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Phytochemical, Mineral Compounds and Anti- Oxidation Studies on Pistacia Lentiscus Shoot Extract

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Keywords: pistacia lentiscus L., phytochemical screen-ing; cortisone, mineral composition, antioxidant activity. tannins, phlobatannins, volatile oil, active components, shoot extract, alkaloid.

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Phytochemical, Mineral Compounds and Anti-Oxidation Studies on *Pistacia Lentiscus* Shoot Extract

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Keywords: pistacia lentiscus L., phytochemical screening; cortisone, mineral composition, antioxidant activity. tannins, phlobatannins, volatile oil, active components, shoot extract, alkaloid.

I. Introduction

Pistacia *lentiscus* (mastic tree), Family Anacardiaceae, an important medicinal plant is widely distributed in Mediterranean Europe, Morocco and Iberian peninsula and in the west through southern France, Turkey, Iraq and Iran. It has a great medicinal value and already has been used in traditional system of medicines. The pharmacology and medicinal use of mastic is diverse. It has been used in cancer, infection, surgical wound adhesion, and ulcers. Studies also document its use as an antioxidant, antiatherogenic, an insecticide, and anti-inflammatory, anti-microbial and for treatment of hypertension and relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Ahida, et al, 2012, Barra, et. al., 2007, Dedoussis et al., 2004; Lamiri et al., 2001, Bonsignore, et al. 1998, Al-Habbal et al., 1984 and Bentley et al., 1980). In addition to its medicinal usage, it has been re- evaluated as a flavoring in chewing gum (Fernandez et al., 2000).

Phytochemicals are chemical compounds that occur naturally in plants. study done by Kumar and Singh (1976) refereed to that phytochemicals are secondary metabolites and are often found in stems, roots, barks, leaves, flowers, fruits and seeds. Common phytochemicals found in plants include tannins, phlobatannins, quinines, alkaloids, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, sugar. They are having potential to affect diseases such as cancer, gout, rheumatism, arthritis (Berboucha et al., 2009; Dimaset al., 2009; Peksel, 2008, Balan, etal, 2007 and Baytop, 1999). Cortisone is a steroid hormone and is used for a variety of ailments. These include endocrine disorders, rheumatic disorders, collagen diseases, dermatologic diseases, allergic states, ophthalmic diseases, respiratory diseases, hematologic disorders, neoplastic diseases, edematous diseases.

Antioxidant - Most phytochemicals have antioxidant activity and protect the cells against oxidative damage and reduce the risk of developing certain types of cancer activity includes: flavonoids, polyphenols.

Anthracene is glycosidic compounds of formula $C_{14}H_{10}$. Anthracene is converted mainly to anthroquinone, a precursor to dyes (Gerd, et al., 2006). It uses in pesticides (Agency for Toxic Substances, 1999). Study by Dembitsky VM (2005) reported that Anthracene and its derivatives, isolated from and identified in plants and microorganisms that demonstrate different biological activities. They are of great interest, especially for the medicinal and/or pharmaceutical industries. These biologically active natural surfactants are good prospects for the future chemical preparation of compounds useful as antioxidant, anticancer, antimicrobial, and anti-bacterial agents (Dembitsky, 2005). This study was conducted to determine some photochemical present in *Pistacia lentiscus* shoot extract. We also evaluated its mineral composition.

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II. Material and Methods

a) Plant material

Shoot of P. lentiscus was collected around from Msallata (northwestern part of Libya). Plants were identified according to (Ali, et al., 1977).

b) Preparation of plant extracts

Leaves and parts of stem (10gm) were subjected to sohxlet extraction using 200ml of water and ethyl-acetate (individually) as solvents. Samples kept for 6 hours at 90◦C for water and 70◦C for Ethyl- acetate.

c) Phytochemical tests

Plant extracts, of both solvent water and ethylactate, were screened for the presence of biologically active compounds like alkaloids, flavanoids, Protenis, Phenol, Phlobatannin, Volte oil, Hydrolysable Tannins, Tannins, Saponins, Glycosides, Anthracanens and Cortisone.

d) Procedure for Phytochemical Analysis

i. *Alkaloid Test*

Equal volumes of the solvent extract (5ml) and the Wagner's reagent, described by Imohiosen et al.(2014), were placed into a clean test tube and observed for some minutes. The presence of alkaloid was indicated by a brown precipitate.

ii. *Saponins Test:* (Froth test)

1g of the sample was weighed into a conical flask in 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

iii. *FlavonoidsTest*

Flavonoids was determined by placing 5ml of the solvent extracts into a test tube and few pieces of magnesium chips were added, followed by concentrated hydrochloric was added drop wise. Appearance of Reddish colouration demonstrated the presence of Flavonoids.

iv. *Proteins Test*

1ml of plant extracts was placed into a test tube then 4ml of foline reagent was add. Appearance of blue colouration demonstrated the presence of proteins.

v. *Phenol Test*

2ml of extract was added to 2ml of ferric chloride solution ($FeCl_3$); a deep bluish green solution is formed with presence of phenols.

vi. *Phlobatannin Test*

3ml of hydrochloride acid and 2ml of solvent extract were placed into a clean test tube and the tube was heated for about 10 minutes. Reddish green coloration indicates the presence of phlobatannins.

vii. *Hydrolysable tannins*

4 ml of the extract was placed and shaken in a test tube. 4ml of 10% ammonia solution was added. Formation of an emulsion on shaking indicated the presence of hydrolysable tannins.

viii. *Tannins Test*

3ml of sample extract was boiled in 50ml distilled water, warmed and filtered. A portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. Green colour indicates the presence of tannins.

ix. *Volite Oil Test*

2.0ml of extract solution was mixed with 0.1 ml dilute sodium hydroxide and a small quantity of dilute HCl. A white precipitate was formed with volatile oils.

x. *Anthracanens test*

3g of sample powder was mixed with 4ml of ammonia solution, heated for 15 min. Red color indicated the presence of Anthracanens.

xi. *Glycosides Test*

25 ml of dilute sulphuric acid was added to 5 ml of the extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5 ml of Fehling solution A and B was added. A brick red precipitate of reducing sugar indicates the presence of glycosides.

xii. *Detection of Cortisone*

a. *Preparation of plant extraction*

10g of leaves and stem were extracted with 250ml of water, petroleum ether, hexan and finally ethyl acetate (respectively) using a soxhlet extractor. Each extraction was carried out for 6 h continuously at 90°C, 70°C, 50°C and 30°C with water, ethyl acetate, and hexan and petroleum ether (respectively).

b. *IR Spectra*

A small amount of each extraction was placed on the diamond attenuated total reflectance (ATR) crystal of the Agilent Cary 630 ATR-FTIR analyzer. The samples were pressed against the diamond crystal using the attached pressure clamp. FTIR spectra were acquired in less than 30 seconds. The spectra of reference storied (cortisone) samples were automatically stored in a dedicated spectral database. The spectra of extractions samples were then analyzed using an automated output pass/fail or percentage (%) similarity. 10 ug of pure cortisone was dissolved in different solvent (water, petroleum ether, hexan and ethyl acetate, respectively) and were used for comparison

c. *UV-Visible spectrophotometer*

5g of leaves and stem was extracted with 125ml of ethyl acetate and ethanol (individually). Cortisone was detected in sample by UV-Visible spectrophotometer (Agilent Cary 60 UV-Vis). Cortisone standard was dissolved in ethyl acetate or ethanol then loaded for comparison.

e) Thin Layer Chromatography (TLC)

i. *TLC analysis*

Chromatography plates were prepared using silica Gel, 60 F254 TLC Aluminum Sheet 20x20cm Merck- Germany. The samples were spotted on the plates with graduated capillary tubes (5 μL). The standard of cortisone was dissolved in different solvents (water, petroleum ether, hexan and finally ethyl acetate) then spotted for comparison. Toluene- ethyl acetate-diethyl-amine (14:2:2) were used as mobile phase.

ii. *Detection*

Cortisone band was observed as follows: * Without treatment using UV (254-356nm). *Universal detection reagents: using both concentrated sulphoric acid and iodine vapor from crystal then comparing its rate of flow (R_f) value with standard.

f) Mineral Compounds Analysis

The dried plant was wet oxidized and the elements were determined by Atomic Absorption Spectrophotometer (Perkin-Elmer model 403, Norwalk Ct, USA). The minerals were reported in ppm. The minerals include sodium, potassium, calcium, magnesium, iron, copper and Zn, Pb, Cd and Cr. Values for, Fe, Cu and Mg were read on Atomic Absorption Spectrophotometer (180-30 Hitachi) after standardizing with respective elements (American Association of Cereal Chemists, 1984).

III. Results and Discussion

a) Phytochemicals analysis

The results illustrated in Table I; the phytochemical analysis conducted on water and ethylacetate shoot extracts of *P. lentiscus* revealed the presence of some bioactive components such as alkaloids, tannins, hydrolysable tannins, phlobatannins, phenol, volatile oil, saponins, glycosides, flavonoids, protein and Anthracanens.

Table 1 : Photochemical compound of water and ethyl acetate shoot extracts of *P. lentiscus*

Phytochemical Compounds	Test	Water and ethyl-acetate shoot extract	color develop
1-Alkaloids	Wagner's	A++ B+	Brown precipitate
2-Portein	Folin test	A+ B++	deep blue
3-Phenol	1% aqueous ferric chloride	A+ B+	deep bluish green
4-Phlobatannin	HCl Heating in 10 minutes	A++ B+	Reddish green
5-Flavonoids	pieces of magnesium chips and HCl	A+ B+	red
6- Volite Oil	0.1 % NaOH + HCl	A+ B+	layer oil
7- Hydrolysable tannins	Amonia solution	A+ B++	Formation of an emulsion
8- Tannin	10% ferric chloride, 50ml distilled water heating 30 min.	A+ B++	Green precipitate
9- Saponins	Froth test	A+ B+	Honeycomb froth
10-Glycosides	Fehlange	A+ B+	red
11- Anthra - canens	Amonia solution	A+ B+	red

A=water extract, B=Athylacetate extract, + Positive, ++ = Abundant, - = Negative.

The presence of some of these bioactive components like Alkaloids and Flavonoids confirms similar research conducted by Rhouma, et al..(2009), while the result obtained showed the presence of: glycosides, phenols, saponins tannins, volatile oils, hydrolysable tannins, protein, Hydrolysable tannins and Anthra-canens.

b) Detection of Cortisone

i. *IR Spectra*

The compatibility studies of cortisone standard alone and along with *P. lentiscus* shoot extractions water; ethyl-acetate; hexan and petroleum-ether) are carried out by using FT-IR. The data of study were shown in Fig. (1). The peaks were recorded in the range of 1000 to 3500 cm^{-1}.

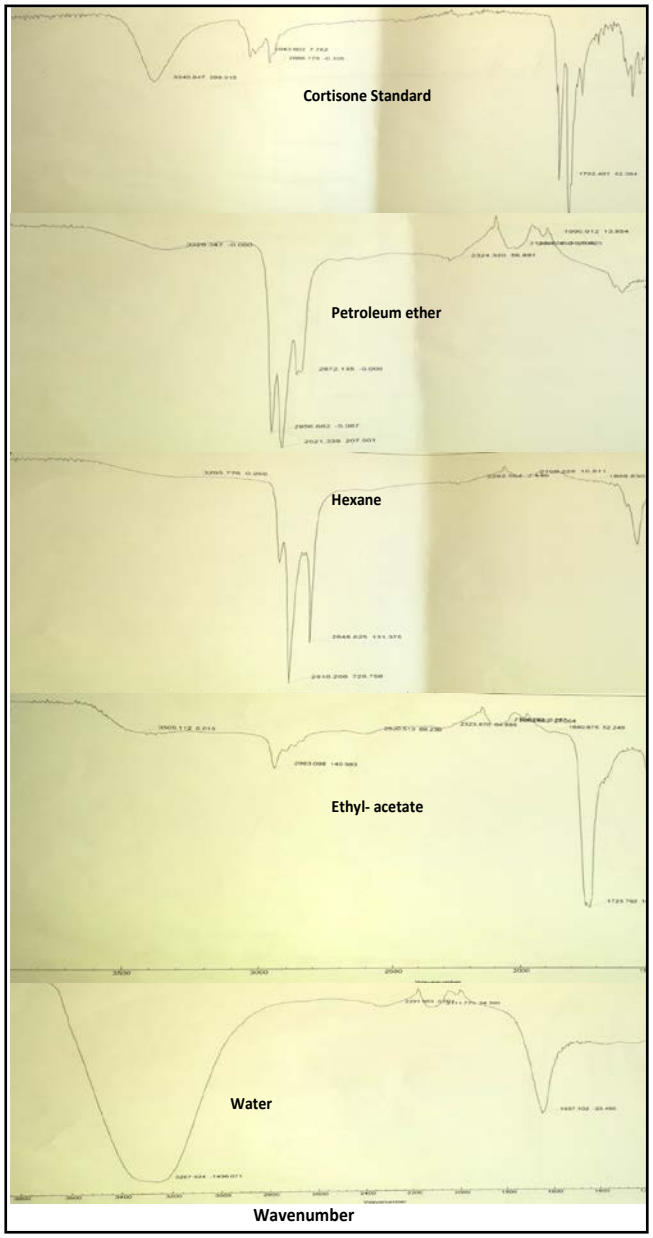

Figure 1 : FT-IR spectra of pure cortisone, Petroleum ether, Haxen, ethyl-acetate and water shoot extracts of P. *lentiscus shoot*

Cortisone isolated from the shoot of *P. lentiscus* corresponds to the infrared spectrum to him by petroleum ether, hexane and ethyl acetate as shown in Figure (1) closely with spectrum of cortisone standard (pouchert, 1981). It pointed out (Nimit et al., 2011 and Harborne, 1973) to the importance of the comparison between the compounds prepared chemically (cortisone and hydrocortisone standard) and those isolated from natural sources as the diagnosis full for several types of plant components, especially when the area fingerprint (Fingerprint region) which is located on the frequencies less than 3000 cm^{-1} and the advantage of this topic area are complex because it produces movement vibratory compound as a whole, well as match frequencies packages described in Fig. (1) with those mentioned by (Nimit et al., 2011 and Tripathi, et.al., 1981) as stated that vibration frequencies of infrared compound effectively isolated from the shoots of *P. lentiscus* intentions and dissolved by petroleum ether and hexane are: (2956.682, 2921.339 , 2872.135 cm^{-1} and2916.208, 2848.625 respectively) either dissolved by and ethyl acetate was so close (2983.098cm^{-1}) and usually show Aliphatic C-H stretch of hydroxy -cortisone and hydrocortisone - at frequency 2943.802cm^{-1} 2916.554 , as shown in Table 2.

Table 2 : Peak of cortisone in FT-IR Spectra

Frequency (cm^{-1}) Cortisone standard	Frequency (cm^{-1}) Shoot extract by petroleum ether	Frequency (cm^{-1}) Shoot extract by haxen	Frequency (cm^{-1}) Shoot extract by ethyl acetate	Description
3340.8847-2900	3328.347	3295.776	3505.112	O-H stretch
2943.802	2921.339	2916.554	2983.098	Aliphatic C-H stretch
1664-1702	1707.808	1701.653	1723.792	C=O stretch
1047.001	1088.545	1048	1043.495	C-O-C bending

The result also illustrated that water extract of *P. lentiscus* did not reveal the presence of cortisone, where infrared absorption peak at 3267.924 and unlike pure cortisone (standard).

ii. *UV-Visible Spectrophotometer*

Ethanol and ethyl acetate extract of *P. lentiscus* shoot were analyzed for cortisone detection using UV-Visible spectrophotometer. The result suggested that cortisone was detected at 284 nm, in ethanol extract and ethyl acetate extract. This result in agreement with study done by Christian, M. which referred that absorption spectra of total 7 steroids at 200 nm, 7 at 254 nm, 6 at 366 nm were obtained from P. fraternus.

iii. *Thin Layer Chromatography (TLC)*

Standard cortisone and *P. lentiscus* shoot extracts (with petroleum ether, haxen and ethyl acetate, individually) were spotted on TLC plates then developed using 18Toluene: 2 ethyl acetate: 2 diethyl amine as a solvent. Spots were detected using iodine vapor, UV and concentrated sulphoric acid (Heftmann, et al., 1966). The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of RF value (Mendham, et. al., 2002). Result of this study, as shown in Table 3 and Fig. 2, indicated that there was match between standard cortisone and plant extracts in terms of spots number and RF values.

Table 3 : TLC results of *P. lentiscus* shoot extracts and cortisone standard

Spots number	Cortisone Standard R_F values	Shoot extract by petroleum Ether RF values	Shoot extract by hexan R_F values
Spot 1	0.93	0.93	0.93
Spot 2	0.84	0.84	0.84
Spot 3	0.72	0.72	0.72
Spot 4	0.62	0.23	0.62
Spot 5	0.51	0.12	0.23
Spot 6	0.45	0.06	0.12

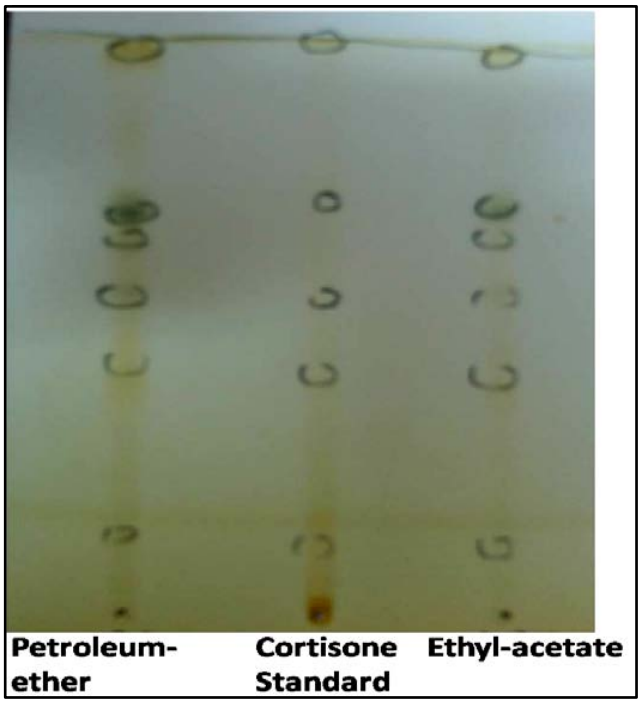

Figure 2 : TLC results of *P. lentiscus* shoot extracts and cortisone standard detected by iodine vapor

TLC profiling of *P. lentiscus* shoot extracts in different solvent system confirms the presence of diverse group of phytochemicals (steroids). Different R_F values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from *P. lentiscus* shoot extract (Das Talukdar et al., 2010).

c) Mineral components

Investigated elements were chosen (Na, Zn, Fe, K, Cu, Ca, Mg, Cd, Cr, and Pb) according to their role and importance in many biological mechanisms. Quantitative determinations were made of the mentioned elements in the shoots of *P. Lentiscus.* The composition in major and minor minerals of the shoots of *P. lentiscus* is detailed in Fig. 3 and 4.

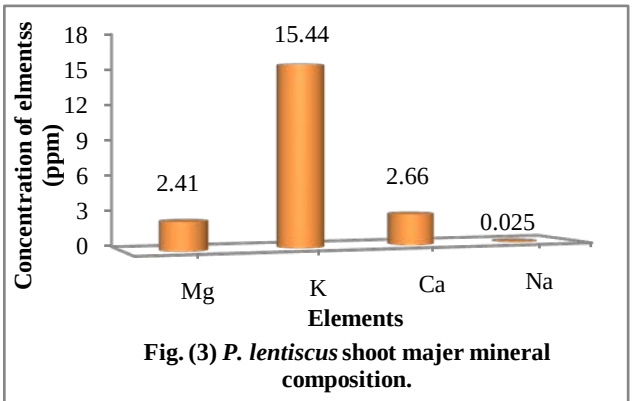

Fig. (3) *P. lentiscus* shoot majer mineral composition.

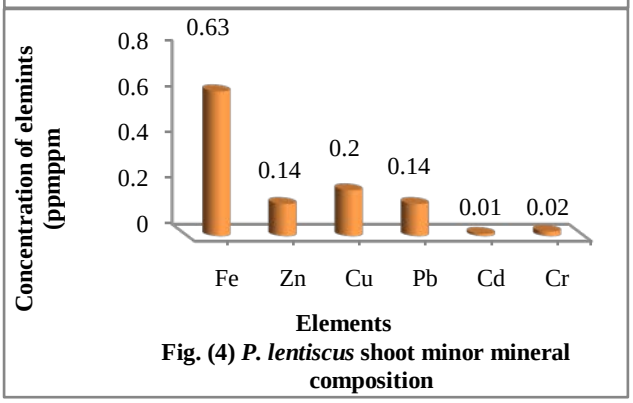

Fig. (4) *P. lentiscus* shoot minor mineral composition

The mineral composition of *P. Lentiscus* shoot revealed its nutritional value for human and/or animal (Omri et. al., 2013).

The importance of mineral composition is due to their nutritional properties and beneficial health effects, as well as their meeting of dietary guidelines required for a healthy diet (Welna et al., 2008). Results of this study revealed to that Potassium had the highest content of (15.44ppm) dry weights this result in agreement with study done by (Aouinti et al., 2014). The level of Ca in the shoots of *P.lentiscus* in this work was found to be higher (2.66 ppm) dry weight compared to Na (0.25 ppm). Calcium is the major component of bone and assists in teeth development (Brody, 1994).

Magnesium is not only essential, but it is also a constitutive element of chlorophyll, so that its highest concentration was found in leaves (Aouinti et al., 2014). In this study, the average concentration of Magnesium in the *P.lentiscus* shoot was 2.41 ppm. **Iron** functions as hemoglobin in the transport of oxygen. Iron functions as essential component of enzymes involved in biological oxidation such as cytochromes (Malhotra, 1989). It is an important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb) and the cytochromes (Chandra, 1990). Though it is an essential element, excess intake can lead to iron toxicity and can damage lipids and proteins (Bothwell, et al., 1979 and Fraga, et al., 2002). Result of this study revealed to that shoots of *P. lentiscus* are reach in iron with a concentration of 0.63 ppm. This result is consistent with the (Aouinti et al. 2014).

Cupper and Lead has been detected in the shoot of *P. lentiscus* with concentration of 0.14ppm. Cupper is component of many redox and lignin-biosynthetic enzymes. In plant, its deficiency symptoms include Chlorosis, dead spots in leaves, stunted growth, terminal buds die, necrosis in young leaves (Soetan, et al., 2010). There are also inter-relationships of iron, copper and cobalt (in vitamin B12) in hemoglobin synthesis and red blood cell formation (Hays, et. al., 1985). Lead is best known for its toxicological properties (Macrae, et al, 1993) there are increased in depressives and schizophrenics but reduced in manic patients (Stanley, et al., 2002). Lead is an ubiquitous environmental and industrial pollutant that has been detected in every facet of environmental and biological systems.

Zinc is distributed widely in plant and animal tissues and occurs in all living cells. Zn dependent enzymes are involved in macronutrient metabolism and cell replication (Hays, et al., 1985). In humans, deficiency disease or symptoms include hypogonadism, growth failure, impaired wound healing, and parenteral nutrition (Murray *et al.,* 2000).

Cadmium and chromium have detected in the shoots of *P. Lentiscus* with low concentration (0.01 and 0.02 respectively. The possible effects of the general population of long term, low level exposure to cadmium have been of concern recently (Asagba, 2009), because cadmium readily distributed in tissues after exposure and it inhibits antioxidant enzymes (Chater et al.,2009; Asagba and Eriyamremu, 2007; Bagchi et al.,1996; Gupta et al., 1991) and this inhibition can lead to increased oxidative stress which may result in membrane damage and loss of membrane-bound enzymes like ATPases (Galazyn-Sidorczuk et al., 2009; Asagba and Obi, 2005; Asagba et al., 2004 and Figueiredo- Pereira et al., 1998). Chromium is an essential element for animals and humans (Frieden, 1984). It has been found in nucleoproteins isolated from beef liver and also in RNA preparations (Uppala et al., 2005). It could play a role in maintaining the configuration of the RNA molecule, because Cr has been shown to be particularly effective as a cross-linking agent for collagen (Eastmond et al., 2008).

IV. Conclusion

The phytochemical tests performed on the shoot extracts of *P. lentiscus* shows the presence of alkaloids, tannins, hydrolysable tannins, phlobatannins, phenol, volatile oil, saponins, glycosides, flavonoids, , protein and Anthracanens. The present study revealed the presence of cortisone in *P.lentiscus* shoot extracts which were confirmed by various techniques studies. Since cortisone contains a wide range of medicine and pharmacological properties, they can be exploited more storied in future for further studies.

V. Acknowledgements

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