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Qualitative and quantities analysis of phytochemicals of various extract for *Ephedra altissima* from Libya

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Abstract

Phytochemical screening is an important step which principals to the isolation of some compounds. Concluded using different organic solvents such as ethanol, chloroform and acetone otherwise using aqueous extracts of leave of *Ephedra (Ephedra altissima)*, The chemical constituents (Qualitative and Quantities analysis) all most presented in crude extracts of plant (Tannins, Saponins, Flavonoids, Cardiac glycosides and Alkaloids) formerly Steroids; Terpenoids and Anthraquinones not presented in some crude extracts were considered. Moreover, results proved that quantities analysis of ethanolic extract of leaves had significant amount of chemical compounds 87% moreover the aqueous crude extract were moderate 82% and the Chloroform 79% and acetone extracts 77% were lowliest.

Keywords: *Ephedra*, Crude Extract, Ethanolic Extract, Qualitative and Quantitate analysis.

1. Introduction

The nature has its credibility. Plants a rich source of chemical ingredients, so that are attractive detects presence of its chemical components, therefore used as natural product for the treatment of various diseases [1-2]. Moreover, Plants have continuously a constituent of natural product for the treatment of various diseases [3]. Furthermore, the demand of medicinal plants is increasing in many plants needs through pharmacological investigations. The medicinal value of these plants can be perceived from the chemical agents they possess which may alter certain physiologic actions in the human body. The most important of these bioactive constituents of plants are terpenes, alkaloids, flavonoids and phenolic compounds [4]. In addition, some medicinal properties are still obscured with in plant which need through scientific investigative. *Ephedra (Ephedra altissima)*, where it is an herbal stimulant drug composed of two active compounds (pseudoephedrine and ephedrine) and that are of ingredients in many over-the-counter products. The synthesized form of ephedra ephedrine can be produced by chemical synthesis and in its pure form (ephedrine sulfate) is an unpleasant tasting. Ephedrine and pseudoephedrine stimulate the opening of air passages in the lungs and are used as decongestants for the short-term treatment of asthma, bronchitis, and certain allergic reactions. Ephedrine and pseudoephedrine are also used in dietary supplements that claim to promote weight loss and enhance athletic performance [5]. Roughly active compounds are structurally related to amphetamines; they play similar, whereas less potent, roles in stimulating the central nervous system. Ephedrine is frequently the primary ingredient found in illegally synthesized drugs, including methamphetamines [6]. *Ephedra (Ephedra altissima)*, known in Libyan by local traditional name Khadraia. *Ephedra altissima* is a species of *Ephedra* that is native to the western Sahara (Libya, Algeria, Tunisia, Morocco, Chad, Mauritania), [7-8]. Thus, main objective of this research work is to consider the phytochemical screening. (Qualitative and Quantities) of the content which is present in different crude extracts.

2. Material and Methods

2.1 Plant collection (Source of plant material)

Fresh leaves samples of *Ephedra altissima* were collected from naturally growing populations located in Missalata district in Libya. The samples were identified at the herbarium section of the Department of Biological Sciences, Faculty of Science El-Mergeb University Alkhums. Libya. The fresh leaves plant samples were washed by tap water, air dried in shadow at room temperature milled well into a fine powder in a mixer grinder and sieved to give particle size of 50-150mm. then stored until needed.

2.2 Preparation of leave extracts

The aqueous and organic extracts (ethanol, chloroform and acetone) of fine pulverized leaves were prepared by taking the weighed amount of fine powdered leaves sample separately in proper volume of distilled water and organic solvent at a 40% (w/v) concentration (200g leaves powder in 500 ml water) for 72h with alternating shaking, and then extracted considerable were filtered through Whatman filter paper No. 1, Subsequently, by using rotary evaporator the solvents were separated. The dried yield were stored in refrigerator at 4 °C until further use

2.3 Qualitative Phytochemical Analysis

Chemical investigations were carried out both on the ethanolic chloroform, acetone and aqueous extract using standard procedures to identify the constituents [9-13].

2.4 Test for Tannins

About 0.5 g of the extract (dry extract) was dissolved in distilled water and about 10 ml of bromine water added. Decolouration of bromine water indicated the presence of tannins.

2.5 Test for saponins

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtered sample was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously to observe for the formation of emulsion.

2.6 Test of Flavonoids (Alkaline Reagent Test)

10 ml of each extracts (separately) were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

2.7 Test of Terpenoids (Salkowski's test)

10 ml of each extracts (separately) were mixed in 4 ml of chloroform, and concentrated H₂SO₄ (6 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show the presence of terpenoids.

2.8 Test of Cardiac glycosides (Keller-Killani test)

10 ml of each extracts (separately) were treated with 4 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated Sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.9 Test for alkaloids

10 ml each extracts (separately) were treated with 8 ml of picric acid solution in a test tube. The formation of orange coloration indicated the presence of alkaloids in the extract.

2.10 Test for Anthraquinones

6 g of the powdered sample (separately) were soaked into 20 ml of benzene in a conical flask. The mixture was allowed to stand for 10 min then filtered. 10 ml of 10% ammonia solution was added to the filtrate and shaken for 30 sec. Pink, red or violet colour in the ammonia phase indicated the presence of Anthraquinones in the extracts.

2.10.1 Quantitative phytochemical analysis

2.10.2 Extraction of Plant Materials

The quantities of the phytochemicals present were determined using the methods of Harborne J.B., 1973 and Obadoni B.O.,

Ochuko B.O, 2001 and D. Krishnaiah 2009. [13-15] as shown below:

The extracts were weighed after separating the solvents by evaporated under reduced pressure and dried using a rotary evaporator at 55 °C then a percentage yield for each extract was calculated as:

$$\text{Yield \%} = \frac{\text{Final weight of extract}}{\text{Total weight of ground plant}} \times 100$$

2.10.3 Alkaloid Determination

6g of the sample were weighed into a 500 ml beaker and 500 ml 25% acetic acid in ethanol was added and covered to stand for 4hrs. This was filtered and the extract was concentrated using a water-bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and weighed [13-14].

2.10.4 Saponin Determination

25g of fine powdered leaves were separate in 250ml of 25% ethanol. The suspension was heated over a hot water bath for 4hrs with continuous stirring at about 60 °C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90 °C. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous fraction was recovered while the ether layer was discarded. The purification process was repeated thrice. 60ml of n-butanol extracts were washed twice with 10ml 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 in percentage [14].

2.10.5 Determination of flavonoids

10 g of fine powdered leaves were frequently extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference [16].

3. Results and Discussion

3.1 Screening of Phytochemicals

Preliminary phytochemical analysis of leaves extracts of plant. Using different solvent (Aqueous, ethanol, chloroform and acetone) revealed the presence of various phytochemical, Phytochemical screening of bioactive compounds for the four extracts have been analyzed in this study and there is wide range of phytochemical compounds present in the four extracts as presented in table 1. Phytochemical analysis exposed the presence of Tannins, Saponins, Flavonoids Steroids, Terpenoids, Cardiac Glycosides, Alkaloids and Anthraquinones in plant leave. In accordance with the table flavonoids were observed to be highly extracted in both Chloroform and acetone followed by ethanol and aqueous system, whereas studies proved that aqueous system was potent in the extraction of saponins when the ethanolic, chloroform and acetone were found to be the least effective solvents in extracting saponins, which could be due lesser amount of compounds in the plant, which could be dissolved in these solvents. Cardiac glycosides were observed to be highly extracted in both ethanolic, Chloroform and acetone when compared with aqueous extract. The ethanolic extract was found to be a significant solvent in

the extraction of Steroids, Terpenoids, Cardiac glycosides and Alkaloids. On another hand, the ethanolic was the poor solvent in extracting of the Saponins. Alkaloids were moderately extracted by aqueous and ethanolic extract in the leaves. Terpenoids and Anthraquinones were found to be absent in both

the Chloroform and acetone extracts. Overall results suggested that Ethanolic was the best solvents, aqueous to be moderate one and Chloroform and acetone were poor solvents in the extraction of the phytochemicals.

Table 1: Phytochemicals analysis in the leaves extracted by various solvents

Chemical Constituent	Aqueous Extract	Ethanolic Extract	Chloroform Extract	Acetone Extract
Tannins	++	+++	+	+
Saponins	+++	+	+	+
Flavonoids (Alkaline Reagent Test)	++	++	+++	++
Steroids	-	+++	+	+
Terpenoids (Salkowski test)	+	+++	-	-
Cardiac glycosides (Keller-Killani test)	++	+++	+++	+++
Alkaloids	+++	+++	++	++
Anthraquinones	-	+++	-	-

+ = low concentration, ++ = moderate concentration, +++ = high concentration, - = absent.

Table 2: Quantification analysis of the leaves extracted

Plants Name	percentage yield %				Flavonoids In 1g %	Saponins In 1g %	Alkaloids In 1g %
	Aqueous extract	Ethanolic extract	Chloroform Extract	Acetone Extract			
<i>Ephedra altissima</i>	82	87	79	77	0.49	0.59	0.52

3.2 Quantification of Flavonoids and Tannin

Alkaloids, Saponins and Flavonoids content and percentage yield were measured in all the extracts of *Ephedra altissima* leaves were presented in Table 2. Determined that the percentage yield of ethanolic, aqueous, chloroform and acetone extracts were 87, 82, 79 and 77% respectively. And showed the presence of saponins, alkaloids and flavonoids were 0.59, 0.52 and 49 in the plant extracts of leaves respectively. Obviously the lowest levels were the flavonoid content and alkaloids were moderate where the percentages of saponins were considerable.

4. Conclusion

In conclusion, leaves from the *Ephedra altissima* plants possessed equally good inhibitory activity against the tested bacteria. Aqueous, Ethanolic, chloroform and acetone extracts of leaves showed almost comparable phytochemical activity, which might be support their traditional use against infectious diseases, also the presence of most general phytochemicals possibly responsible for their therapeutic effects. It further imitates optimism for the advance of many more novel chemotherapeutic agents or templates from such a plant which in future may serve for the production of synthetically improved therapeutic agents.

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