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Comparative phytochemical and antimicrobial investigation of some plants growing in Al Jabal Al-Akhdar

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ABSTRACT

El-Jabal Al Akhdar area possesses unique physiographic and climatic conditions that provides an excellent ecological niche and contributed to the restriction of many plant species. Among the most widely used plants are Arbutus pavarii [family Ericaceae], Pistacia lentiscus [family Anacardiaceae] and Myrtus communes [family Myrtaceae]. Although the fruits of these plants are commonly considered as an edible fruits for human and animals, yet their nutritive values are not extensively studied, as well as their leaves. The results of chemical analysis of their fruits indicated that there is a high amount of carbohydrates, 50 %, 42.14% and 4.73% for Arbutus, Myrtus and Pistacia respectively. The total percentages of protein in the three studied plants were present with variable quantities; the highest protein contents were found to be for Myrtus 5.63% followed by Pistacia 5% and the lowest was for Arbutus 2.18%. Determination of sodium, potassium, calcium and phosphorus contents revealed that there is a high level of both sodium, and potassium 0.46 % and 2.67% in Pistacia fruits, while the highest calcium content was for both Myrtus and Pistacia 0.38%&0.37% respectively .Myrtus fruits were found to contain the high level of phosphorus 0.13 mg/one gram powdered plant. The percentages for moisture [gravimetrically] and total phenolic contents were determined using ferricyanid complex assay [spectrophotometric prussion blue method]. At the same time, preliminary photochemical screening of their fruits and leaves was carried out .The total alcohol extracts of their leaves exhibited a significant antimicrobial activity against both tested bacteria and fungi.

INTRODUCTION

Libya has tremendous wealth of medicinal plants scattered in all over a vast area. These plants are used in Linyan folklore medicine for their medical as well as nutritive values. **Myrtus comunus**, **Arbutus pavarii** and **Pistacia lentiscus** are three widely used plants in Al -Jabal Al -Akhdar region .

Myrtus comunus is known as **common myrtle**. The plant is native to southern Europe and north Africa, commonly cultivated as an ornamental garden shrub and widespread in the Mediterranean region .The extract of the myrtle herb is used as antiseptic; aromatic; astringent; balsamic; carminative; haemostatic; hair tonic and in the treatment of internal ulceration and rheumatism. The plant is taken internally in the treatment of urinary infections, digestive problems, bronchial congestion, sinusitis and dry coughs. Externally, it is used in the treatment of acne (the essential oil is normally used here), wounds, gum infections and hemorrhoids. An essential oil obtained from the plant is antiseptic and is used locally in the treatment of rheumatism. The fruit is carminative.

Pistacia lentiscus (**Mastic**) is an evergreen shrub or small tree cultivated for its aromatic resin. It is native throughout the Mediterranean region, from Morocco and Iberia in the west through southern France and Turkey to Iraq and Iran in the east. Mastic resin is a relatively expensive kind of spice, that has been used, principally, as a chewing gum, for at least 2,400 years. Mastic gum is principally used either as a flavouring or for its gum properties, as in mastic chewing gum. In Lebanon and Egypt, the spice is used to flavour many dishes, ranging from soups to meats to desserts, The resin is used as a primary ingredient in the production of cosmetics such as toothpaste, lotions for the hair and skin, and perfumes. People in the Mediterranean region have used mastic as a medicine for gastrointestinal ailments Regular consumption of mastic has been proven to absorb cholesterol, reduce the risk of heart attacks. Mastic oil also has anti-bacterial and anti-fungal properties, and as such is widely used in the preparation of ointments for skin disorders. It is also used in the manufacture of plasters. Mastic can heal peptic ulcers by killing Helicobacter pylori, which causes peptic ulcers, gastritis, and duodenitis. Mastic gum is also used in the production of high grade varnish.

Arbutus is a genus of at least 14 species of flowering plants in the family Ericaceae, native to warm temperate regions of the Mediterranean, western Europe, and North America. North American members of the genus are called strawberry tree. **Arbutus** species are used as food plants, several species are widely cultivated as ornamental plants . Arbutus bark and leaves used as medicines for colds, stomach problems, and tuberculosis. The fruit is edible but has minimal flavour. **Arbutus pavarii** is a member of the genus Arbutus used for honey production, as food due to its berries, as ornament trees as well as its medicinal uses as it is used in the treatment of kidney diseases. It is recorded as an endemic medicinal species with a high relative importance value.

The current literatures concerned with these studied plants revealed the presence of essential oil obtained from both leaves and flowers of **Myrtus comunus** which exhibited antimicrobial and anti-oxidant activities [1-4] as well as poly-phenol compounds [5-7]. More over many published reports demonstrated various activities of different Myrtle leaves extracts viz.: the antimicrobial [8-10], antioxidant [11], anti-hnflammatory [12], antidiabetic [13], anti ulcerative [14] and insecticidal activity [1].

Previous researches mentioned the presence of resin in **Pistacia lentiscus** known as mastic resin,which is effective in the treatment of duodenal ulcer. Essential oil obtained from leaves, gum and twigs of the plant showed an antimicrobial activity .

Few reports were traced concerned with **Arbutus pavarii** , the most available literatures revealed the presence of different phyto-constituents in the leaves and fruits of **Arbutus unedo** [American strawberry tree] viz.: triterpenes and irridoid glycosides, organic acids, tannins, flavonoids, sterols, phenolic compounds and amino acids.

While only one available report proved the presence of amyrin, lupeol, oleanolic acid, arbutin, catechin, isoquercitrin, myricetin and ferulic acid in the leaves of the Libyan strawberry tree **Arbutus pavarii**. The aim of this study is to highlight on these three plant species dealing with their nutritive values.

MATERIALS AND METHODS

Plant materials

The leaves and fruits of Arbutus pavarii, Myrtus communus and Pistacia lentiscus were collected from Al –Jabal Al Akhdar- El – Bieda city – Libya during 2010 . Leaves and fruits of the plants under investigation were separately air-dried, powdered and kept in tightly closed amber colored containers.

1-Preliminary phytochemical screening

The air-dried powdered fruits and aerial parts [stems and leaves] of each studied plant were screened for their contents of volatile oils, carbohydrates and/or glycosides, tannins, free and combined flavonoids, unsaturated sterols and/or triterpenes , alkaloids, anthraquinones and cardiac glycosides .The results are recorded in table(1)

2-Determination of Moisture

moisture content was determined gravimetrically (AOAC , 1980) (2) .

3-Preparation of total alcohol extract of the leaves of each studied plant

30gm of the air dried leaves of each studied plant were separately extracted with alcohol 90% using soxhlet apparatus till exhaustion. Each of the resulted extract was concentrated under vacuum by rotary evaporator. The residues left after distillation of solvent were weighed and kept in a desiccators.

4-Determination of Total Carbohydrates

It was carried out as follows : -

- One gram of each powdered fruit was defatted with petroleum ether, then extracted with hot 80% ethanol twice .
- The combined extracts were evaporated till dryness, the dried residues were dissolved in 10 ml of 10% aqueous isopropanol in a volumetric flasks.
- One ml of sample containing the equivalent of 20 – 100 µg glucose was pipetted into thick-walled test tubes of 16 – 20 mm diameter. A reagent blank containing 1ml of water , and a set of glucose standards (e.g . , 25 , 50 and 75 µg glucose , in a volume of 1 ml) were prepared at the same time . One ml of 5.0% (W/ V) phenol was added to all tubes and mixed then from a fast flowing it was added 5 ml of concentrated sulphuric acid , directing the stream of acid on the surface of the liquid and shaking the tube simultaneously , to effect fast and complete mixing .
- The tubes were allowed to stand 10 min. , shaken and placed in water bath 25 to 30 ^{o}C for to 20 min. before readings were taken . The colour was stable for several hours .
- The absorbance of characteristic yellow colour measured at 490 nm and followed Beer's law up to optical densities of at least 1.0 Construction of standard calibration curve of glucose Fig.(1)**.**The percentages of total carbohydrates present in the fruit of each plant under investigation were calculated table (3) .

5-Extraction and Determination of total Phenols

The total phenol contents were extracted and determined as follows-

One gram of defatted samples [fruit] were extracted with 10 ml each of methanol (twice) , and acidic methanol (1% v/v) . After stirring for 20 min. at room temperature. The two methanol extracts , and the acidic methanol extracts were combined for analysis.Evaporate of the combined extracts till residues, then dissolve these residues in 10ml. methanol in a volumetric flasks. Each of the combined extracts was separately analyzed for total phenols using spectrophotometric prussion blue method.Samples (0.5 ml.) of each extract were diluted with 3.0 ml distilled water and then 3.0 ml of 0.008 M $K_3Fe(CN)_6$ was added, followed immediately by the addition of 3.0 ml of 0.1 M HCL.After 5 minutes the color developed was measured spectrophotometrically at 720 nm. Against blank of identical composition without the examined samples.The concentration of phenolic contents in each examined fruit extract was determined from the calibration curve constructed using known concentration of tannic acid [10-100 µg/ml].fig.(2) and table (4).

6-Determination of Proteins

Total nitrogen was determined using Kjeldahl method (AOAC , 1965) . Kjeldahl method is the standard method of nitrogen determination. The method consists of three main steps:

1)-digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia.

2)-distillation of the ammonia into a trapping solution.

3)- quantification of ammonia by titration with a standard solution.

Procedure

Place 0.5-1g. of each fruit powdered sample in a Kjeldahl tube, add 2.5g. of potassium sulfate , 0.25g. copper sulfate and finally sulfuric acid.Heat the tubes in Kjeldahl instrument (Kjeldatherm, Gerhardt, Germany) for 1-2 hours at 350 C till transparent solution.After cooling, transfer the digested tube to the evaporation system (Vapdest 30, Gerhardt) Program the system to add 30% sodium hydroxide solution to the digested solution for 10 seconds, reaction time 2 minutes and steam evaporation for 3 minutes.The ammonia evaporated is transferred to conical flask containing 25ml. boric acid and add few drops of methyl red indicator (0.1%).Titrate the ammonium borate against 0.25 M hydrochloric acid to the end point [from yellow to red color] table (5).

7-Determination of the percentages of macro elements [sodium, potassium, calcium and phosphorus]

One gram of each dried powdered fruits were ashed, the resulted ash samples were extracted with 2 ml. of dilute hydrochloric acid. The volume of each sample was adjusted to 100ml.Sodium, potassium and calcium were determined quantitatively using **Jeneway flame photometer clinical PFP 7.** The results were demonstrated in table (6)

8-Anti-microbial activity for each prepared total alcohol extract

The antimicrobial screening was performed applying the disc agar diffusion method using nutrient agar media for bacteria and potato dextrose agar media for fungi, the following microorganisms were tested: **Gram positive bacteria** [Bacillus subtilis, Staphylococcus aureus], **Gram negative bacteria** [Pseudomonas aeruginosa, Escherichia coli] , **fungi** [Candida albicans, Asperagillus flavus], susceptibility test discs of Cefotrioxan [30µg/disc] HIMEDIA laboratories Pvt. Limited. Nystatin I.U. [17 unit/disc] were used as standard antibiotic and antifungal drugs. The total alcohol extract was given in a concentration of 0.4mg/disc. After incubation, the zones of inhibition were recorded in mm. Diameter less than 5mm. indicates no effect. A disc impregnated with alcohol is used as a negative control as well as discs of Cefotrioxan, and

Nystatin were used as a positive control for each micro-organism. Results are illustrated in table (7).

RESULTS AND DISCUSION

Table (1) recorded the results of preliminary phytochemical screening for the three plant species under investigation. This screening revealed the following :-

1.Presence of carbohydrates and/or glycosides, unsaturated sterols and/or triterpenes, tannins and flavonoides [both aglycones and glycosides] in the studied plants.

2.The presence of volatile oil was shown in both plant organs of P istacia lentiscus and Myrtus communus.

3.Absence of alkaloids, cardiac glycosides, saponins and anthraquinones

Table (1) :- preliminary phytochemical screening of the leaves and fruits of *each studied plant*

Name of the test	**Pistacia lentiscus**		**Myrtus communus**		**Arbutus pavarii**	
	leaves	***fruits***	***leaves***	***fruits***	***Leaves***	***fruits***
Carbohydrates /and or glycosides	+	+	+	+	+	+
Saponins	-	-	-	-	-	-
Unsaturated sterols and/or triterpenes:						
a-Leibermann's and Burchard's test	+	+	+	+	+	+
b-Salkwask's test	+	+	+	+	+	+
Tannins	+	+	+	-	+	+
Flavonoids:						
a-aglycones	+	+	+	+	+	+
b-glycosides	+	+	+	+	+	+
Anthraquinones:						
a-aglcones	-	-	-	-	-	-
b-glycosides	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-
Alkaloides and /or nitrogenous bases	-	-	-	-	-	-
Volatile oil	+	+	+	+	-	-

Table (2) Percentage of moisture in each studied fruit

Name of the plant	*%Moisture*
Myrtus communus	***55.15%***
Pitacia lentiscus	***36.4%***
Arbutus pavarii	***60.56%***

The high moisture content of each studied fruits was recorded in table (2)

Table(3)Percentage of total carbohydrates present in each studied fruit

Name of the plant	*% Carbohydrates*
Myrtus communus	***42.14%***
Pitacia lentiscus	***4.73%***
Arbutus pavarii	***50.5%***

The high percentage of total carbohydrates was observed 50.5% table (3) in Arbutus pavarii fruits followed by that of Myrtus communus (42.14%) . Pistacia lentiscus fruits were found to be the least carbohydrate content (4.73%). Carbohydrates play several vital roles in living organisms. They can be oxidized to yield energy to drive metabolic processes. Carbohydrates polymers act as energy storage molecules , carbohydrates derivatives are found in a number of biological molecules, including some coenzymes and the nucleic acids Oraby,(2005) . The above results showed that the fruits of both Myrtus communus and Arbutus pavarii contain a high amount of carbohydrates so, they can be considered as a good nutritive edible fruits.

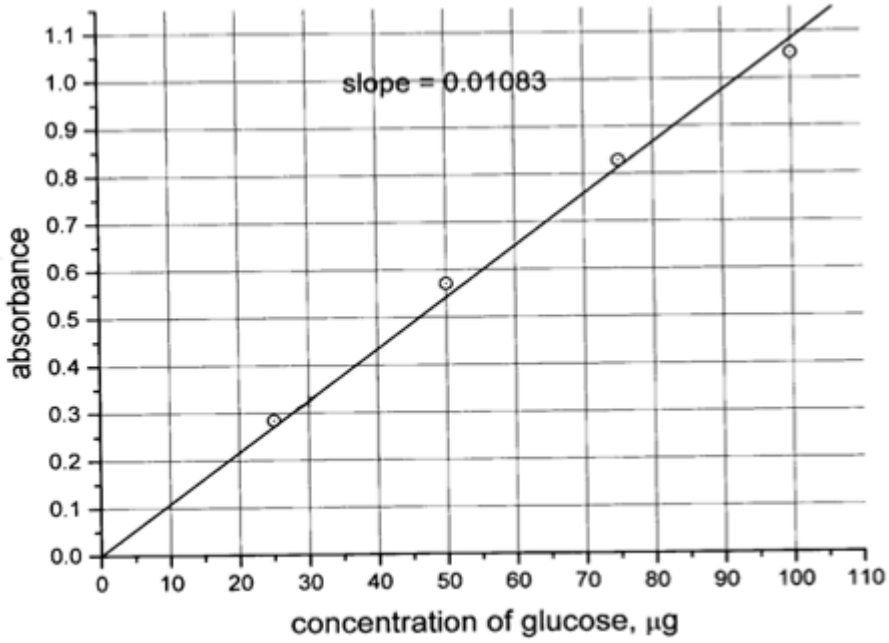

Fig. 1-Standard calibration curve of glucose

Table(4)Percentage of total phenols present in each studied fruit

Name of the plant	Phenol(mg/gm)
Myrtus communus	7O
Pistacia lentiscus	89
Arbutus pavarii	50

Table (4) demonstrated the high quantity of phenols in all studied fruits, this is represented in the following order Pistacia lentiscus (89 mg/g powdered plant) > Myrtus communes (70mg/g powder drug) >Arbutus pavarii (50mg/g powder). These results revealed the possible anti-oxidant activity of the studied fruits as well as other pharmacological activities, which required further investigation in the near future in order to enrich knowledge about this valuable plant.

Table (5) Percentage of protein present in each studied fruit

Name of the plant	Protein%
Myrtus communus	5.63
Pistacia lentiscus	5
Arbutus pavarii	2.18

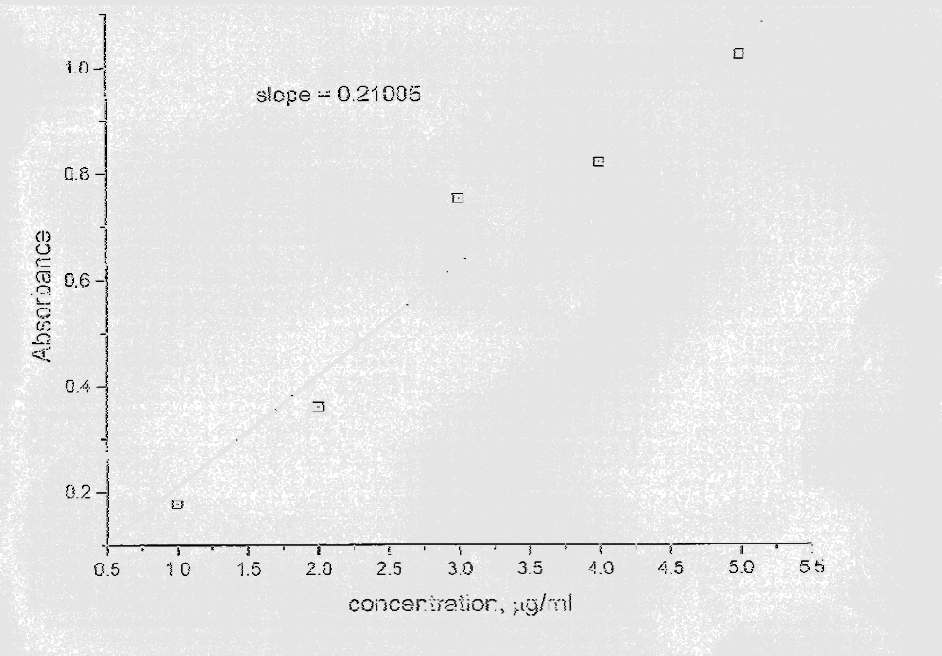

Fig. 2- Standard calibration curve of tannic acid

Table (5) illustrated the presence of protein in the three studied plants with variable quantities. Fruits of Myrtus communus was found to be the highest followed by Pistacia lentiscus and the lowest was for Arbutus pavarii fruits. The above results showed that these three fruits contain a high amount of carbohydrates and a moderate percentage of protein , so it can be considered as nutritive edible fruits. Moreover, the presence of high percentages of phenolic compounds revealed the anti-oxidant activity of these fruits as well as other pharmacological activities, which required further investigation in the near future in order to enrich knowledge about these valuable plants.

Table (6) Percentage of macro elements present in each studied fruit

Macro element	Myrtus communus	Pistacia lentiscus	Arbutus pavarii
Sodium	**0.31**	**0.46**	----------
Potassium	**2.44**	**2.67**	**1.29**
Calcium	**0.38**	**0.37**	**0.12**
Phosphorus	**0.013**	**0.004**	----------

Table (6) demonstrated the high quantity of Potassium, calcium and sodium were observed in Pistacia lentiscus fruits followed by of Myrtus communes , while Arbutus pavarii fruits revealed lowest potassium and calcium contents with the absence of sodium. Moreover, Myrtus and Pistacia fruits were found to contain low percentage of phosphorus 0.013 and 0.004 % [0.13&0.04 mg/one gram powdered drug respectively], while Arbutus fruits were devoid of it.

These macro elements play an important and vital role in our bodies; potassium and sodium regulate the osmotic and acid base balance of body fluid and they are the major cations of intracellular fluid. Sodium shows a special role in nervous transmission and considered as an active transport of sugars and amino acids. While potassium play a special role in ionic basis of muscle excitability and also as cofactor for several reactions in carbohydrate metabolism. Moreover, calcium is an essential cofactor for many enzymes including those needed for nervous transmission and muscle contraction. It also plays an important role in skeleton and teeth

structure and in blood coagulation. Though, Pistacia lentiscus fruit with its higher contents of these three macro elements could play an effective role in the treatment of nutritional deficiencies.

Anti microbial activity:-

-From table (7) we can conclude that: Ethanol extract prepared from the three studied plants possessed a broad spectrum effect against both the tested Gram positive and Gram negative bacteria , in addition to their moderate inhibitory effect against both tested fungal strains Candida albican and Asparagillus flavus

-Ethanolic extract obtained from **Myrtus communus** showed the least antimicrobial activity against Klebsiella pneumonia with the high activity against the other tested gram negative bacteria. At the same time this extract showed a moderate activity against the tested gram positive organisms. In addition to its moderately high anti fungal effect against the tested fungi.

-Ethanolic extract obtained from **Pistacia lentiscus** showed moderate antibacterial activity against most of the tested gram positive bacteria , with the low activity against Pseudo.areugenosa. Moreover, this extract showed a moderately high activity against the tested gram negative organisms. In addition to , the negative effect on Asparagillus flavus.and moderately high activity against the tested Candida strain.

-Ethanolic extract obtained from **Arbutus pavarii** showed the least antibacterial activity against most of the tested gram negative bacteria [Klebsiella pneumonia and E.coli] with the high activity against Pseudo.areugenosa. Otherwise this extract showed a moderately high activity against the tested gram positive organisms with a low anti fungal effects. These results agreed with what is mentioned in the previous studies dealing with both the antibacterial and antifungal activities of the entitled plant species. From table (7) we can conclude the following:-

Table (7) :-Antimicrobial activity of different extracts prepared from the three species under investigation

Total alcohol extract / Micro-organism	Myrtus communus	Pistacia lntiscus	Arbutus pavarii	Ceftrioxan	Nystatin
Gram- positive					
Staphylococcus aureus	18 ++	18 ++	15 +	20 ++	--
Bacillus subtilis	16 ++	17 ++	20 ++	13 +	--
Gram negative					
E. Coli	21 ++	15 +	6 +	30 +++	--
Pseudomonas aeruginosa	25 ++	5 -	25 ++	23 ++	--
Klebsiella pneumonia**e**	6 +	19 ++	--	26 +++	--
Fungi					
Candida albican	13 +	16 ++	6 +	--	21 ++
Asparagillus flavus	15 +	--	--	--	13 +

Inhibition zones = <5 [-], 6-15[+] , 16-25[++] , >25[+++]

The three plant species exhibited a significant anti bacterial effect against the tested Gram positive and Gram negative bacteria under investigation in comparison with the standard antibiotic Ceftrioxan and moderate antifungal effects in comparison with the standard anti fungal Nystatin.

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