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Phytochemical Screening of *Abelmoschus esculentus* From Leptis area at Al-Khums Libya

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Abstract
Abelmoschus esculentus L. is used in Libya as a nutrient and as a medicinal plant for treating many diseases. The coarse powders of *Abelmoschus esculentus* L fruits and leaves were subjected to successive extraction with Distilled water and Ethyl Alcohol solvents (each separately) by maceration method. Qualitative and quantitive phytochemical analysis of fruits and leaves of aqueous and ethanolic crude extracts of this plant showed the presence of various phytochemical constituents such as tannins, steroid, flavonoids, saponin, alkaloids, anthraquinones, phenol, terpenoids and cardiac glycosides, while resin is present in the leaves aqueous crude extracts and absent in ethanolic crude extracts of fruits. Some of these secondary metabolites like alkaloids, phenolic and terpenoids were examined by visualizing tests were for more predictable intended for a presence of each within using TLC analysis. The quantitative results of the fruits and leaves crude extracts of *Abelmoschus esculentus* L. revealed the percentage yield of the chemical constituents 31.6 and 20.2 for leaves aqueous and ethanolic crude extracts and for fruits was 17.6 and 13.4 % respectively. While the 11.7, 16.4 and 9.4 each for flavonoids, saponin and alkaloids for fruits and 33.6, 28.21 and 18.7 for leaves respectively. Furthermore, there is no previous preliminary phytochemical, and evaluation of their therapeutic properties and other evidence about this medicinal plant in Libya.

Keywords: *abelmoschus esculentus* l., maceration, crude extracts, phytochemical constituents, secondary metabolites

1. Introduction
The presence of chemical constituents in plants is important for consuming as nutrition and treatment against several diseases. These phytochemicals are essentially classified into two groups and which are primary and secondary metabolites based on the utility in plant metabolism. The primary metabolites are included common Carbohydrates, Amino acids, Proteins and Chlorophylls while secondary metabolites included of Flavonoids, Saponins, Alkaloids, Tannins, Steroids, and etc [1, 2]. Likewise, the phytochemical components are the source for various pharmaceutical productions. As such constituents are performing a vital role in the classification of crude drugs [3]. Though the using and advantage of plants and its crop for treating of many diseases since old time. The World Health Organization (WHO) found that 80% of the people of developing countries still rely on conventional medicines, often plant drugs, for their primary health care obligations [4]. The diversity in the plant crude extracts has different usages of its potentialities in treating ailments. Additionally, it could use as food preservative also [5]. Mostly, fruits and vegetables showed the significant useful properties, mainly in maintaining a brilliant health and nutritional potentials in the area of prevention and delay in the beginning of chronic diseases and the providing of enzymes and vitamins necessary for suitable body function [6]. *Abelmoschus esculentus* L., Okra or Lady's Finger is known by various local names in different parts of the world. E.g. it is called Bamia in Libya; which is distributed widely in Africa, Asia, Southern Europe, and America [7]. The mucilage of *Abelmoschus esculentus* L. binds cholesterol and bile acid carrying toxins deposited into it by the liver. Correspondingly, *Abelmoschus esculentus* L., the furthermost commonly vegetable yields in Asia and Africa and was carried through North Africa and the areas bordering the Mediterranean and eastward [8]. Moreover, is the vegetable of high value due to its high nutritional importance [9], and contains a great proportion of fibres, which supports to stabilize blood sugar by improving the rate as a result of which sugar is absorbed from the intestinal tract. As well as the presence of these fibres together with other nutrition, which manifests it's advantageous for lowering blood sugar levels within the body, assisting along with diabetes and the fibres, hence improves the concentration of the blood sugar level by reducing sugar absorption through the intestines [10]. *Abelmoschus esculentus* L. has a beneficial against spermatorrhoea, chronic dysentery and genitourinary illnesses [11], its therapeutic importance in relief from haemorrhoids and healing of ulcers [12]. The main purpose of the present study was the phytochemical investigation of *Abelmoschus esculentus* L. material and screening for the presence of various phytochemicals in this medicinal plant.

2. Materials and methods
2.1 Collection of plant sample
The fresh, mature, healthy fruits and leaves of *Abelmoschus esculentus* L. were collected locally from the farmland of Leptis area in Alkhums (Libya). The collected plant samples were botanically identified and confirmed by the Biology

Department Science College, El Mergeb University Al-Khums Libya. The samples of the selected plant were used for the purpose of their qualitative and quantitative phytochemical analysis by carried out using standard methods in the Chemistry Department, Science College Al-Khums University of El-Mergeb Al-Khums Libya. The collected samples of fruits and leaves, washed via tap water, then with distilled water after that drying in shadow and finish drying in oven 45 ºC, after that grounded the dry samples separately into a fine powder using an electric mill, while the dried samples were crushed using a porcelain mortar and pestle (trituration method) to enhance the surface area for absorption of the solvents [13-14].

2.2 Preparation of the extracts

The coarse powders of *Abelmoschus esculentus* L fruits and leaves were subjected to successive extraction with Distilled water and Ethyl Alcohol solvents (each separately) by maceration method and soaked in the appropriated solvents at room temperature for 72 h while continuing shaking. Extracts were filtered through No. 1 Whatman filter paper. The filtrate was then vacuumed rotary evaporated to concentrate the extracts. The extracts were subjected to qualitative and quantitative phytochemical analysis (examined by thin-layer chromatography (TLC) on silica gel using various solvents (Dragendorff's, Vanillin, Ferric Ferro cyanide) systems and visualized using standard methods i.e. UV-254/364 nm and sprayed with suitable reagents) [13].

2.3 Qualitative Method of Analyses of Phytoconstituents

The different qualitative chemical examinations were carried out for establishing the producing extract and for finely powdered samples to identify numerous Phyto-constituents present in them [13, 15, 16].

2.3.1 Test for tannins

1 g of the each of each of the dried powdered samples (separately) was boiled separately in 40 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration.

2.3.2 Test for steroids

4 ml of acetic anhydride was added to 1 g of each of the crude extract (separately) with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples, indicating the presence of steroids.

2.3.3 Teat for flavonoids

Three procedures were used to determine the presence of flavonoids in the plant sample:

1. About 0.5 of each of the powdered plant sample (separately) was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed, indicating a positive test for flavonoids.
2. 5 ml of dilute ammonia solution was added to about 0.25 of the aqueous filtrate of each plant extract (separately) followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.
3. 3- 4 drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed, indicating the presence of flavonoids.

2.3.4 Test for Saponin

About 1 g of each of the powdered sample (separately) was boiled in 10 ml of distilled water in a water bath and filtered. 5 ml of the filtrate was mixed with 2-3 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 1-2 drops of olive oil and shaken vigorously, then observed in the formation of emulsions.

2.3.5 Test for alkaloids

5 ml of each of the crude extracts (separately) was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate was treated separately with both reagents (Maeyer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate development [17, 18, 19].

2.3.6 Test for Anthraquinone

5 ml of each of the crude extracts (separately) was boiled with 10 ml of sulfuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was a pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes [15, 16].

2.3.7 Test for Phenols

To 2ml of various crude extracts of samples, 4ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution were added. Development of blue or green colour indicated the presence of phenols [17, 20, 21].

2.3.8 Test for Resin

2 ml of various crude extracts were treated with a few drops of acetic anhydride solution followed by 2 ml of concentrated Sulphuric acid (H_2SO_4). Resins give colouration ranging from orange to yellow [17, 18, 21].

2.3.9 Test for Terpenoids: (Salkowski's test) 10ml of the various crude extract was mixed in 4 ml of chloroform followed by the careful addition of 5ml concentrated (H_2SO_4). A layer of the reddish brown colouration was formed at the interface, thus indicating a positive result for the presence of terpenoids [17, 20, 21].

2.3.10 Test for Cardiac Glycosides: (Keller-Killani test) 5 ml of various crude extracts was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, after that the addition of 1 ml concentrated Sulfuric acid. Brown ring was formed at the interface which indicated the presence of deoxy sugar of cardenolides. A violet ring may appear below the brown ring, though in the acetic acid layer, a greenish ring may also form just progressively throughout the layer [15, 17-20].

2.3.11 Preparations of Thin Layer Chromatography Plates (TLC)

Plates were established in the laboratory by covering 20cm x 20cm plates with silica gel. About 50g of silica gel UV 254 nm was weighed in a weighing balance and using a filter funnel poured into 250ml of Mayer's flask, 90mls of distilled water was added and the mixture agitated carefully for 5minutes until uniform slurry was obtained, the slurry was rapidly decanted into the spreader and rapidly swept across the plates with 0.02mm thickness, the plates were spread out to dry at room temperature for about 6 Hours.

2.3.12 The Chromate-Plates (samples spotting on)

The chromate-plates were allowed to develop till the solvent front was about 5/6 of the plate. The plates were developed in the following solvents; chloroform: hexane (75:25; 50:50; 20:80), chloroform: methanol: hexane (1: 1:2), MeOH: CHCL 2: EtOAc (1:1:2), MeOH: EtOAc: H2O: Acetic acid (10:30:1 Drop: 5 Drops). They were then removed and allowed to dry in open air after marking the solvent front; the chromate-plates were then observed under UV light at a wavelength of 254 nm and 365 nm then sprayed with reagents. The best mobile phase giving best results was presented [13, 14].

2.3.13 Preparation of the development basins mobile phases

Four development basins (6x12x15) cm, the solution of each developing solvent was then decanted into the basins and covered to attain equilibrium for a period of 15minutes. Development was allowed to ensue until the solvent obverse. The plate was then removed from the chamber and the solvent front directly noticeable with a pointed object. The plate was then allowed to dry in a fume cupboard. The location of the separate solutes was positioned by various methods. Coloured constituents can be seen accurate when observed in contradiction of the stationary phase whilst colourless species were perceived by spraying the plate with a suitable reagent, which produced coloured zones in the areas, which they inhabit [3].

2.3.14 Preparation of reagents

2.3.14.1 Preparation of Mayer's reagent

0.71 g of mercuric chloride was dissolved in 120 ml of distilled water. 1 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions were mixed and volume was raised to 200 ml with distilled water.

2.3.14.2 Preparation of Dragendorff's reagent

Solution A: 3.4 g of basic bismuth nitrate and 40 g of tartaric acid were dissolved in 160 ml of distilled water.

Solution B: 32 g of potassium iodide was dissolved in 80 ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

2.3.15 Detection of Alkaloids, Phenolics and Terpenoids using TLC

The chromate-plates were activated in an oven at 100 ^{o}C for about 15minutes. After complete cooling, they were spotted using the dry aqueous extracts and ethanol extracts samples. Each sample was spotted 2 cm apart using a very thin capillary tube. An air blower was used to dry the samples spots so as to control the spot size; ethanol in four beakers was used to clean the thin capillary tube before spotting the next sample. After the plates were developed, they were left to dry for about 15minutes, then viewed under U.V fluorescence light at wavelength 254 nm and 365 nm, and finally sprayed with the required detection reagent either Dragendorff's, Ferro-cyanide, and Vanillin respectively) to determine the compounds present and the solvent system which gave the best observation and results is presented [3].

2.3.16 Detection of Alkaloids (Dragendorff's s test)

The dry samples were dissolved in dichloromethane and then spotted on a thin layer chromatography plate which was developed in 20 % hexane in ethyl acetate. The presence of alkaloids in the developed chromatogram was detected by spraying with freshly prepared Dragendorff's reagent in a fume chamber. A positive reaction on the chromatogram indicated by an orange or darker coloured spot against a yellow background is confirmatory evidence that the plant extract contained alkaloids.

2.3.17 Detection of Phenolics (and preparation of Ferric Ferro cyanide reagent)

10% of iron chloride ($FeCL_3$ (Aqus) was mixed with iron cyanide ($FeCN_6$) (1g/100ml) or 0.1g/10ml) 0.1g of ferric chloride and 0.1g of potassium Ferro cyanide ($K_3F_3CN_3$) was freshly prepared by dissolving in 10ml of distilled water. Equal portions of 1 and 2 were mixed, sprayed to the plates and heated at 110°C. Change of colour to blue (instant) indicates the presence of Phenolics [3, 4].

2.3.18 Detection of Terpenoids (and preparation of Vanillin reagent)

10% of vanillin was dissolved in Ethanoic acid – concentrated Sulphuric acid in a ratio of 2:1 mixed and sprayed onto the plates and then they were put in the oven for 15mins. Presence of terpenoids was indicated by the separation into different colors; brown, dark green and purple colour [13, 14].

2.3.19 Detection of flavonoids

TLC plate was exposed to ammonia. The presence of flavonoids is indicated by coloured spots such as yellow, pink, grey and brown spots [13].

2.3.20 Detection of Anthraquinones

TLC plate is sprayed with a solution of 5 ml CH_3OH and 5 g potassium hydroxide (KOH). Change of the original yellow, brown colour to purple shows a positive test [13].

2.4 Quantities analysis of Phytoconstituents

2.4.1 The percentage yield: Successive value

Accurately weighed of the fresh, mature, healthy fruits and leaves of the selected plant were coarse and air dried material was subjected to maceration extraction 72 hours with appropriate solvents (ethanol and water) separately. The extracts were filtered, concentrated and the solvent was removed by vacuum distillation. The extracts were dried in the vacuum desiccator and the residues were weighed [18]. Which contain maximum chemical compound are these sorts as

depending upon the solvent nature and categories. Empty bottles were weighed using an analytical balance and their weights noted and labelled. The extracts from the distillation flask were then transferred to the bottles using a spatula and then weighed again. The bottles were labelled appropriately, stating the name of the extract, the name of the plant, the solvent used, and the weight stored. The tight bottles were stored at fridge until further use [13, 14]. The percentage yield of each crude extract was calculated by (1).

$$\text{Percentage Yield \%} = \frac{\text{Wt. of dried extract}}{\text{Wt. of sample}} \text{X} 100 \quad (1)$$

2.4.2 Quantitative Method of Analyses of Phytoconstituents

2.4.2.1 Flavonoids Determination: 20 g of the coarse powders of *Abelmoschus esculentus* L fruits and leaves (separately) were extracted recurrently with 200 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath; the dry content was weighed to a constant weight [22, 23].

2.4.2.2 Alkaloids Determination

10 g of the coarse powders of *Abelmoschus esculentus* L fruits and leaves were weighed (separately) into a 500 ml beaker and 400 ml of 10% acetic acid in ethanol was then added, the reaction mixture were covered and allowed to stand for 4 hours. This was filtered, extracted and concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue was the alkaloid, which was dried and weighed to a constant mass [16].

2.4.2.3 Saponins Determination

10 g of the coarse powders of *Abelmoschus esculentus* L fruits and leaves were weighed (separately) into a conical flask after which 50 ml of 20 % aqueous ethanol were added. The mixture was heated using a hot water bath. At about 55°C, for 4 hours with continuous stirring, after which the mixture was filtered and the residue re-extracted with a further 100 ml of 20% ethanol. The combined extracts were reduced to 20 ml over a water bath at about 90°C. The concentrate was transferred into a 100 ml separatory funnel and 10 ml of diethyl ether were added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 30 ml of n-butanol were added. The combined n-butanol extracts were washed twice with 5 m1 of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the Saponin content was calculated as the percentage of the starting material [24].

3. Results and discussion

The phytochemical constituents are accountable for therapeutic activity of plant species. Hereafter in the present research the qualitative and quantitive phytochemical analysis of fruits and leaves of *Abelmoschus esculentus* L was carried out and the results as showed at following tables 1 and 2.

Table 1: Quantitative analysis of fruits and leave crude extracts of *Abelmoschus esculentus* L

Plant's Name		Percentage Yield (%)				
		Aqueous Extract (%)	Ethanolic Extract (%)	Flavonoids (%)	Saponins (%)	Alkaloids (%)
Abelmoschus esculentus L	Leaves	31.6	20.2	33.6	28.21	18.7
	Fruits	17.6	13.4	11.7	16.4	9.4

Analysis of the quantitative analysis of fruits and leaves crude extracts of *Abelmoschus esculentus* L. revealed the percentage yield of the chemical constituents 31.6 and 20.2 for leaves aqueous and ethanolic crude extracts and for fruits was 17.6 and 13.4 % respectively. While the 11.7, 16.4 and 9.4 each for flavonoids, saponin and alkaloids for fruits and 33.6, 28.21 and 18.7 for leaves respectively.

Table 2: Qualitative phytochemicals analysis of the fruits and leaves crude extracts of *Abelmoschus esculentus* L:

Chemical Components	Plant Extracts			
	Aqueous Extract		Ethanolic Extract	
	Leaves	Fruits	Leaves	Fruits
Tannin	+++	++	+++	+++
Steroids	+++	++	+++	++
Flavonoids	+++	+++	+++	+++
Saponnins	+	++	+	+
Alkaloids	+	+++	+++	+++
Anthraquinons	+	+	+	+
Phenols	+++	++	+++	+
Resin	++	-	++	-
Terpenoids	+++	++	+++	++
Cardiac Glycosides	+++	+++	+++	+++

+++ = Reach, ++ = Moderate, + = good, - = Absent.

The results of the qualitative phytochemical analysis of aqueous and ethanolic crude extracts of fruits and leaves as showed above in table 2 the presence of various secondary metabolites like tannins, steroid, flavonoids, Saponin, alkaloids, anthraquinones, phenol, terpenoids and cardiac glycosides, and absent of resin in aqueous and ethanolic extracts of fruits. Consequently, in this research, the phytochemical analysis and the visualizing tests were for more predictable the presence of each alkaloid, phenolic and terpenoids using TLC analysis to propose that the properties of chemical components have a potential for remedial various diseases. For instance, the main objective of diabetes regulatory is to maintain blood glucose level to prevent diabetes induced complications, and traditionally, it was used as an alternative cure for diabetes [25] and when taken regularly as a part of diet has shown a protective effect against Diabetes [26]. Also, the *Abelmoschus esculentus* may be considered by means of medicinal plant used as a nutritional for enhancement in dipping the blood glucose level in hyperglycemia induced by diabetes and as an important component of preventive therapy in the management of diabetes and its related complications. Moreover, the fruit mucilage is used to indulgence diarrhoea in acute inflammation, intestines and dysentery and kidneys catarrhal infections, ardour urine, dysuria irritation of the stomach and gonorrhoea [27].

4. Conclusions

Regardless of rarer various studies on medicinal plant resources of Libya, plenty of medicinal plants and associated indigenous users nevertheless wait for proper documentation and evaluation of their therapeutic properties. Phytochemical screening of aqueous and ethanol extract of *Abelmoschus esculentus* L leaves was revealed the presence flavonoids, tannins, terpenoids, saponins, steroids, alkaloids by the positive reaction with the respective test reagent. Results obtained in this investigation indicate that *Abelmoschus esculentus* L leaf extract, rich in the chemical components exhibited highest activities.

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