Hot Water Extract of Acacia nilotica (L.) Pods

After polysaccharide precipitation the filtrate was evaporated to dryness under vacuum using rotatory evaporator (yield 92.97 w/w % of dry extract).
The residue was dissolved in least amount of water and partitioned several time till complete exhaustion against diethyl ether, ethyl acetate, butanol and residue (water soluble fraction), the yields were 24.7%, 12.5%, 21.6% and 34.17% w/w of dry extract respectively.

**In Vitro screening for the Hypoglycemic Activity Using α-Amylase Inhibition Technique**

The α-amylase inhibition assay was performed using the chromogenic method adopted from Sigma–Aldrich. Pancreatic α-Amylase was dissolved in ice-cold distilled water to give a concentration of 4 unit/ml solution. Potato starch (0.5%, w/v) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride, was used as a substrate solution. 40 µl of plant extract or mucilage (20 mg/ml in DMSO), 160 µl of distilled water and 400 µl of starch were mixed in a screw-top plastic tube. The reaction was started by the addition of 200 µl of the enzyme solution. The tubes were incubated at 25 °C for a total of 3 min. Final concentrations in the incubation mixture were plant extract, 1 mg/ml, 0.25% (w/v) starch and 1 unit/ml enzyme. After 3 minutes 200 µl mixtures was removed and added into a separate tube containing 100 µl color reagent solution (96 mM 3, 5-dinitrosalicylic acid, 5.31M sodium potassium tartrate in 2M NaOH) and placed into 85 °C water bath. After 15 min, this mixture was diluted with 900 µl distilled water and removed from the water bath. α- amylase activity was determined by measuring the absorbance of the mixture at 540 nm. Control incubations, representing 100% enzyme activity were conducted in an identical fashion replacing plant extract with DMSO (40 µl). For blank incubations (to allow for absorbance produced by the plant extract), the enzyme solution was replaced with distilled water and the same procedure was carried out as above.

Percent (%) of α-amylase inhibition = \[
\frac{\text{Absorbance control}-\text{Absorbance test}}{\text{Absorbance control}} \times 100
\]

**In Vivo screening for the Hypoglycemic Activity of Hot Water Extract of Acacia nilotica (L.) Pods**

**Animals**

Adult male albino rats weighing 100–120 g obtained from the laboratory animal house of the National Researches Center, Dokki, Egypt. Rats were housed in clean cages and acclimatized to the laboratory condition with temperature (22–24 °C), 12-h light: 12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and to tap water.

**Preparation of Diabetic Rat**

Adult male albino rats were injected with alloxan purchased from Sigma–Aldrich chemical company (USA) intraperitonealy (120 mg/kg.). Nine rats were used in each group. Each animal was used once only in all of experiments. The food and water were removed from cages 12 h before blood sampling.

**Dose Calculation**

Dose for of hot water extract of Acacia nilotica (L.) pods was used as mentioned in 7.
The Dose of Fractions was Calculated as Follow

\[ \text{Dose} = (\text{yield \% of fraction}) \times \text{dose of extract}/100 \]

**Determination of the Median Lethal Dose (L.D_{50}) of the Extract**

Adult male albino rats with average weight of (100-120) were used for the determination of median lethal dose (L.D_{50}) of the crude extract was a single oral dose through a stomach tube.

The lethal dose fifty (L.D_{50}) of hot water extract of *Acacia nilotica* (L.) pods was determined and the mortality was recorded for 72 hours.

The determination of the lethal dose fifty (L.D_{50}) for the extract was carried-out through two important successive steps according to [11] as follow:

**Preliminary Test for LD_{50} of Hot Water Extract of Acacia nilotica (L.) Pods**

Five groups each group contains 4 rats, where the animal groups receiving the following doses of plant extract:

1\textsuperscript{st} group: receive 500 mg/kg of plant extract, 2\textsuperscript{nd} group: receive 1000 mg/kg of plant extract, 3\textsuperscript{rd} group: receive 2000 mg/kg of plant extract, 4\textsuperscript{th} group: receive 3000 mg/kg of plant extract and 5\textsuperscript{th} group: receive 4000 mg/kg of plant extract.

The minimum dose that killed animals in the group called maximum tolerated dose (MTD). The following dose that killed animals in the group called lethal dose (L.D_{100}), where the approximate L.D_{50} (App, LD_{50}) is calculated and this dose is used for the determination of median lethal dose (L.D_{50}) in the following steps.

The Approximate L.D_{50} (App. LD_{50}) = (MTD) + L.D_{100}/2

**Estimation of the Median Lethal Dose (L.D_{50})**

The dose of approximate L.D_{50} is multiplying with constant factor (1.1) and the following groups (6 rats each) taken the following dose:-

1\textsuperscript{st} group: received the dose of (approximate L.D_{50} \times 1.1) and number of rats died in the group was estimated for 72 hours, 2\textsuperscript{nd} group: received the dose of (approximate L.D_{50} \times 1.1^{2}) and number of rats died in the group was estimated for 72 hours, 3\textsuperscript{rd} group: received the dose of (approximate L.D_{50} \times 1.1^{3}) and number of rats died in the group was estimated for 72 hours, 4\textsuperscript{th} group: received the dose of (approximate L.D_{50} \times 1.1^{4}) and number of rats died in the group was estimated for 72 hours, 5\textsuperscript{th} group: received the dose of (approximate L.D_{50} \times 1.1^{5}) and number of rats died in the group was estimated for 72 hours.

Using the method of [14], a curve can be obtained between the administrated doses and number of animal died in the groups. From the curve, the dose which has the ability to kill half number of animals in the group is called the median lethal dose fifty (L.D_{50}) can be calculated.
Study of the *In vivo* Chronic Treatment Hypoglycemic Activity of the Crude Extract

Three days after alloxan injection, the rats with fasting blood glucose higher than 180 mg/dl were used for this experiment. Rats were subdivided into 3 groups:

**Group 1:** Control group: non diabetic rats that orally administered of a daily dose of 1 ml 3% tween 80 in water solution for 60 days.

**Group 2:** Diabetic control group: alloxanized diabetic rats that orally administered of a daily dose of 1 ml 3% tween 80 in water solution for 60 days.

**Group 3:** Diabetic-hot water extract of *Acacia nilotica* (L.) pods group: alloxanized diabetic rats that orally administered of a daily dose of 300 mg/kg b.w. for 60 days.

**Blood Sampling**

Blood samples were drawn from the retro-orbital plexus of the overnight fasted rats (12 hours) as described by 21.

**Determination of Blood Glucose Level**

Plasma glucose was determined using enzymatic colorimetric kit (from Vitro Scient, Egypt) according to 23.

**Safety Profiles of the Hot Water Extract of *Acacia nilotica* (L.) Pods**

**Determination of Glutamic Pyruvic Transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT)**

The determination of GPT and GOT activities were performed according to 19.

**Determination of Alkaline Phosphatase (ALK)**

The determination of ALK activity was performed according to 5.

**Determination of Urea activity**

Measurements were made according to the method designated by diamond Kits and referred to 17.

**Determination of Creatinine**

The determination of creatinine was carried out using the biomérieux reagent kits according to 4.

**Statistical Analysis**

Differences between vehicle control and treatment groups in biological screening were statistically analyzed by one-way ANOVA technique followed by the least significant difference (L.S.D). Results are expressed as mean ± S.E. Methods of statistical analysis were done according to 3.
The Hypoglycemic Activity of Fractions of the Hot Water Extract of *Acacia nilotica* (L.) Pods (Acute Treatment)

Albino mice were injected with alloxan (120 mg/kg, i.p.). Three days after injection, the mice with fasting blood glucose higher than 170 mg/dl were used for the experiments. Eight mice were used in each group. The extracts were administrated orally then blood sampling was carried out after one and two hours.

Mice were subdivided into 7 groups:

**Group 1:** Diabetic control group: alloxanized diabetic mice that orally administered a dose of 1 ml 3% tween 80 in water solution.

**Group 2:** Diabetic-hot water extract of *Acacia nilotica* (L.) pods group: alloxanized diabetic mice that orally administered a dose of 300 mg/kg b.w.

**Group 3:** Diabetic-mucilage of *Acacia nilotica* (L.) pods group: alloxanized diabetic mice that orally administered a dose of 55.5 mg/kg b.w.

**Group 4:** Diabetic-diethyl ether fraction of *Acacia nilotica* (L.) pods group: alloxanized diabetic mice that orally administered a dose of 68.1 mg/kg b.w.

**Group 5:** Diabetic-ethyl acetate fraction of *Acacia nilotica* (L.) pods group: alloxanized diabetic mice that orally administered a dose of 4.6 mg/kg b.w.

**Group 6:** Diabetic-butanol fraction of *Acacia nilotica* (L.) pods group: Alloxanized diabetic mice that orally administered a dose of 58.8 mg/kg b.w.

**Group 7:** Diabetic-water soluble fraction of *Acacia nilotica* (L.) pods group: alloxanized diabetic mice that orally administered a dose of 86.1 mg/kg b.w.

**Phytochemical Study of the Hot Water Extract of *Acacia nilotica* (L.) Pods**

**Investigation of the Diethyl Ether Fraction of the Hot Water Extract of *Acacia nilotica* (L.) Pods**

**Paper Chromatography of the Diethyl Ether Fraction of the Hot Water Extract of *Acacia nilotica* (L.) Pods**

Quantitative fractionation was carried out by applying 1 gm of the ether fraction on 3 mm paper chromatography alongside with the available authentic and subjected to mobile phase Butanol : Acetic acid: Water  4: 1: 5. Further purifications were done by using 15% acetic acid as mobile phase alongside with available authentics. The band no.1 and band no. 2 are 100 mg and 123 mg respectively.

**Identification of the Main Components of the Diethyl Ether Fraction by Data Dependent Mixture Triple Play ms/ms Technique**

A major band represented (44.44%) of the ether fraction. Therefore, isolation and identification of this band has been carried out using data-dependent mixture triple play ms/ms technique. Band 6 was eluted from PC using methanol and dried under vacuum. One milligram of the residue was dissolved in HPLC grade methanol and 1 µl was injected. This
technique helps in finding useful structural information about compounds in the fraction automatically.

There is no need to specify parent ions. The MS scan range was determined; the LCQ series MS detector can collect full scan MS data, pick the most intense parent ion in the spectrum, and fragment the ion to generate product ions which compared with reported data by $^{22}$.

Investigation of the Ethyl Acetate Fraction of the hot Water Extract of Acacia nilotica (L.) Pods

Fractionation of the ethyl acetate fraction of the hot water extract of Acacia nilotica (L.) pods has been carried out by applying 1 gm of the ethyl acetate fraction on 3 mm paper chromatography and subjected to mobile phase Butanol: Acetic acid: Water 4: 1: 5.

Two bands been isolated and purified by paper chromatography, then weighed. (87 & 130 mg respectively). Further purifications of 2 bands have been done using BAW 4:1:3 as mobile phase alongside with available authentic.

Investigation of the Butanol Fraction of the Hot Water Extract of Pods of Acacia nilotica (L.)

Two dimension PC using 15% acetic acid then BAW 4: 1: 5 have been used for isolation of major compounds of the butanol fraction alongside with the available authentics.

Quantitative fractionation of butanol fraction has been done by using 1 gm. The separated bands have been eluted by methanol then dried under vacuum and weighed.

Investigation of the Amino Acids of the Water Soluble Fraction of the Hot Water Extract of Acacia nilotica (L.) Pods:

Acid Hydrolysis of Water Soluble Fraction for Amino Acids Using HPLC

Three hundred mg of water soluble fraction was put in hydrolysis tube. Add 1ml of 6N HCl, the solution was freeze with a mixture of dry ice and ethanol and the tube was evacuated with a vacuum pump. The hydrolysis tube was closed by melting the glass with a suitable gas-burner. The material was hydrolyzed in an oven with a uniform temperature distribution of 110°C for 72 hour, and then the tube is cooled down in an ice-bath after hydrolysis. Afterwards, the solution was centrifuged in order to separate the insoluble components. The supernatant of the centrifuged solution was evaporated at 40°C in a rotary evaporator, dissolved in 1 ml distilled water and evaporated once again in order to remove traces of acids. The sample was dissolved with 2 ml of the sample diluting buffer.

HPLC Analysis of Amino Acids:

The HPLC analysis was performed on a model Eppendorf-Germany LC 3000 Amino acid analyzer. The flow rate was 0.2 ml/ min, the pressure of buffer was from 0 to 50 bars, the pressure of reagent was from 0 to 150 bars and the reaction temperature was 50°C.
RESULTS AND DISCUSSION

In vitro screening for the hypoglycemic activity using α-amylase inhibition technique:

The hot water extract of *Acacia nilotica* (L.) pods and its mucilage did not inhabit the activity of α-amylase. Thus; their hypoglycemic activity cannot be due to inhibition of the activity of α-amylase.

Determination of the Median Lethal Dose (L.D50) of the Water Extract

The median lethal dose fifty (L.D50) of the hot water extract of *Acacia nilotica* (L.) pods are found to be 3.2 g/kg of body weight.

In vivo Screening for the Hypoglycemic Activity of the Hot Water Extract of *Acacia nilotica* (L.) Pods and/or its Fractions:

In vivo Screening for the Hypoglycemic Activity of the Hot Water Extract

It can be concluded from Table (1) and Fig.1 that: Alloxan elevated the plasma glucose level in all animals. The change in the plasma glucose levels in both the control and diabetic control groups are insignificant. The decreases in plasma glucose levels by treatment with extract under investigation are significant, which proved the hypoglycemic activity of this plant. Also, it can be seen that the plasma glucose level decrease at percentage of 70% by the hot water extract of *Acacia nilotica* (L.) pods.

Table (1): Study of the In vivo treatment hypoglycemic activity of the crude extract

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg b.w</th>
<th>Mean ± S.E</th>
<th>% of change</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1 ml 3% tween 80</td>
<td>107±1.4</td>
<td>99±2</td>
<td>7%</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>1 ml 3% tween 80</td>
<td>391±1.36</td>
<td>407±2.6</td>
<td>-4%</td>
</tr>
<tr>
<td>Diabetic+ The hot water extract of</td>
<td></td>
<td>391±1.36</td>
<td>116±1.72</td>
<td>70%**</td>
</tr>
<tr>
<td><em>Acacia nilotica</em> (L.) pods</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Results are expressed as mean ± S.E (n=9).
- * Significant & ** High significant.
- Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with pot-hoc SPSS computer program.
Fig. 1: Blood sugar levels (mg/dl) of diabetic rats before and after treatments with water extract.

Safety profiles of the Hot Water Extract of *Acacia nilotica* (L.) Pods:

Determination of the Liver Functions in Treated Rats with the Water Extract

Analysis of the liver enzymes (GOT, GPT and ALT) proved that the changes are insignificant relative to the control group which proved the safety of the water extract on liver as shown in Table (2) and illustrated in Fig. 2.

Table (2): Effect of the crude extract on the liver enzymes in plasma of the treated rats

<table>
<thead>
<tr>
<th></th>
<th>GOT</th>
<th>GPT</th>
<th>ALK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.14±0.70</td>
<td>35±0.50</td>
<td>74.86±1.47</td>
</tr>
<tr>
<td>Diabetic</td>
<td>60.89±0.52</td>
<td>36.13±0.79</td>
<td>91±0.90</td>
</tr>
<tr>
<td>Hot water extract of <em>Acacia nilotica</em> (L.)</td>
<td>61.96±0.47</td>
<td>35.20±0.56</td>
<td>88.86±0.71</td>
</tr>
</tbody>
</table>

- Results are expressed as mean ± S.E (n=9).
- *Significant

Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with post-hoc SPSS computer program.
Fig. 2: Effect of the Water Extract on the Liver Enzymes of Plasma of the Treated Rats.

Determinations of the Kidney Functions in Treated Rats with the Water Extract

Analysis of urea and creatinine proved that the changes are insignificant relative to the control group which proved the safety of the crude extract on kidney as shown in Table (3) and illustrated in Figs. 3.

Table (3): Effect of the water extract on creatinine and urea in plasma of the treated rats

<table>
<thead>
<tr>
<th></th>
<th>Urea</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.57±0.90</td>
<td>0.76±0.035</td>
</tr>
<tr>
<td>Diabetic</td>
<td>19.86±0.60</td>
<td>0.89±0.03</td>
</tr>
<tr>
<td>Hot water extract of</td>
<td>18.29±0.39</td>
<td>0.80±0.036</td>
</tr>
<tr>
<td>Acacia nilotica (L.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Results are expressed as mean ± S.E (n=9).
- * Significant.

Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with post-hoc SPSS computer program.
Fig. 3: Effect of the Water Extract on Creatinine and Urea in Plasma of the Treated Rats.

The Hypoglycemic Activity of Fractions of the Hot Water Extract of *Acacia nilotica* (L.) Pods (Acute treatment).

As shown in table (4) and Fig.4, It can be concluded that the hot water extract of *Acacia nilotica* (L.) decreases the plasma glucose level by 39% after 1 hour and by 12% after 2 hours. The mucilage fraction of *Acacia nilotica* (L.) extract decreases the plasma glucose level by 24% after 1 hour and raises it by 21% after 2 hours, while the diethyl ether fraction raises the plasma glucose level by 2% after 1 hour and decreases it by 26% after 2 hours. The ethyl acetate fraction decreases the plasma glucose level by 37% after 1 hour and 10% after 2 hours. Also, the butanol fraction decreases the plasma glucose level by 21% after 1 hour and 22% after 2 hours and the water soluble fraction decreases the plasma glucose level by 41% after 1 hour and 28% after 2 hours.

Finally, the hot water extract of *Acacia nilotica* (L.) pods produces hypoglycemic effect more than its fractions.

**Table (4): Blood sugar levels (mg/dl) of diabetic mice after one hour and two hours of administrations of treatments with fractions of *Acacia nilotica* (L.) (n=8)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg b.w</th>
<th>Mean ± S.E</th>
<th>% of change from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 ml 3% tween 80</td>
<td>174±10.36</td>
<td>172±9.89</td>
</tr>
<tr>
<td>Diabetic + the hot water extract of <em>Acacia nilotica</em> (L.)</td>
<td>300</td>
<td>107±8.94</td>
<td>151±4.13</td>
</tr>
</tbody>
</table>
Diabetic+ Mucilage of *Acacia nilotica* (L.)

<table>
<thead>
<tr>
<th>Fraction Type</th>
<th>Mean</th>
<th>Standard Error</th>
<th>Mean ± Standard Error</th>
<th>Percentage Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic+ Ether fraction of <em>Acacia nilotica</em> (L.)</td>
<td>68.1</td>
<td>177±5.13</td>
<td>152±6.80</td>
<td>-2%</td>
</tr>
<tr>
<td>Diabetic+ Ethyl acetate fraction of <em>Acacia nilotica</em> (L.)</td>
<td>4.6</td>
<td>109±0.59</td>
<td>155±12.79</td>
<td>37%</td>
</tr>
<tr>
<td>Diabetic+ Butanol fraction of <em>Acacia nilotica</em> (L.)</td>
<td>58.8</td>
<td>138±5.23</td>
<td>135±4.29</td>
<td>21%</td>
</tr>
<tr>
<td>Diabetic+ Water soluble fraction of <em>Acacia nilotica</em> (L.)</td>
<td>86.1</td>
<td>103±4.86</td>
<td>124±5.88</td>
<td>41%</td>
</tr>
</tbody>
</table>

- Results are expressed as mean ± S.E (n=8).
- * Significant & ** High significant.
- Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with pot-hoc SPSS computer program.

**Phytochemical Study of the Hot Water Extract of *Acacia nilotica* (L.) Pods**

**Investigation of the Diethyl Ether Fraction of the Hot Water Extract of *Acacia nilotica* (L.) Pods**
Paper Chromatography of the Diethyl Ether Fraction of the Hot Water Extract of *Acacia nilotica* (L.) Pods

Paper chromatography of the isolated bands alongside with the available authentic either separately or as mixtures emphasized that one band is a methyl gallate 10% and another one is a gallic acid 12.3%. A reported data about the hypoglycemic activities of gallic acid and methyl gallate proved that they are potent inhibitor of brush border sucrase and other disaccharidases and thus could potentially interfere with the digestion of disaccharides causing hypoglycemia.

**Identification of the Main Components of the Diethyl Ether Fraction by Data Dependent Mixture Triple Play ms/ms Technique**

The obtained results are presented in Table (5) and Fig.5. We can conclude that:

Data-dependent mixture triple play ms/ms technique helped in discover this band of the ether fraction of the hot water extract of *Acacia nilotica* (L.) by comparing the obtained mass spectra of each of the detected component with that reviewed by. Six compounds were identified, representing 88.43% of the total compounds in band 6. These compounds are methyl gallate (36.13%) as the major compound, followed by Epicatechin-5-gallate (29%), Epicatechin-5, 7-digallate (13.6%), Epigallocatechin-5, 7-digallate (8.7%), Epicatechin (0.86%) and Epicatechin 3, 5, 7-trigallate (0.14%).

It is to be mentioned that polyphenols having galloyl residues interacted with sodium-dependent glucose transporter as antagonist-like molecules, playing a role in controlling the dietary glucose uptake in the intestinal tract producing hypoglycemic effect. Therefore, the obtained result of hypoglycemic activity of this fraction which appears to be rich in polyphenols having galloyl residues may be due to this fore mentioned mechanism.

Fig. 5: Chromatogram of Mass Scan of Band (6) of the Ether Fraction of the Hot Water Extract of *Acacia nilotica* (L.).
Table (5): Identified Compounds in the Ether Fraction of *Acacia nilotica* (L.).

<table>
<thead>
<tr>
<th>Compound</th>
<th>MS-1</th>
<th>Major Fragments</th>
<th>%</th>
<th>RT</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl gallate</td>
<td>183</td>
<td>183,169</td>
<td>36.1</td>
<td>2.28</td>
<td></td>
</tr>
<tr>
<td>Epicatechin</td>
<td>289</td>
<td>289, 245, 179, 125</td>
<td>0.86</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Epicatechin-5-gallate</td>
<td>441</td>
<td>289, 289, 169</td>
<td>29</td>
<td>9.05</td>
<td></td>
</tr>
<tr>
<td>Epicatechin-5,7-digallate</td>
<td>593</td>
<td>441, 289, 169</td>
<td>13.6</td>
<td>13.03</td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin-5,7-digallate</td>
<td>609</td>
<td>456, 305, 288, 179, 169</td>
<td>8.7</td>
<td>13.45</td>
<td></td>
</tr>
<tr>
<td>Epicatechin 3,5,7-trigallate</td>
<td>745</td>
<td>593, 441, 289, 169</td>
<td>0.14</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>
Investigation of the Ethyl Acetate Fraction of the Hot Water Extract of *Acacia nilotica* (L.) Pods

Yellow crystalline powder (18 mg) represent 1.8% of the fraction, melting point was 249 °C. It appeared as dull spot under UV and gave yellow fluorescence on spraying with AlCl$_3$ reagent.

The compound proved d to be naringenin on the basis of its chromatographic behavior, fluorescence under UV, melting point and mass spectrum. On complete acid hydrolysis no sugar was obtained in the aqueous phase and naringenin as an aglycone in the organic phase. Naringenin was previously isolated from *Acacia nilotica* (L.) [6]. A reported data about the hypoglycemic activities of naringenin proved that it improved insulin signaling and sensitivity and thereby promotes the cellular actions of insulin [10].

Also, yellowish-white crystal (23mg) representing 2.3%, melting point was 252 °C. It appeared as purple spot under UV. The compound proved to be gallic acid on the basis of its chromatographic behavior.

Investigation of the Butanol Fraction of the Hot Water Extract of *Acacia nilotica* (L.) Pods

White crystals (11mg) representing 1.1%, melting point was 202 °C. It appeared as purple spot under UV.

The compound was expected to be methyl gallate by comparing the chromatographic behavior with that of authentic.

Gray to slightly beige crystalline powder (12mg) representing 1.2%, melting point was 360 °C [20]. It appeared as purple spot under UV. The compound was identical in the chromatographic behavior to authentic ellagic acid. A reported data about the hypoglycemic activities of ellagic acid proved that it has inhibitory activity against aldose reductases so that taking it might be able to relieve diabetic complications [24].

Moreover, white crystal (10mg) representing 1% melting point was 203 °C; it appeared as purple spot under UV. The compound was expected to be epicatechin gallate by comparing the chromatographic behavior with that of authentic. The hypoglycemic of epicatechin gallate activity is due to its interaction with sodium-dependent glucose transporter as antagonist-like molecules, playing a role in controlling the dietary glucose uptake in the intestinal tract [12].

Furthermore, yellow amorphous powder (15 mg) represents 1.5% of the fraction. It appeared as brown spot under UV and gave yellow fluorescence on spraying with AlCl$_3$ reagent. The compound was expected to be quercetin 3-O-glycoside by comparing the chromatographic behavior with that of authentic [15, 16]. A reported data about the hypoglycemic activities of quercetin proved that it improved insulin sensitivity and tyrosine phosphorylation in fructose-fed animals and the effect was comparable with that of metformin [10].

Investigation of the Amino Acids of the Water Soluble Fraction of the Hot Water Extract of *Acacia nilotica* (L.) Pods

HPLC analyses of amino acids are illustrated in Table (6). It can be seen that:
The total amino acids represent 1.9% of ware water soluble fraction of *Acacia nilotica* (L.). Fifteen amino acids are identified. Proline acid is appeared as a major amino acid and represents 71.8% of total amino acids. Methionine and Tyrosine are present as a minor amino acid of and each represents 0.20% of total amino acids.

**Table (6): Amino Acids of the Water Soluble Fraction of the Hot Water Extract of *Acacia nilotica* (L.)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Time (min)</th>
<th>Percent in total amino acid</th>
<th>Percent in water soluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic</td>
<td>10.22</td>
<td>6.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Threonine</td>
<td>13.52</td>
<td>1.4</td>
<td>0.029</td>
</tr>
<tr>
<td>Serine</td>
<td>14.72</td>
<td>1.8</td>
<td>0.037</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>15.35</td>
<td>4.3</td>
<td>0.037</td>
</tr>
<tr>
<td>Glycine</td>
<td>23.23</td>
<td>0.93</td>
<td>0.019</td>
</tr>
<tr>
<td>Alanine</td>
<td>24.08</td>
<td>3.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Proline</td>
<td>24.17</td>
<td>71.8</td>
<td>1.43</td>
</tr>
<tr>
<td>Valine</td>
<td>29.50</td>
<td>0.73</td>
<td>0.015</td>
</tr>
<tr>
<td>Methionine</td>
<td>35.33</td>
<td>0.20</td>
<td>0.004</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>37.53</td>
<td>3.3</td>
<td>0.019</td>
</tr>
<tr>
<td>Leucine</td>
<td>38.65</td>
<td>0.95</td>
<td>0.025</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>41.32</td>
<td>0.20</td>
<td>0.004</td>
</tr>
<tr>
<td>Phenyllalanine</td>
<td>42.85</td>
<td>0.48</td>
<td>0.01</td>
</tr>
<tr>
<td>Histidine</td>
<td>50.65</td>
<td>3.18</td>
<td>0.06</td>
</tr>
<tr>
<td>Lysine</td>
<td>54.10</td>
<td>1.77</td>
<td>0.03</td>
</tr>
<tr>
<td>Arginine</td>
<td>62.85</td>
<td>1.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. It affected about 171 million people worldwide in 2000 and the number is projected to increase to at least 366 million by 2030. 26

This study proved that folk medicine is still widely practiced by the population in Egypt, and the use of medicinal plants constitutes the common legacy of all Egyptians. Despite the
penetration of the modern medicine, traditional medicine continues to be a viable health alternative for the large underprivileged section of the Egyptian population. Thus, it is important to document and restore the remains of ancient medical practices that still exist in Egypt and other parts of the world, and preserve this knowledge for future generations.

This study shows the potential hypoglycemic activity of one of the most important plants in Egyptian traditional medicine. In conclusion, our results have shown that the hot water extract of *Acacia nilotica* (L.) pods; possess hypoglycaemic effect on alloxan-induced hyperglycemic rats orally. Thus, the folk use of these plants may be validated by this study. However, controlled clinical trials will be required to confirm its hypoglycemic action and general safety.

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