Original Article- BIOLOGY AND ENVIRONMENTAL SCIENCE

CHANGES IN SOME PHYSIOLOGICAL CHARACTERISTICS IN THE LIBYAN STRAWBERRY - TREE PLANT (*ARBUTUS PAVARII L*) IN SELECTED LOCATION OF THE GREEN MOUNTAIN (AL -JABEL AL-AKHDAR) – LIBYA

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Abstract

The present research was carried out to study changes in some physiological characteristics in the Libyan Strawberry -Tree plant (*Arbutus Pavarii*L) in selected location of the Green Mountain (Al -Jabel Al-Akhdar) - Libya. The plant material was selected using random sampling technique from the EL-Gabal EL-Akhdar area (Asulntah area). Chemical tests and assays were carried out to determine the stored essential oils, total flavonoids content and total phenolic content. The photosynthetic pigments and microbial number was estimated as well. The study also made use of gas chromatography and mass spectrophotometric methods.

Keywords: *Arbutus pavaraii L*, Green mountain, Libya, essential oils, photosynthetic pigments, phenolic compounds.

How to Cite:

Introduction

Arbutus pavarii Pamp. (Ericaceae) is one of the endemic species in Libya and it distributes naturally as wild plant in El-Gabel El-Akhdar area, which characterized with Mediterranean climatic conditions (Elshatshat 2009; Elshatshat et al 2009; and Elabidi and Elshatshat 2017). It is- evergreen shrub, 1.5 to 3 m tall reddish brown peeling bark flowering season [late October to February] flowers are a good source of nectar for bees used for folk medicinal purposes drought tolerant The fruit takes around 8 months to ripen, and they are spherical and warty, and turn from yellow to orange to scarlet as the autumn progresses (figure 1). There are a number of factors for the difficulty of germination and growth of these plants naturally in the wild land. Drop in rate of rainfall annually it is the most important environmental factor which has made the wild lands drier and decreased significantly seeds germination (Elmaghrabi at al. 2017). In addition, overgrazing and the use of lumber as firewood and also expansion of new farms, which contributed of deterioration sharply of edible and medical wild plant resources which led these species to endanger (Elmaghrabi et al 2017). There were about six species of Arbutus grows spontaneously around the Mediterranean basin. The essential oil of

Haplophyllum tuberculatum was reported by (Al-Rehaily et al, 2014) which prepared by hydrodistillation of the fresh flowering aerial parts of the plant collected from wild types . The oil was subsequently analyzed by GC and GC-MS. Thirty seven compounds, accounting for 96.4 % of the oil composition were identified in this study (Al-Rehaily et al, 2014). The antimicrobial and activity of the essential oil was also evaluated against various human pathogens, where a relatively low inhibitory range was observed. The purpose of this study was to develop an efficient protocol for in vitro propagation of endanger edible strawberry tree (Arbutus pavarii) and medical plant Haplophyllum tuberculatum. Thiswork,basedonHPLC-DAD-   MS/MSanalysisof strawberry tree (Arbutus unedo L.) honeys, previously selected by sensory evaluation and melissopalynological analysis, showed that, in addition to the above-mentioned acid, there were other high levels of substances useful for the botanical classification of this unifloral honey. The results clearly demonstrate that a promising level of chemically-determined antioxidant activity of a plant extract is not necessarily correlated with biological significance, as assessed by the effect of A. unedo fruit in a neurodegeneration cell model.

Objectives

Identify some of the physiological and morphological characteristics of in the developing *Arbutus Pavarii* in the Jabal ALAkhdar-Eco-region – Libya area.

Determine the chemical composition (essential oil contents and antioxidant activity) of the *Arbutus Pavarii*.

Materials and methods

Materials

1.1. Plant material:

The samples of *Arbutus pavarii* were collected from the EL-Gabal EL-Akhdar area (Asulntah area) and then three random plants were choosen that included (Sample1= plant intact and naturally grown, sample2= plant grown almost normal and sample 3= plant not grown naturally). All samples were collected from the same location.

1.2. Chemicals:

1, 1-Diphenylpicrylhydrazyl (DPPH·), methanol, ethanol and acetone were supplied by Sigma and Merck company. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, anhydrous sodium sulfate and pyrogallol were obtained from the biochemistry laboratory of Chemistry department-Benghazi University.

2. Methods:

2.1. Extraction of essential oil from leaves of Arbutuspavarii (sample 1, sample 2 and sample 3):

The dry powdered leaves of *Juniperus phoenicea*(500g) were subjected to hydro distillation using Clevenger apparatus. The isolation of volatile oils was completed within 6 hours **(Clevenger, 1928).**

2.1.1. Store essential oils:

The oil samples were stored at 7°C in dark air-tight containers after drying over anhydrous sodium sulfate and filtered before injecting to GC-MS analysis.

2.2. Oil analysis:

The oil samples extracted from leaves of *Arbutus pavarii*were subjected to the following tests:

2.2.1. Gas chromatography/ Mass spectra

Thermo Scientific, Trace GC Ultra & ISQ Single Quadruple MS, DB-5 bonded-phase fused-silica capillary column was used in for GC/MS analysis of essential oils. This experiment has been conducted in the central laboratory at Cairo University-Egypt.

2.2.2. Antioxidant activities assays and quantitative analysis:

All of these experimental assays have been conducted in biochemistry laboratory at Benghazi University.

2.2.2.1. Total phenolic content (TPC):

Total concentration of phenolic compound in all oil extracts obtained from leaves of *Arbutus pavarii*was estimated using the colorimetric method based on Folin-Ciocalteu reagent (**Sawsan *et al.* 2010).** 0.05 ml of the oils at different concentrations "100,200,300,400,500 μg/ml" were mixed separately with 0.05 ml of Folin-Ciocalteu reagent. Then 0.5 ml of 15% sodium carbonate solution was added to the mixture and then the adjusted to 1 ml with 0.4 ml of distilled water. The reaction was allowed to stand for 10 min, after which the absorbance were recorded at 725 nm by UV-visible spectrophotometer.Quantification was done with respect to standard calibration curve of pyrogallol.The results were expressed as pyrogallol "μg/ml" Fig (6). Estimation of the phenolic compounds was carried

out in triplicates. The results were mean values (standard deviations).

Concentration of Pyrogallol " µg/ml "	Mean (Standard Deviation)
100	0.410 (0.032)
200	0.799 (0.0220)
300	1.333 (0.0045)
400	1.828 (0.0117)
500	2.105 (0.0225)

Table (1): Total phenolic content (TPC) of pyrogallol

$y = 0.0044x - 0.0307$
$R^2 = 0.9908$

figure (6)
Total phenolic content of pyrogallol
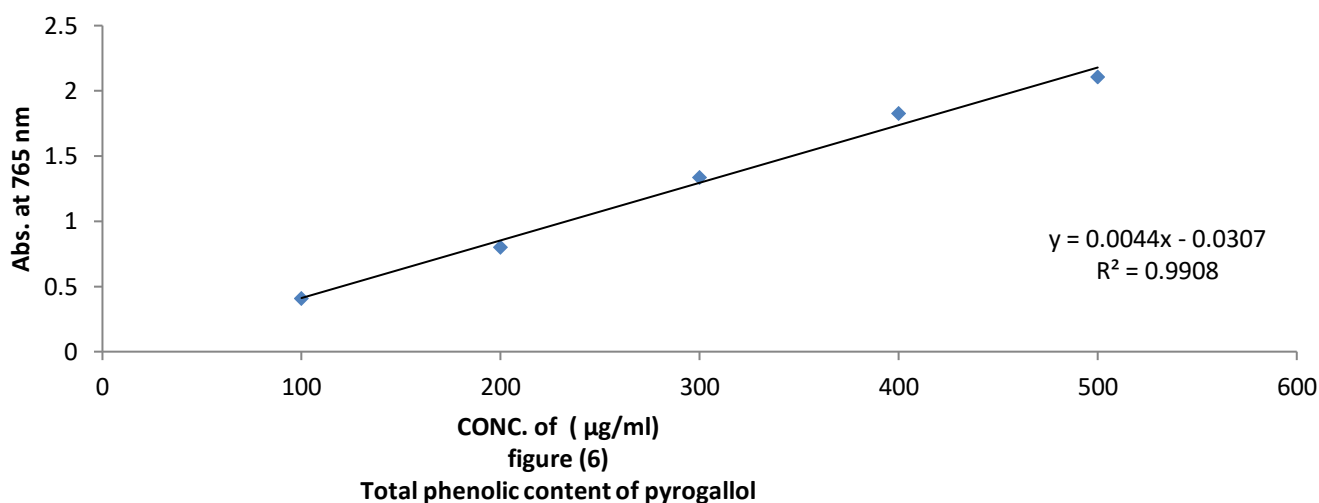
2.2.2.2. Total flavonoids content (TFC):

Aluminum chloride colorimetric method was used for determination of total flavonoids **(Chang *et al.*, 2002)**. 2 ml of different concentration "100, 200, 300, 400, 500 µg/ml " of oil extracts mixed with 0.1ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. The calibration curve was obtained by preparing different quercetin solutions in methanol at concentrations "100 to 500 µg/ml" Fig (7).

Concentration of qurecetin " µg/ml "	Mean (Standard Deviation)
100	0.279(0.092)
200	0.560 (0.035)
300	0.834 (0.003)
400	1.066 (0.009)
500	1.300 (0.006)

Table (2): Total flavonoids content of qurecetin

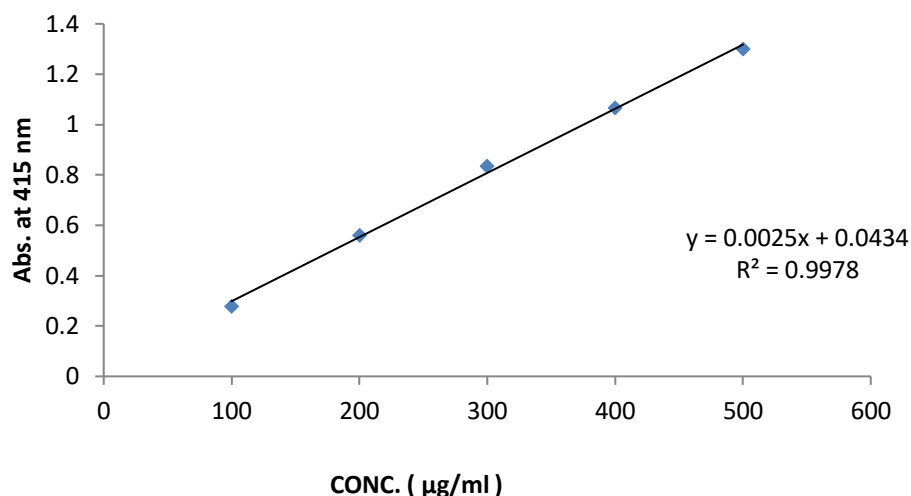$$y = 0.0025x + 0.0434$$
$$R^2 = 0.9978$$

Figure (7)
Total flavonoids content of qurecetin

2.2.2.3. Reducing power assay (RPA):

The reducing power was determined according to (**Naznin and Hasan, 2009**). 2ml of the all oil extracts with different concentrations "100,200,300,400,500µg/ml" were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide then mixture was incubated in water bath at 50 C⁰ for 20 minutes and 2.5 ml of trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 ml

of the supernatant was mixed with 2.5 ml of distilled water and 1 ml $FeCl_3$, substances, which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm by UV-Visible spectrophotometer. Quantification was done with respect to standard calibration curve of ascorbic acid the results were expressed as ascorbic acid "µg/ml" Fig (8).

Potassium ferricyanide + Ferric chloride antioxidant Potassium ferricyanide + Ferrous chloride

Concentration of vitamin C " µg/ml "	Mean (Standard Deviation)
100	0.201 (0.0280)
200	0.495 (0.0350)
300	0.697 (0.0087)
400	0.992 (0.0727)
500	1.201 (0.0305)

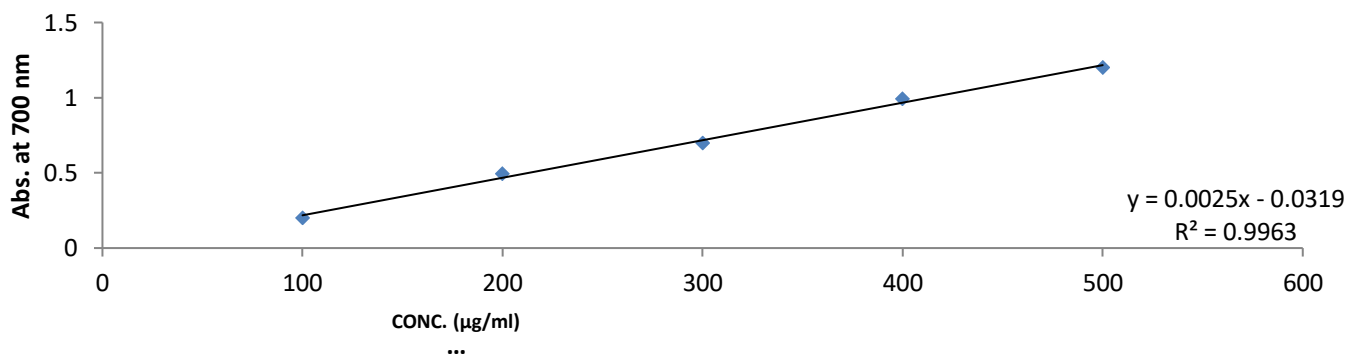
$$y = 0.0025x - 0.0319$$
$$R^2 = 0.9963$$

Table (3): Reducing power assay of vitamin C

2.2.2.4. DPPH free radical scavenging activity (RSA):
The antioxidant activity of all oil extracts were measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH ˙method as modified by (**Potapovich and Kostyuk 2003)**. The reaction mixture containing 2 ml of all extracts at different concentrations "100,200,300,400,500µg/ml" and 2ml of DPPH˙ (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. When the DPPH˙ reacted with an antioxidant compound in oil that can donate hydrogen, it was reduced and resulting in decrease of absorbance at 517nm using UV-visible spectrophotometer, and the mean values were obtained from triplicate experiments. The percentage of the remaining DPPH˙ was plotted against the sample concentration. A lower value indicates greater antioxidant activity. Radical scavenging activity was expressed as percent ages of inhibition and was calculated using the following formula:- **%DPPH "RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100**

2.3. Growth characteristics of (*Arbutuspavarii*):
The study samples were divided into lots of different trees, depending on the appearance of exterior of the tree; three replicates in each tree were made to find out the quantitative and morphological differences.

2.4. Estimation of photosynthetic pigments:
The photosynthetic pigments were extracted from a known fresh weight of leaves in 85% aqueous acetone to a certain concentration for spectrophotometric measurements. The photosynthetic pigments (chlorophyll a, b and carotenoids) were determined by, spectrophotometric method as described by **Metzner *et al*. (1965).** The pigments extract were measured against a blank of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm. Taking into consideration the dilution factor, it was possible to determine the concentration of pigment fractions (chl. a, b and carotenoids) as mg/ml using following equations:

Chlorophyll a = $10.3\ E_{663} - 0.918\ E_{644}$ = mg/ml
Chlorophyll b = $19.7\ E_{644} - 3.87\ E_{663}$ = mg/ml

$$\text{Carotenoids} = 4.2\ E_{452.5} - \left\{ \begin{array}{c} 0.0264\ \text{chl. a} \\ + \\ 0.4260\ \text{chl. b} \end{array} \right. = \text{mg/ml}$$

Finally, these pigment fractions were calculated as mg/g fresh matter.

2.5. The Estimation of microbial number:
2.5.1. Nutrient Agar medium
    23 g of nutrient agar medium were suspended in 1-liter ml distilled water and then heat to boiling to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 min.

2.5.2. Sample preparation:
2.5.2.1. Plant:
    Leaves (brown and green) werecollected from *Arbutus pavarii.* 2 grams of each part was put in a test tube containing 8 ml of distilled water and shaked for 30 seconds. 1 ml of sample was put in a petri dish and then poured by nutrient agar medium under sterile conditions and incubated at 37.5°C forthree days. Total number of microbial colonies of each plate was estimated.

2.5.2.2.Soil:
    Two samples were collected from sifted soil. 1 gram of each sample from different sites (under the sieve and above the sieve) was added to test tube containing 9 ml of distilled water and shaked for 30 seconds. 1 ml of each sample was put in a petri dish and then set up the nutrient agar medium incubated at 37.5 °C forthree days. Total number of microbial colonies of each plate was estimated.

2.5.3. Pure Culture:
    A single bacterial colony from each mixed culture was taken by loop, streaked on nutrient agar medium, and incubated at 37.5 ° C for three days.

2.5.4. Gram stain
    Differential staining (Gram stain)was carried out to confirm that the bacteria were gram negative and rode shaped. Smears from single colony bacteria grown on EMB at 37 ˚C and 44.5˚C were prepared and covered by primary stain(Crystal violet) for 1 min and then Mordant (Iodine) for 1 min to made CV-I complex after that decolorized agent (70% ethanol) was washed the slide to remove the CV-I complex in the thin layer of peptidoglycan of gram negative bacteria. Wall. Drops of counter stain (safranin)were added for a half minute.was The slide then washed in The distilled water and dried by filter paper after that examined by microscope using an oil immeration lens . In summary, gram-positive cells retain the dye and remain purple, Gram-negative cells did not retain the dye; they were colorless until counterstained with a red dye (pink cells).

2.5.5. Endospore staining:
A film was made on a clean slide by emulsifying part of a colony in loopful of distilled water. The film was then air-dried and stained with malachite green for 4-5 min, using a flame. The smear was rinsed rapidly with water and stained with safranin solution for 30 sec. The slide was washed with water and allowed to dry. On microscopic examination the endospores appeared green and the cells were pink (**Abualdahab and Gorani, 1983**).

2.5.6. Motility test:

The test was used to distinguish between motile and non-motile bacteria, using the hanging-drop preparation. First a little oil immersion was placed

around the edge of the slide, then with a wire loop a small loopful of the culture was transferred to a clean dry covered slip. After that, the cover slide was inverted over the cover slip, so that the drop was in the center of the cavity and the slide was pressed down gently but slimly so that the oil seals the cover slip in position. The slide was inverted quickly and smoothly and the drop of culture was placed in the form of the hanging-drop, and the preparation was examined quickly. It is necessary to distinguish between Brownian movement (a continuous agitation of very small particle suspended in a fluid which is called unbalanced impacts with molecules of the surrounding fluid) or drift in one direction caused by the slide being slightly tilted and true motility (Abualdahab and Gorani, 1983).

Result and discussion

Antioxidant evaluation of essential oils extracted from leaves of *Arbutus pavarii* Pamp. (Ericaceae).

The antioxidant activities of essential oils extracted of three samples from leaves of *Arbutus pavarii* Pamp. (Ericaceae)growing in Al-Jabal Al Akhdar were evaluated by:

1.1 Total phenolic content (TPC):

Figure (9) show the total phenolic content found in essential oils where the essential oil of sample number (1) contained high total phenolic content, the

results expressed according to pyrogallol as phenolic compound in figure (6).

1.2 Total flavonoids content (TFC):

The results obtained in this study as shown in figure (10) indicated that the essential oil of healthy sample contain high amounts of flavonoids compounds as compared with the qurecetin in figure (7) which was used as standard.

1.3 Reducing power assay (RPA):

As shown in figure (11) the reducing power assay of essential oil of sample 1and sample 2 exhibit higher reducing activity than the ascorbic acid.

1.4 The DPPH· radical scavenging activity:

The result of the DPPH· radical scavenging activity of essential oil are shown in figure (12), this result compared with the well-known antioxidant ascorbic acid were the percent of the inhibition is 97% at 500 µg/ml of the essential oil of sample 1 and 92% at 500 µg/ml of the essential oil of sample 2, while the percent of the inhibition is 38% at 500 µg/ml of the essential oil sample 3.

Table (4): Total phenolic content (TPC) of essential oils extracted of three samples from leaves *Arbutus pavarii*(Sample1= plant intact naturally grow, sample2= plant growth almost normal, sample 3= No plant grows naturally) and % variation from the corresponding control (pyrogallol as standard) .

Concentration " µg/ml"	Mean phenolic content (Standard Deviation)						
	Pyrogallol	Sample1	% var.	Sample2	% var.	Sample3	% var.
100 (1)	0.410 (0.032)	0.711(0.03)*	73.4↑	0.699 (0.05)*	70.5↑	0.025 (0.0162)***	97.5↓
200	0.799 (0.0220)	0.799 (0.01)†	Zero	0.782 (0.011)†	2.13↓	0.085 (0.020)**	89.4↓
300	1.333 (0.0045)	1.435 (0.02)†	7.65↑	0.988 (0.002)*	31.2↓	0.222 (0.022)**	84.5↓
400	1.828 (0.0117)	1.78 (0.01)†	2.63↓	1.56 (0.016)†	12.4↓	0.754 (0.036)*	57.6↓
500	2.105 (0.0225)	2.69(0.07)†	27.8↑	2.21 (0.009)†	17.8↓	1.061 (0.037)**	60.6↓

† Insignificant difference from the corresponding control at $P > 0.1$
* Significant difference from the corresponding control at $P < 0.05$
** Highly sig. difference from the corresponding control at $P < 0.01$
*** Very highly sig. difference from the corresponding control at $P < 0.001$
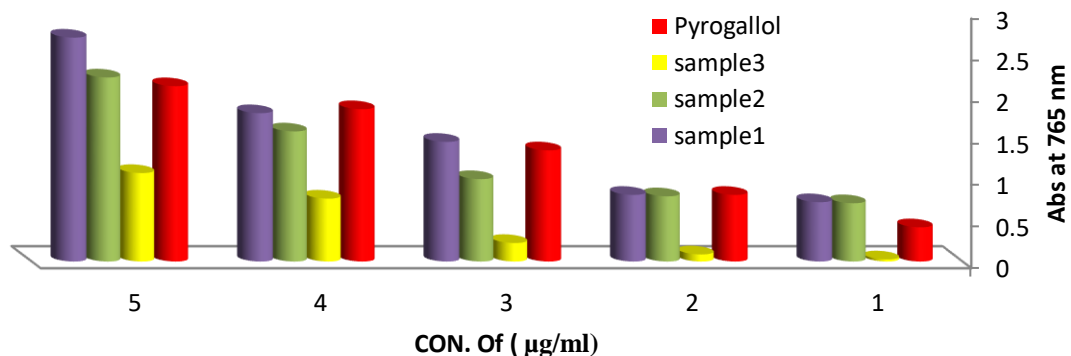↓ Decrease
↑ Increase

Fig.(9): Total phenolic content (TPC) of essential oils extracted from leaves *Arbutus pavarii*and Pyrogallol as phenolic compound.

Table (5): Total flavonoids content essential oils extracted of three samples from leaves*Arbutus pavarii*(Sample1= plant intact naturally grow, sample2= plant growth almost normal , sample 3= No plant grows naturally) and % variation from the corresponding control(qurecetin as standard   ).

Concentration " µg/ml"	Mean phenolic content (Standard Deviation)						
	qurecetin	Sample1	% var.	Sample2	% var.	Sample3	% var.
100	0.279 (0.092)	0.297 (0.032)†	6.45 ↑	0.276 (0.002)†	1.07 ↓	0.0845 (0.055)**	69.7↓
200	0.560 (0.035)	0.497 (0.011)†	11.25↓	0.375 (0.001)*	33.0 ↓	0.095 (0.045)**	83.0↓
300	0.834 (0.003)	0.664 (0.002)†	20.4 ↓	0.609 (0.008)†	27.0 ↓	0.141 (0.075)**	83.1↓
400	1.066 (0.009)	0.820 (0.090)†	18 ↓	0.869 (0.003)†	18.4 ↓	0.423 (0.071)**	60.3↓
500	1.300 (0.006)	1.690 (0.011)*	30 ↑	1.340 (0.001)†	3.08 ↑	0.866 (0.085)*	33.4↓

†Insignificant difference from the corresponding control at P > 0.1

* Significant difference from the corresponding control at P < 0.05

** Highly sig. difference from the corresponding control at P < 0.01

*** Very highly sig. difference from the corresponding control at P < 0.001
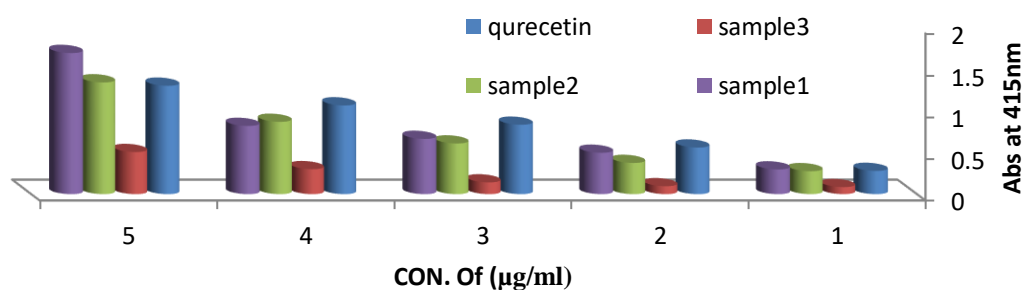
↓ Decrease

↑ Increase

Fig.(10): Total flavonoids content of essential oils extracted from leaves *Arbutus pavarii* and qurecetin as flavonoid compound .

Table (6): Reducing power assay essential oils extracted of three samples from leaves *Arbutus pavarii*(Sample1= plant intact naturally grow, sample2= plant growth almost normal , sample 3= No plant grows naturally) and % variation from the corresponding control(vitamin C)

Concentration "μg/ml"	Mean phenolic content (Standard Deviation)						
	Vitamin C	Sample1	% var.	Sample2	% var.	Sample3	% var.
100	0.201 (0.0280)	0.503 (0.0448)***	150.3↑	0.564 (0.07)***	180.6↑	0.044 (0.006)**	78.1↓
200	0.495 (0.0350)	0.875(0.0965)**	76.8↑	0.871 (0.0)**	75.9↑	0.104 (0.002)**	79↓
300	0.697 (0.0087)	1.293 (0.0471)**	85.5↑	1.321 (0.003)**	89.5↑	0.432 (0.005)*	38.0↓
400	0.992 (0.0727)	1.563 (0.0266)*	57.6↑	1.899 (0.01)***	91.4↑	0.902 (0.016)†	9.07↓
500	1.201 (0.0305)	2.339 (0.0401)***	94.8↑	2.486 (0.01)***	107.0↑	1.052 (2.965)†	12.41↓

† Insignificant difference from the corresponding control at $P > 0.1$
* Significant difference from the corresponding control at $P < 0.05$
** Highly sig. difference from the corresponding control at $P < 0.01$
*** Very highly sig. difference from the corresponding control at $P < 0.001$
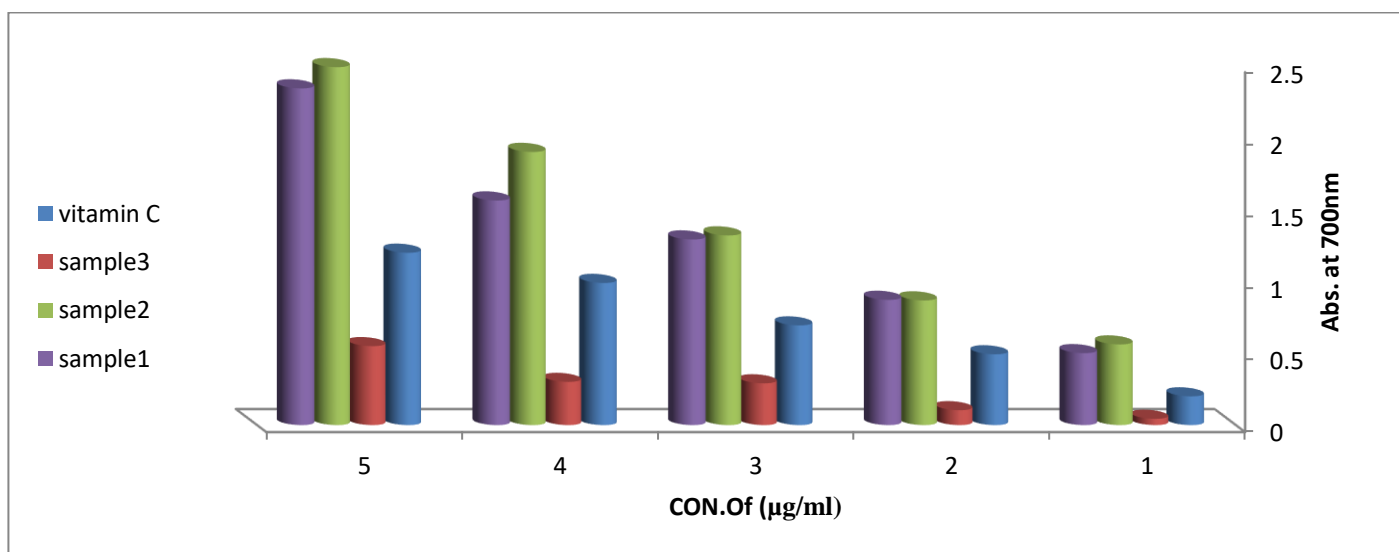↓ Decrease
↑ Increase

Fig.(11): Reducing power assay of methanol , ethanol and acetone extracted from leaves *Arbutus pavarii* and vitamin C.

Table (7): DPPH radical scavenging activity of vitamine C, and essential oils extracted of three samples from leaves *Arbutus pavarii* according to % inhibition.

Concentration "µg/ml"	Percent of inhibition (%)						
	vitamin C	Sample1	% var.	Sample 2	% var.	Sample3	% var.
100	92.3%	57% *	38.2↓	39% *	57.7↓	11% **	88.1↓
200	93.8%	79% †	15.8↓	59% *	37.1↓	19% **	79.7↓
300	95.1%	85% †	10.6↓	75% †	21.1↓	27% **	71.6↓
400	95.8%	89% †	7.1↓	87% †	9.19↓	33% **	65.6↓
500	96.7%	97% †	0.31↑	90% †	6.93↓	38% **	60.7↓

† Insignificant difference from the corresponding control at P > 0.1
* Significant difference from the corresponding control at P < 0.05
** Highly sig. difference from the corresponding control at P < 0.01
*** Very highly sig. difference from the corresponding control at P < 0.001
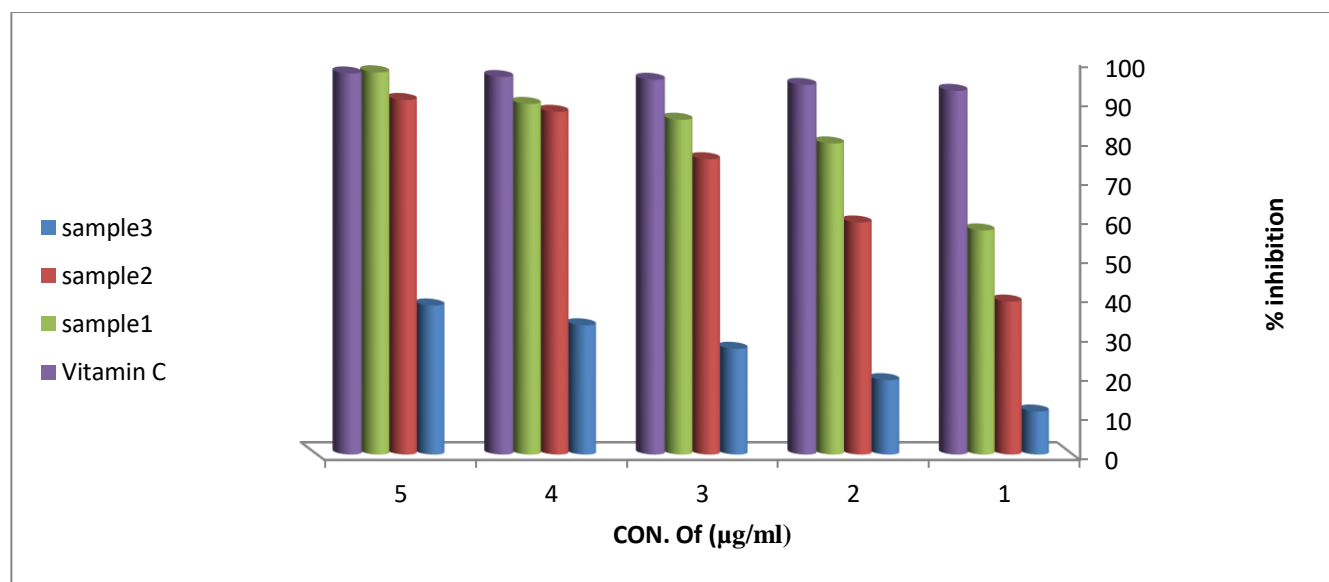↓ Decrease
↑ Increase

Fig. (12): DPPH radical scavenging activity of vitamin C and essential oils extracted from leaves *Arbutus pavarii* according to % inhibition.

2. The GC-MS analysis of the essential oils extracted from leaves *Arbutus pavarii*:

The retention time of the different compounds in the essential oils of *Arbutus pavarii* are presented in Table 8. Thirty-four volatile compounds, representing 88.29% of the total oil composition in sample 1, were identified in the leaves oils (Table 8). Monoterpene hydrocarbons were found to be the major group of compounds. The most abundant component found in the leaf oil was α pinene (20.85%) followed by Germacrene D (16.49%), while α pinene and Germacrene D in sample 2 and sample 3 were (12.45%, 3.09%) and (9.19% ,1.054%) respectively. Surprisingly, there were decrease of these compounds in the sample diseased (table 8).

Table (8): The main components of essential oils of *Arbutus pavarii*. leaves collected from Al-Jabal Al Akhdar area (A sulnta)

*Rt: Retention time obtained by chromatogram.
Sample1= plant intact naturally grow, sample2= plant growth almost normal and sample 3= No plant grows naturally.

Constituents	Area %			*Rt (min)
	sample 1	Sample 2	Sample 3	
α –pinene	20.85	12.45	3.09	4.16
β –myrcene	1.36	0.23	-	5.06
Terpinolene	0.07	-	-	5.26
β –phellandrene	3.84	1.79	0.02	5.91
Trans-Caryophyllene	5.44	5.00	2.087	14.66
4,7,10-Cycloundecatriene,1,1,4,8 tetramethyl-,cis,cis,cis	5.31	4.13	0.12	15.37
Germacrene D	16.49	9.19	1.054	16.05
Germacrene B	2.73	1.72	-	17.46
α – cedrol	0.36	-	-	18.27
+. alpha.-longipinene	2.98	2.082	0.471	18.08
Naphthalene,1,2,3,4,4a,5,6,8a,-octahydro-7-methl-4-methylene-1- (1-methylethyl)-(1.alpha,4a.alpha.,8a.alpha)	5.22	2.011	0.57	16.23
Camphene	0.29	-	-	4.32
β-pinene	0.49	-	-	4.81
1-phellandrene	0.39	-	-	5.33
delta.3-carene	0.40	-	-	5.46
α – terpinolene	0.23	-	-	7.10
Terpinolene	5.13	5.13	2.093	13.11
Linalool	0.47	-	-	7.37
Citronellol	0.63	-	-	10.30
α-cubebene	0.39	-	-	13.60
β-bourbonene	0.22	-	-	13.80
β-elemene	0.93	-	-	13.94
Widdrene	1.24	0.53	-	14.83
α-gurjunene	0.35	-	-	16.40
Zingiberene	0.43	-	-	16.92
delta.-cadinene	5.63	5.00	3.73	16.81

α-muurolene	0.43	-	-	17.01
α-calacorene	0.24	-	-	17.10
Elemol	0.61	-	-	17.28
caryophyllene oxide	2.06	1.890	0.281	17.95
Fonenol	0.36	-	-	18.58
α-ylangene	1.33	0.072	-	19.03
β- selinene	0.30	0.30	0.30	19.54
vulgarol B	1.09	0.873	-	19.88

1. The results of morphological and growth characteristics are given in table (9). From this table, the sample number (1) reflected the highest mean value of plant height and stem diameter. They were 222.67 cm and 16.42 cm, respectively.

Table (9) :Growth in term of plant height (cm) ,stem diameter (cm) and leaf dry weight (mg) of *Arbutus pavarii* . in EL-Gabal EL-Ahkdar area.

Sample Name	Plant height (cm)		Stem diameter (cm)		Leaf dry weight (mg)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Healthy sample	222.67	(21.35)	16.42	(5.90)	3.0234	(0.19)
Semi healthy sample	216.55	(17.93)	11.22	(3.14)	3.2058	(0.23)
Sample sick	214.23	(23.47)	9.89	(2.05)	3.8240	(0.11)

2. The results obtained in this study as shown in table(10) indicate that the healthy sample contain high amount of chlorophyll A, chlorophyll B and carotenoids as compared with the semi healthy sample and Sample disease.

Table(10): Estimation of photosynthetic pigments of *Arbutus pavarii* leaves in EL-Gabal EL-Ahkdar area.

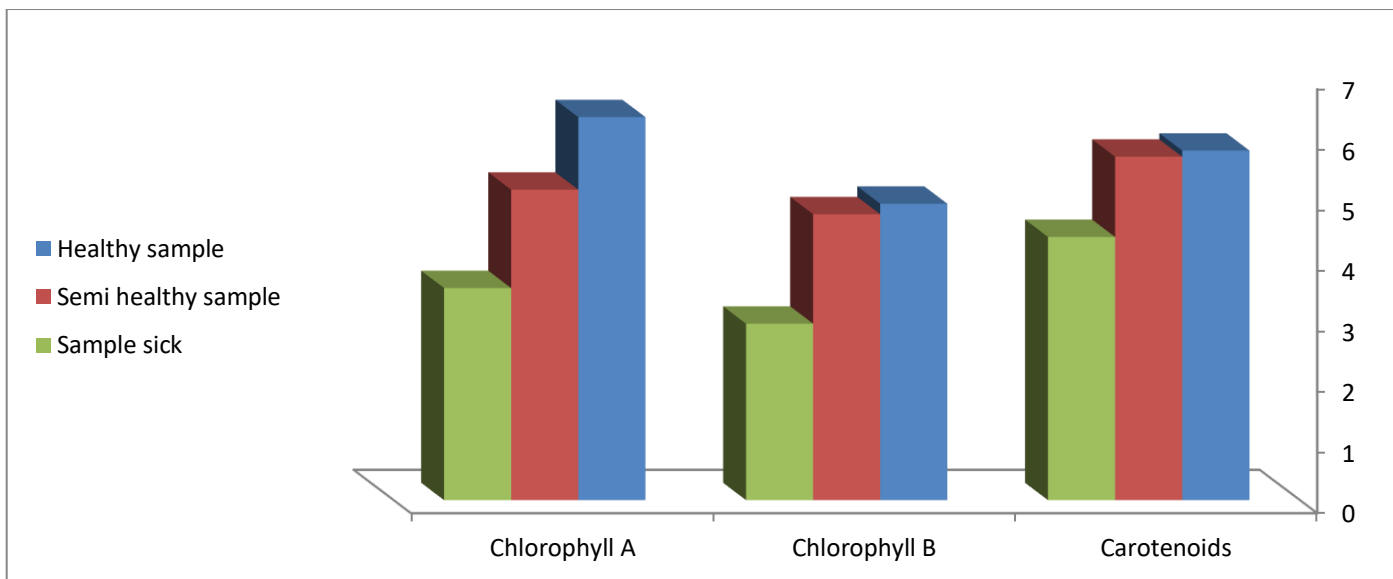| Sample	Chlorophyll A (mg/ml)	Chlorophyll B (mg/ml)	Carotenoids (mg/ml)
Healthy sample	5.7708	4.894	6.323
Semi healthy sample	5.670	4.721	5.126
Sample sick	4.346	2.915	3.503

Fig. (13)Estimation of photosynthetic pigments of *Arbutus pavarii*. leaves in EL-Gabal EL-Ahkdar area

3. As shown in Fig (14) decreased the number of bacteria in the sub- branches of Semi- healthy sample but increased the number of bacteria in diseased sample. The number of bacteria in the sample healthy at the main branch of the highest less than the bottom, but in the semi-healthy sample, the number of bacteria from the top down and more of them there are more in the center, while in diseased sample, the number of bacteria is too much and this shown in Fig (15). In addition, the number of bacteria in lichens The number of bacteria in three samples and an equal number was more than 300, as shown in the Fig (16). Through our results was observed that the number of bacteria in the green leaves of the sample healthy sample more than semi-healthy sample, as shown in Fig (17), while the number of bacteria in the brown leaves in all samples were large Fig (18). Fig (19) indicated that the number of bacteria taken of selected locations of Arbutus pavarii tree in an environment actinomycetes were a few specific sites, but the number of bacteria is high in lichens and green leaves and soil
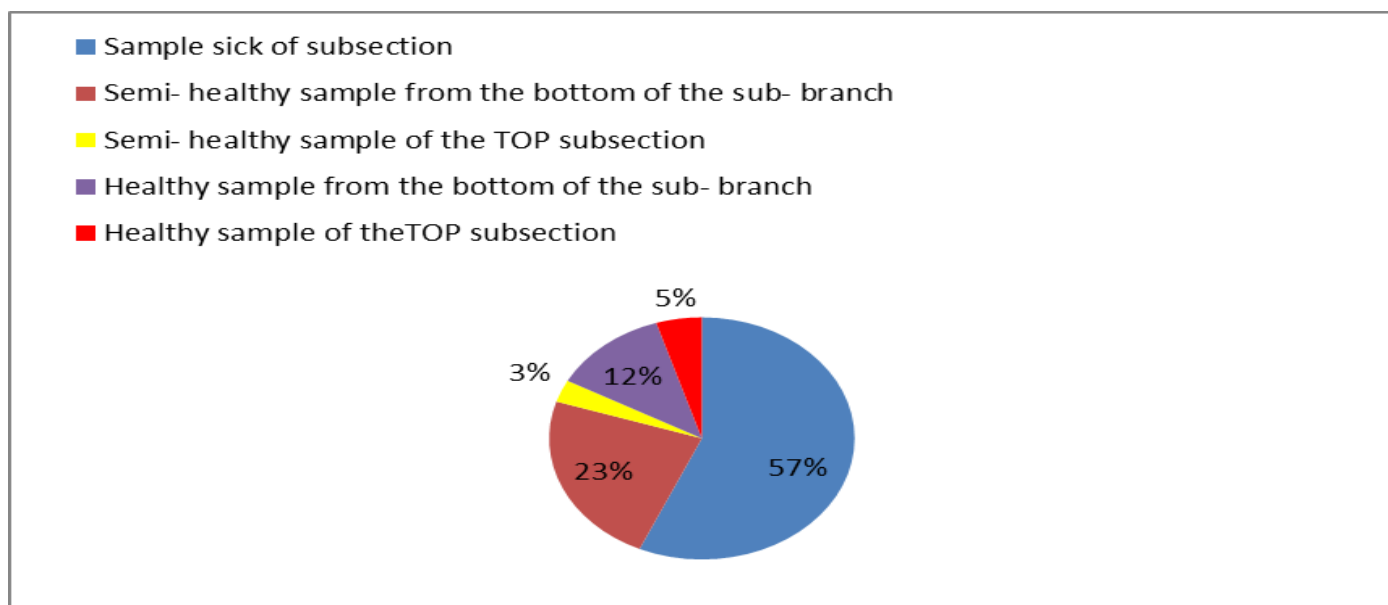

Fig. (14): The number of bacteria on the sub- branches in *Arbutus pavarii*.
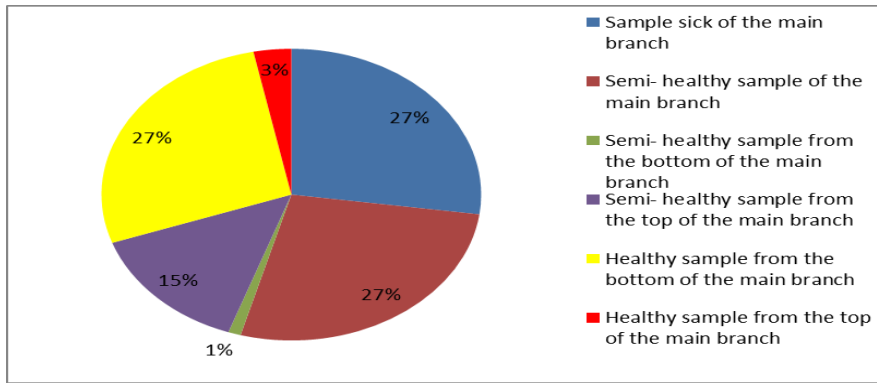
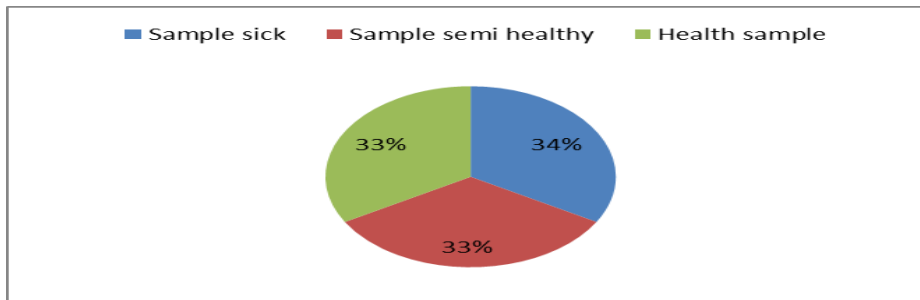**Figure (15): The number of bacteria on the main branch in *Arbutus pavarii*.**

Figure (16): The number of bacteria on lichens in *Arbutus pavarii* plant.
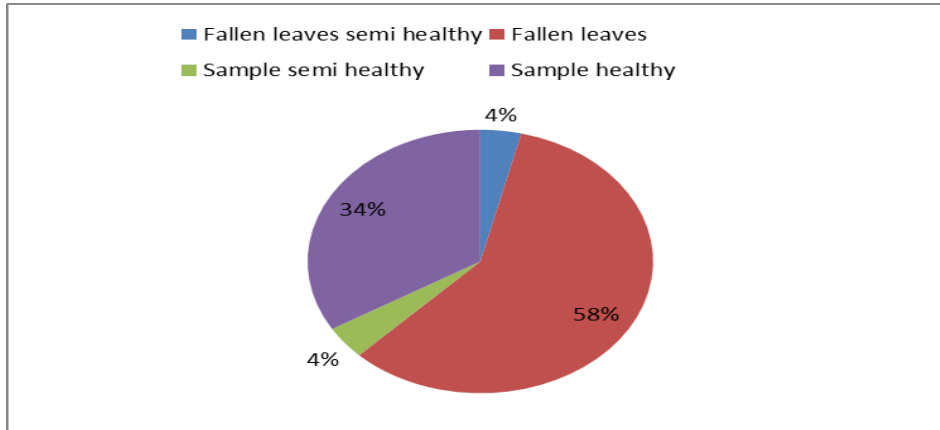

Figure (17): The number of bacteria on green leaves in *Arbutus pavarii*plant**.**
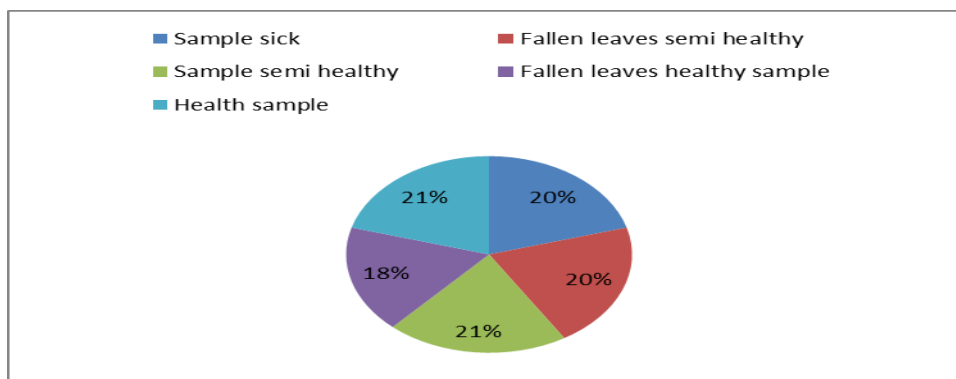
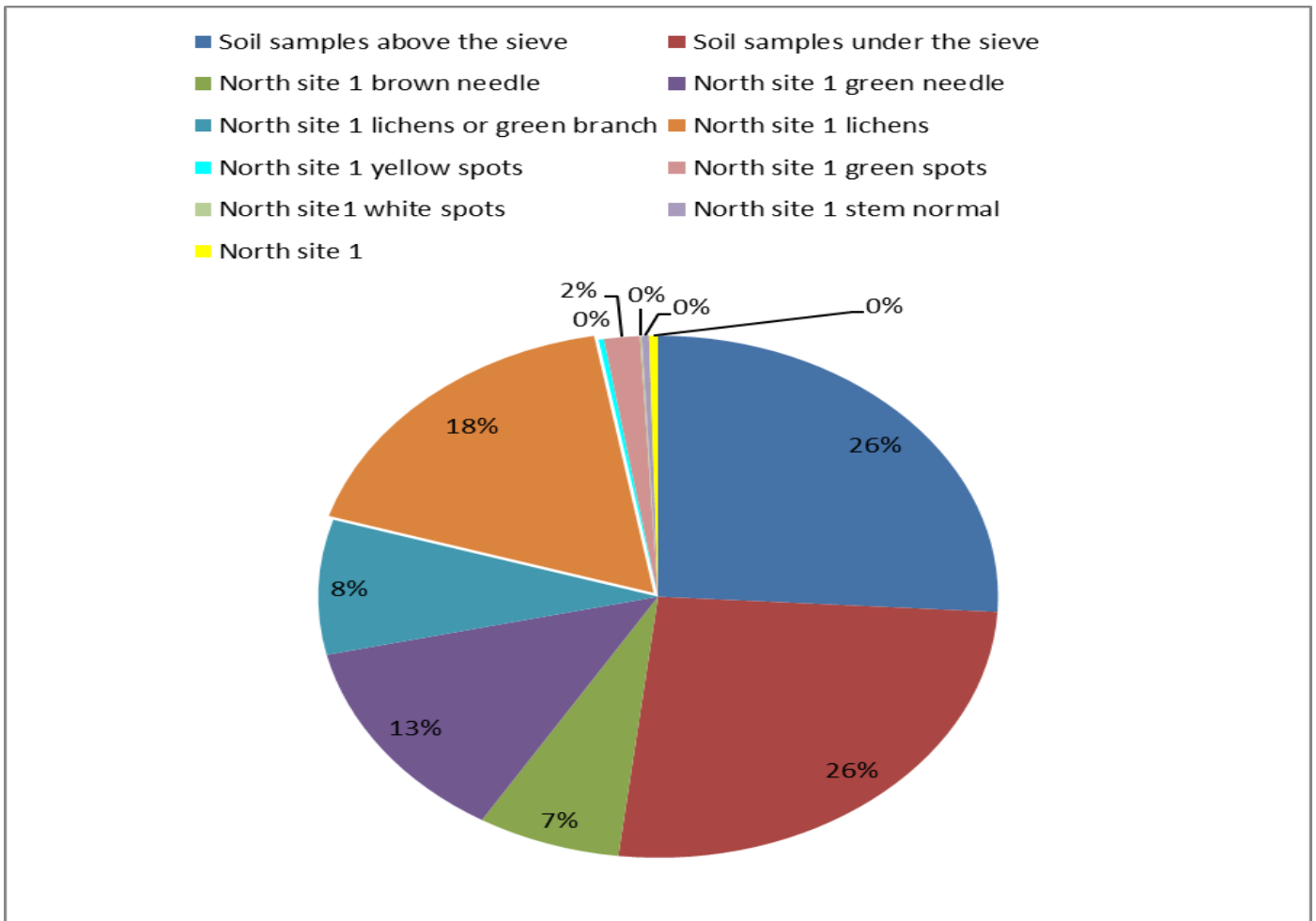**Fig. (18):** The number of bacteria on brown leaves in *Arbutus pavarii* plant**.**

Figure (19): The number of bacteria and total Actinomycetes mass.
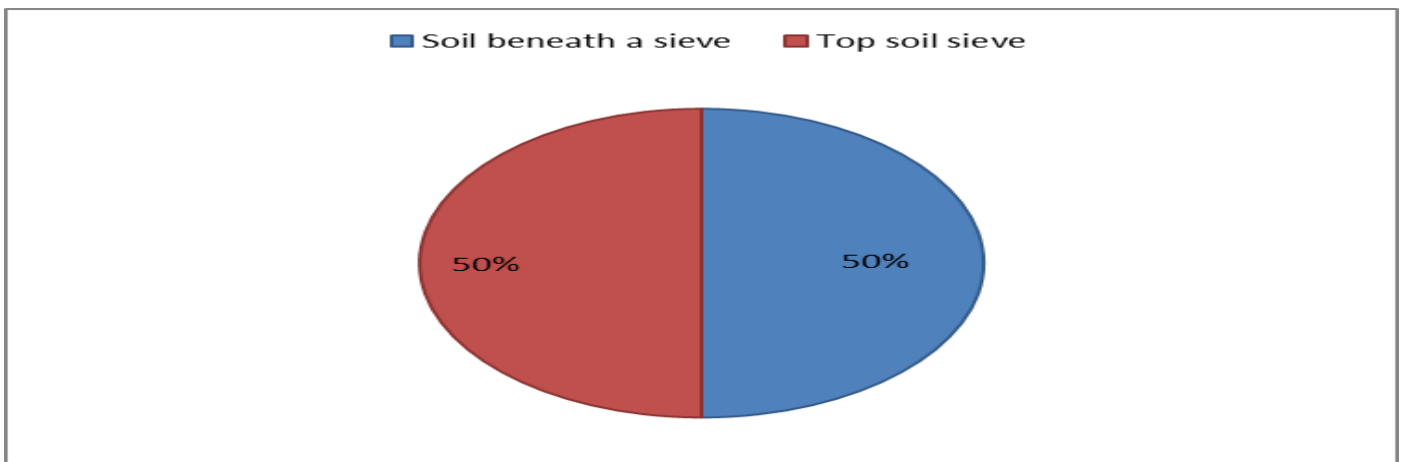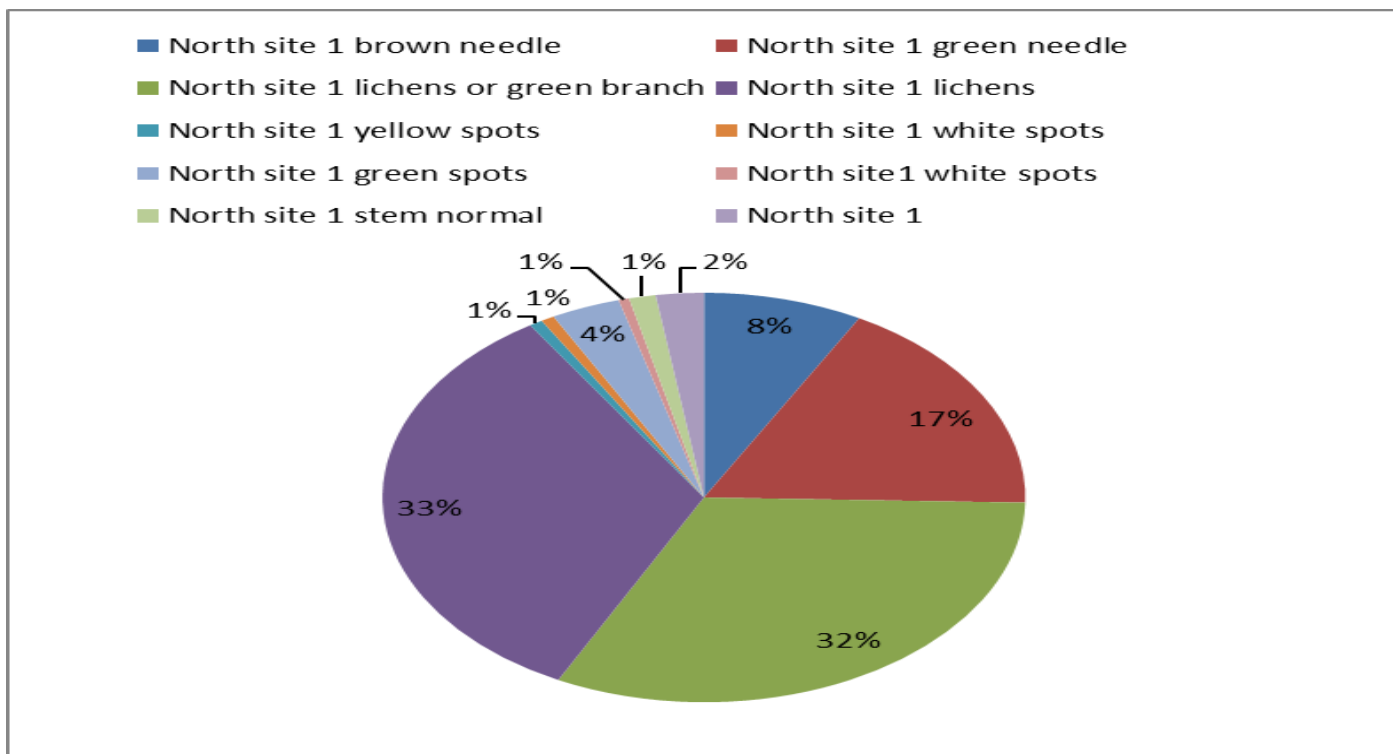
Figure (20): The number of bacterial colonies in soil samples.

Figure(21): The number of bacteria in the Nutrient Agar media taken by Wipes.

Table (11): The Gram stain test, Shape, Endo spores test and Motility test of bacteria on green leaves of *Arbutus pavarii* plant.

Sample Name	Number bacteria	Gram stain	Shape	Endo spores test	Motility test
Sample healthy	141	gram negative	Bacillus	Negative	positive
Fallen leaves healthy	246	gram negative	Bacillus	Negative	positive
Sample semi healthy	17	gram negative	Bacillus	Negative	positive
Fallen leaves semi healthy	16	gram negative	Bacillus	Negative	positive

Table (12): The Gram stain test, Shape, Endo spores test and Motility test of bacteria on brown leaves of *Arbutuspavarii* plant.

Sample Name	Number bacteria	Gram stain	Shape	Endo spores test	Motility test
Sample health	>300	gram negative	Bacillus	Negative	Positive
Fallen leaves healthy sample	261	gram negative	Bacillus	Negative	Positive
Semi healthy sample	>300	gram negative	Bacillus	Negative	Positive
Fallen leaves semi healthy	>300	gram negative	Bacillus	Negative	Positive
Sample sick	>300	gram negative	Bacillus	Negative	Positive

Table (13): The Gram stain test ,Shape, Endo spores test and Motility test of bacteria on lichens of *Arbutus pavarii* plant.

Sample Name	Number bacteria	Gram stain	Shape	Endo spores test	Motility test
Sample health	>300	gram negative	Bacillus	Negative	Positive
Sample semi healthy	>300	gram negative	Bacillus	Negative	Positive
Sample sick	>300	gram negative	Bacillus	Negative	Positive

Table (14): The Gram stain test ,Shape , Endo spores test and Motility test of bacteria on the sub- branches of *Arbutus pavarii*.

Sample Name	Number of bacterial colony	Gram stain	Shape	Endo spores test	Motility test
Healthy sample of the highest subsection	26	gram negative	Bacillus	Negative	Positive
Healthy sample from the bottom of the sub- branch	66	gram negative	Bacillus	Negative	Positive
Semi- healthy sample of the highest subsection	16	gram negative	Bacillus	Negative	Positive
Semi- healthy sample from the bottom of the sub- branch	125	gram negative	Bacillus	Negative	Positive
Sample sick of subsection	>300	gram negative	Bacillus	Negative	Positive

Table (15): The Gram stain test ,Shape, Endo spores test and Motility test of bacteria on the main branch of *Arbutus pavarii*.

Sample Name	Number of bacterial colony	Gram stain	Shape	Endo spores test	Motility test
Healthy sample from the top of the main branch	36	gram negative	Bacillus	Negative	Positive
Healthy sample from the bottom of the main branch	>300	gram negative	Bacillus	Negative	Positive
Semi- healthy sample from the top of the main branch	160	gram negative	Bacillus	Negative	Positive
Semi- healthy sample from the bottom of the main branch	12	gram	Bacillus	Negative	Positive

		negative			
Semi- healthy sample of the main branch	>300	gram negative	Bacillus	Negative	Positive
Sample sick of the main branch	>300	gram negative	Bacillus	Negative	Positive

Table (16): The Gram stain test ,Shape, Endo spores test and Motility test of bacteria in the environment Actinomycetes

Sample Name	Number of bacterial colony	Gram stain	Shape	Endo spores test	Motility test
North site 1	5	gram negative	Bacillus	Negative	Positive
North site 1 normal stem	4	gram negative	Bacillus	Negative	Positive
North site1 white spots	1	gram negative	Bacillus	Negative	Positive
North site 1 green spots	20	gram negative	Bacillus	Negative	Positive
North site 1 yellow spots	3	gram negative	Bacillus	Negative	Positive
North site 1 lichens	>200	gram negative	Bacillus	Negative	Positive
North site 1 lichens or green branch	98	gram negative	Bacillus	Negative	Positive
North site 1 green needle	200>	gram negative	Bacillus	Negative	Positive
North site 1 brown needle	80	gram negative	Bacillus	Negative	Positive
Soil samples under the sieve	>300	gram negative	Bacillus	Negative	Positive
Soil samples above the sieve	>300	gram negative	Bacillus	Negative	Positive

DISCUSSION:

1. Antioxidant evaluation of essential oils extracted from *Arbutus pavarii Pamp. (Ericaceae):*The antioxidant activities of essential oils extracted from leaves of Juniperus were evaluated by:

Total phenolic content (TPC) and total flavonoids content (TFC): Data in figure (9) showed that total phenolic content found in essential oils , were the essential oil of sample number 1(plant intact naturally grow) contain high total phenolic content, the results expressed according to pyrogallol as phenolic compound in figure (6). The total flavonoid obtained in this study as shown in figure (10) indicate that the essential oil of sample number 1contain high amount of flavonoids compounds as compared with the qurecetin in figure (7) whish was used as standard. The results of the current study using the *Arbutus pavarii*correlated with the findings of other investigators (**Mansouri *et al*., 2011, Nakanishi *et al.,* 2005**). Phenols and polyphenolic compounds such as

flavonoids are widely found in food products derived from plant sources and they have been shown to poses significant antioxidant activities (**Van Acker*et al.,* 1996**). **Reducing power assay (RPA) and The DPPH˙ radical scavenging activity:** Fe (II) reduction is often used as an indicator of electron- donating activity, which is an important mechanism in phenolic antioxidant action (**Nabavi *et al.,* 2009).** In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of Fe+3 to Fe+2 by donating an electron. The amount of Fe+2 complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. As shown in figure (11) the reducing power assay of essential oil of sample 1and sample 2 exhibit higher reducing activity than the ascorbic acid. This results obtained from the in vitro antioxidant screened showed that essential oil of sample number 1 and 2 from leaves*Arbutus Pavarii* has considerable

amounts of polyphenols and flavonoids compounds which are responsible for the antioxidant properties. And also they gave the reductive potential due to reducing capacity and DPPH free radical scavenging activity which serves as strong indicator of antioxidant activities (Figure 12). **Fatma and Violet (2013)** found that essential oils of *Arbutus Pavarii* has the highest radical-scavenging activity followed by all medicinal Plants. The genus *Arbutus Pavarii* is an important component of arid and semi-arid ecosystems throughout the northern hemisphere (**Farjon, 1992; Adams, 2008**). Previously, from the genus *Juniperus* some terpenoids have been isolated (**Barrero et al., 2006; Mansouri et al., 2010**), neolignans (**Nakanishi et al., 2004**) and flavonoids (**Inatomi et al., 2005**).The species of *Arbutus Pavarii* is considered as an important medicinal plant largely used in traditional medicine. The anti-inflammatory activity of some diterpenoids of leaves*Arbutus Pavarii*(**Mansouri et al., 2010**) and several studies about the essential oil of leaves Arbutus Pavarii have been published (**Adams, 2008**). All oils of*Arbutus Pavarii* of five localities from eastern Algeria have a high content of α- pinene , Δ3-carene, limonene, terpinolene and the α-terpinyl acetate (**Messaoud et al., 2013**).

The phenolic and the flavonoids compounds are groups of secondary metabolites with broad range of biological properties such as: antioxidant, antibacterial ,anti-atherosclerosis, cardiovascular protection and improvement of the endothelial function, it has been reported that antioxidant activity of the phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors play an important role by adsorbing and neutralizing reactive free radicals, and chelating ferric ions which catalyses lipid peroxidation, and regarded as promising therapeutic agent for free radical-linked pathologies (**Nyangono et al., 2012**).

2.The GC-MS analysis of the essential oils extracted from leaves *Arbutus Pavarii*:

The retention times of the different compounds in the essential oils of *Arbutus Pavarii* are presented in Table 8. A total of thirty four volatile compounds, representing 88.29% of the total oil composition in sample 1, were identified in the leaves oils (Table 8). Monoterpene hydrocarbons were found to be the major group of compounds. The most abundant component found in the leaf oil was α pinene (20.85%) followed by Germacrene D (16.49%), while α pinene and Germacrene D in sample 2 and sample 3were (12.45%,3.09%) and (9.19% ,1.054%) respectively. Surprisingly, there was a decrease in these compounds in the diseased sample (Table 8).

        The results are consistent with those obtained by (**Angioni et al., 2013**) which identified that α pinene and Germacrene D were among the main components of the essentials oils extracted from *Arbutus Pavarii* spp. Turbinate. The results were also consistent with those reported on the analysis of other

juniper oils (**Adams et al., 2015, Adams, 1998**) in which α pinene was more common. (**Achak et al., 2008**) . The main chemical components of Arbutus Pavarii oil are a-pinene, camphene, b-pinene, sabinene, myrcene, a-phellandrene, a-terpinene, y-terpinene, 1,4-cineole, b-phellandrene, p-cymene, terpinen-4-ol, bornyl acetate, cayophyllene and trace amounts of limonene, camphor, linalool, linalyl acetate, borneol and nerol (http://www.essentialoils.co.za). Through the results we note that these compounds are very few and may be non-existent in diseased sampl**e.**the results confirm that some compounds may play an important role as a signal molecule based on signal transduction pathway and cross-talk phenomenon (**P. A. Thomas et al., 2007**). The interesting compound we found α – pinene has been found in healthy plant in amount 20.85% where in the others were 12.45 and 3.09-respectively. Thus , from biochemical points of view this compound may be the target and the key factor for the retardation of such endogenous plants species. More work is needed to understand the actual mechanisms for that compounds and it's derivatives produced during the plant life cycle.

Terpenes have been particularly well studied. Oil extracted from *Arbutus Pavarii* is known to be diverse: **Butkiene et al. (2006)** identified 149 different constituents making up 94.9–99.2% of the oils from Lithuanian material. Monoterpenes and sesquiterpenes make up the bulk of this (92–99%) although the balance between these two groups is very variable (monoterpenes: 55– 90%; sesquiterpenes: 13–42%) (**Butkiene et al., 2006; Buhl and Duso, 2006**). Sixteen different diterpenes have also been isolated from juniper leaves, including ssp. hemisphaerica (**Pascual Teresa et al., 1980**). Through our results we note that the weight of Leaf after drying (mg) of diseased sample is more than in the Healthy sample . The length of the Healthy sample was 222.67 cm while diseased sample was 214.23 cm and We found Stem diameter of 16.42 cm Healthy sample while diseased sample and semi-sample (11.22cm and 9.89 cm)respectively. The results obtained in this study as shown in table(10) indicate that the healthy sample contain high amount of Chlorophyll A, Chlorophyll B and Carotenoids as compared with the Semi healthy sample and Sample disease**.** This leads us to the link between the chemical content and shape of the outer plant **Mariam and Salem (2015)**showed *Arbutus Pavarii* plants in EL-Gabal EL-Akhdar area are exposed to huge stress which caused by climatic changes. During obvious years, the rates of precipitation in the study area were clearly decreased, while temperature levels and seasonal drought period were increased. The phenomena of "plant-climate interactions" was clear in our results and it was reflected by increasing damage and death of juniper plants because of shortage of water (rainfalls). The healthy growth, good vegetation cover, and increasing plant frequency in study site number Not only has the

nature played rule in this damage, but anthropogenic factors. Due to over population in the area during few last years, the pressure on vegetation composition was increased rapidly and subsequently, huge number of plant species like juniper were affected. The activities those noticed and recorded in the study area included mining, overgrazing, fire wood collection and fire making, expansion of urbanization, agricultural processes, medicinal plants collection, and effect of weekenders(**Mariam and Salem, 2015**) . The data showed to be the first report gave the scientific communities especiallywere the plant canopy exists specifically in Mediterranean region and Al-Jabal Al-Akdar area. Many researchers attempt to find out what is going on .But no clear data can interpreted the problems even some plants in the same location with the same environmental and soil conditions but you will see some plant totally destroyed and some fully healthy, this lead us to go deeper and to put our hypothesis taken all other studies under consideration. Although chemical characteristics has been conducted on this species, additional research is still required to analyses the essential oils of this species in the studied region

Conclusion

Summary and conclusion:

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids; tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants.

The aim of this study was to investigate and identify some of the physiological characteristics of the *Arbutus Pavarii* L, determine the chemical composition and antioxidant activity.

1. Antioxidant evaluation of essential oilsextracted from *Arbutus Pavarii*:

Total phenolic content that found in essential oils were the essential oil of sample number (1) (intact plant grow naturally) contain high total phenolic content, the results expressed according to pyrogallol as phenolic compound.

This results obtained from the in vitro antioxidant screened showed that essential oil of healthy sample and semi healthy sample from leaves*Arbutus Pavarii*has considerable amounts of polyphenols and flavonoids compounds which are responsible for the antioxidant properties. In addition, they give the higher reductive potential due to reducing capacity and DPPH free radical scavenging activity, which serves as strong indicator of antioxidant activities (Figure 12**).

2. The GC-MS of the essential oils extracted from leaves *Arbutus Pavarii*:

The retention time of the different compounds in the essential oils of *J. phoenicea* are presented in Table 8.

A total of thirty four volatile compounds, representing 88.29% of the total oil composition in sample 1, were identified in the leaves oils (Table 8). Monoterpene hydrocarbons were found to be the major group of compounds. The most abundant component found in the leaf oil was α pinene (20.85%) followed by Germacrene D (16.49%), while α pinene and Germacrene D in sample 2 and sample 3were (12.45%,3.09%) and (9.19% ,1.054%) respectively**.** Surprisingly, there were decrease of these compounds in the sample diseased (table 8). The main chemical components of *Arbutus Pavarii* oil are a-pinene, camphene, b-pinene, sabinene, myrcene, a-phellandrene, a-terpinene, y-terpinene, 1,4-cineole, b-phellandrene, p-cymene, terpinen-4-ol, bornyl acetate, cayophyllene and trace amounts of limonene, camphor, linalool, linalyl acetate, borneol and nerol..(http://www.essentialoils.co.za).

Through the results we note that these compounds are very few and may be non-existent in diseased sampl**e.** The interesting compound we found α –pinene has been found in healthy plant in amount 20.85% where in the others were 12.45 and 3.09- respectively. Thus, from biochemical points of view this compound may be the target and the key factor for the retardation of such endogenous plants species. Through the results, we note that the weight of Leaf after drying (mg) of diseased sample is more than in the healthy sample. The length of the healthy sample was 222.67 cm while diseased sample was 214.23 cm and We found Stem diameter of 16.42 cm Healthy sample while diseased sample and semi-sample (11.22 cm and 9.89 cm)respectively. The results obtained in this study as shown in table (10) indicate that the healthy sample contained high amount of **chlorophyll** A, chlorophyll B and carotenoids as compared with the semi healthy sample and disease sample**.** This leads us to the link between the chemical content and shape of the outer plant.

Conclusion

We conclude from the results that reduction of the chemical compounds of *Arbutus Pavarii* content plays a role in the extinction of this plant. Although chemical characteristics has been conducted on this species, additional research is still required to analyses the essential oils of this species in the studied region to support this result.

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