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PHYTOCHEMICAL INVESTIGATION, CYTOTOXICITY, ANTIBACTERIAL AND ANTI-OXIDANT ACTIVITY OF *OLEA EUROPEA FRUITS* GROWN IN OMAN

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ABSTRACT
Olea europaea (syn. Zaytoun) belonging to the family *Oleaceae* is a small evergreen tree, from 12 to 20 feet high. It is reported to contain various flavonoids, steroids, phenolic compounds proteins and lipids. Biological assay of petroleum ether extracts of *Olea europaea* fruits showed good cytotoxic activity against brine shrimp larvae. *Olea europaea* fruits showed good antibacterial activity against gram negative bacteria. *Olea europaea* fruits showed very good antioxidant activity with hydroalcoholic, chloroform and petroleum ether extracts. This plant has lots of medicinal value in traditional system of medicine all over the world. So these investigations will add more awareness regarding the therapeutic value of this plant.

KEYWORDS: *Olea europaea, Oleaceae,* cytotoxic activity, antibacterial activity, antioxidant activity, Zaitoon.

INTRODUCTION
Natural products are currently in demand and their acceptance is increasing day by day. Near about 500 plants with therapeutic use are mentioned in ancient literature, and 800 plants have been used in traditional systems of medicine.[1] As mentioned in holy Quran, the fruits like olive, grape, date, fig, and pomegranate are gifts and heavenly fruits of God.[2] Olives are used in the treatment of different disease, like fever, infectious diseases such as malaria, and the treatment of arrhythmia, and inhibition of intestinal spasms.[3] Zaitoon (*Olea europaea*) family *Oleaceae* is evergreen tree or shrub that is cultivated throughout the whole Mediterranean region. The olive plant is very good source of pharmaceutical raw material and food products.[4] Because of presence of phenolic compounds in olive, the medicinal properties of olive oil, fruit and its leaves have been recognized as important components of medicine.[5] *Olea europaea* trees are 8-10 m tall and tolerate shallow, stony soil, with little amount of fertilizer, and flourish in areas with dry hot summers and also do well in coastal areas. They can survive in temperatures down to -10 degree Celsius.[6] *O.europaea* crude leaf extracts have several pharmacological properties like anti-inflammatory, anti-cancer anti-oxidant, anti-atherogenic, anti-viral, anti-bacterial, in addition oleuropein has been found cardio protective against acute doxorubicin induced cardiotoxicity and has been shown to exhibit anti-ischemic and lipid lowering activities.[7] Olive leaf extracts exhibit antiviral activity by blocking virus-specific system in the infected host.[8] Oleuropein is the major component present in olive responsible for large number of health benefits in humans. Free radical scavenging activity is one of these benefits.[9] Most of all olive leaf extracts are known for its cardiovascular effects since long. Oleuropein, a bitter component of leaf which gets metabolized to calcium elenolate in the body, is found to be responsible for its antiarrhythmic, coronary vasodilator and antihypertensive activities.[10] Olive oil is used to treat gallstones. Olive fruit is used topically as a cosmetic. Water decoction of plant part is taken for bronchitis. Fresh leaf infusion is taken orally to reduce inflammation.[11]

MATERIALS AND METHODS
Materials
The chemicals and solvents used in this project were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl, was obtained from Sigma company. Gram negative bacteria *Escherichia coli* (ATCC 9637), *Pseudomonus aeruginosa* (ATCC 9027), *Proteus vulgaris* (ATCC 13315) and gram positive bacteria *Staphylococcus aureus* (ATCC 29213) were obtained from microbiology department, CAS Nizwa University. Filter papers were obtained from Whatman, UK. Brine shrimp eggs (ARTEMIA CYSTS) use purchased from Taiwan. Sea salt was obtained from Al-Qurum, Muscat.

Plant sample collection
The *Olea europaea* fruits were collected from Jabal Al Akhdar, Oman. The fruits were identified by Dr. Amina

Al-Farsi a taxonomist at the herbarium of Sultan Qaboos University. A specimen of the plant has been deposited at the Herbarium of SQU. The olive fruits were transported to the lab and washed with tap water then dried in shade at room temperature for one week.

Extraction of the plant materials

Powdered drug of *Olea europaea* fruits have soaked in ethanol for seven days (24h x 7). The solvent then decanted out and filter under vacuum using Buchner apparatus to give clear solution. The ethanol evaporated at low pressure using rotary evaporator to obtain crude extract. The ethanolic extract then suspended in ethanol and water mixture (1:1) and extracted with petroleum ether and chloroform (50ml x 2). All solvents later removed using rotary evaporator to give petroleum ether, chloroform and hydro-alcoholic extracts.

Preliminary Phytochemical Screening

The significance of this parameter is to examine the presence or absence of phytoconstituents in different extract. The presence or absence of different phytoconstituents has seen as per the method described.[12]

Cytotoxicity test

(a) Brine Shrimp Test

Cytotoxicity assay was done as described by Mclaughlin and his coworkers using brine shrimp (*Artemia Salina* Leach) larvae as indicator organisms.[13]

(b) Hatching of shrimp larvae

About 50 mg of brine shrimp eggs sprinkled in artificial sea water placed in a dark compartment of a small polyethylene tank divided into two chambers. The sea water prepared by dissolving 38 g of sea salt in one liter of distilled water. The other compartment was illuminated to attract the shrimp larvae from the dark chamber once are hatched within 24 hours.

(c) Brine shrimp lethality test

Stock solution (10mg/ml) of each extract in dichloromethane was diluted to prepare testing concentrations corresponding to 250, 500 and 1000µg/ml in five difference vials. The solvent was then evaporated out by leaving the vials overnight in the fume hood. About 3 ml of artificial seawater was added in each vial. A total of 10 shrimp larvae were then transferred in each vial and the solution was diluted to 5 ml by using the artificial sea water. The vials were illuminated and maintained at room temperature for 24 hours. Each experiment was done in triplicate. The numbers of survival were counted and the percent mortality of the larvae after 24 hours were calculated.

(d) Data analysis

LC_{50} values and 95% confidence intervals of each sample were generated by probit analysis of the % mortality data using a computer program, EPA Probit Analysis Version 1.5.[14]

Antibacterial test

Antibacterial activity tested by the disc diffusion method. Both gram positive and gram negative bacteria used in antibacterial assay. Whatman filter paper discs diameter 6 mm impregnated with crude extracts and standard drug. The discs placed on agar plates and incubated at 37^{o} C, for 24 hours. Each test repeated three times. Control is blank discs impregnated with solvent dimethyl sulfoxide.[15]

Free radical scavenging activity using DPPH method

Method described by Blois with minor modification. Four concentrations (25, 50, 100 and 200 µg/ml) using methanol as solvent prepared for each extract petroleum ether, chloroform and hydro-alcohol. About 4 ml from each concentration place in a test tube to which one milliliter of 0.1 mM methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) have shaken vigorously. After that all the test tubes allowed to stand at 27^{o}C in dark for 45 min.The absorbance of the prepared samples measured using Ultraviolet spectroscopy at 517 nm.[16]

RESULTS AND DISCUSSION

Phytochemical screening of fruits of *Olea europaea*

Table 1: Detection of Phytoconstituents in *Olea europaea fruits.*

S. No.	Constituents	Result
1	Alkaloids	-
2	Carbohydrate	+
3	Phenolic compound and tannins	+
4	Flavonoides	+
5	Proteins	+
6	Steroidal glycoside	+
7	Resin	-
8	Lipids/ fats	+
9	Anthraquinone	-

Phytochemical screening indicates the presence of primary and secondary metabolites in the fruits of Olive. Carbohydrate, phenolic compound, flavonoids, steroidal glycoside proteins and Lipids/ fats were reported.

Cytotoxic activity

Table 2 showed percentage of mortality of brine shrimp larvae exposed to various concentrations of organic extracts obtained from *Olea europaea* fruits.

Table 2: Mean mortality of brine shrimp larvae when exposed to petroleum ether chloroform, and hydroalcoholic extracts from *Olea europaea* fruits. (n = 10 larvae per treatment).

Concentration µg/ml	Mortality%		
	Petroleum ether	Chloroform	Hydro alcoholic
250	50	10	0
500	50	30	20
1000	60	40	30

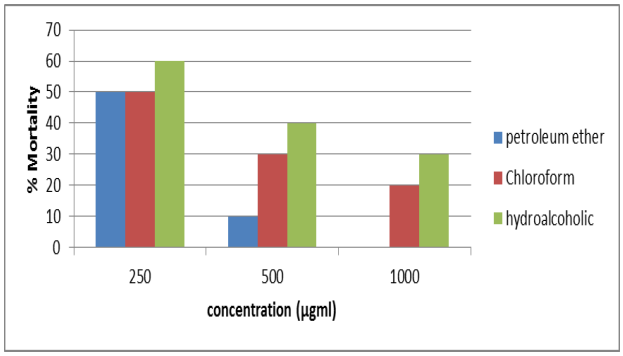

Figure 1: Mean mortality of brine shrimp larvae when exposed to different concentration of crude extract of *Olea europaea* fruits.

It is clear from the above result that middle polar chloroform and polar hydro-alcoholic extracts are less active against the brine shrimp larvae. Hydro-alcoholic and chloroform extracts have killed 30% and 40% of the shrimp larvae at higher concentration of 1000 µg/ml. Non polar fraction like pet.ether extract displayed cytotoxic activities and killed 60% of shrimp larvae.

LC_{50} of petroleum ether, chloroform and hydro alcoholic extract against brine shrimps larvae

The LC_{50} values for the three extracts deduced from the probity analysis results are shown in Table 3. As it can be seen from the table that petroleum ether extract showed some activity against the tested organism.

Table 3: Probity analysis of mortality (LC_{50}) of petroleum ether, chloroform, and hydro alcoholic extracts of *Olea europaea* fruits against brine shrimp larvae (n = 10).

Extract	LC_{50}
Petroleum ether	349.65
Chloroform	1212.94
Hydro alcohol	1617.25

LC_{50} value of petroleum ether, chloroform and hydro alcoholic extract were 349.65, 1212.94 and 1617.25; Out of these three extracts, petroleum ether showed minimum LC_{50} value of 349.65, it means that petroleum ether extract has cytotoxic activity. Hydro alcoholic extract and chloroform extract showed no cytotoxic activity because LC_{50} values was very high.

Antibacterial activity of *Olea europaea* fruits extract

Table 4: Results of antibacterial activity of three extracts against four organisms, *E-coli*, *S. aureus*, *P. aeruginosa*, and *P.vulgaris*.

Extracts	Concentration µg/ml	*S. aureus*	*E. coli*	*P.aeruginosa*	*P. vulgaris*
Petroleum ether	1000	10	12	10	12
	500	8	9	9	11
	250	7	7	7	9
Chloroform	1000	ND	10	12	11
	500	ND	8	9	9
	250	ND	7	7	7
Hydro alcoholic	1000	9	ND	ND	17
	500	ND	ND	ND	15
	250	ND	ND	ND	12
Augmentin	30	30	32	26	31
Control DMSO		0	0	0	0

(diameters of inhibition zones in mm).

ND: Not detected

In this experiment different bacterial strains have used against the plant extracts of *Olea europaea* fruit. All the three extracts were showing good activity against gram negative bacteria at different concentrations as compare to the standard drug Augmentin. Gram positive bacteria *S.aureus* has inhibited only by Petroleum ether extracts. Medicinal potential of *Olea europaea* fruits and its exploitation can certainly support achieving indigenous medical treatment in Oman.

Antioxidant activity

Antioxidant activity of different organic extracts of *Olea europaea* fruits

Table 5: Antioxidant activity of different organic extracts of *Olea europaea* fruits.

Concentration µg/ml	% Inhibition		
	Pet.ether	Chloroform	Hyd.alcohol
25	78.15	98.68	95.15
50	81.68	98.9	95.4
100	82.2	98.9	95.7
200	87.9	98.98	97.5

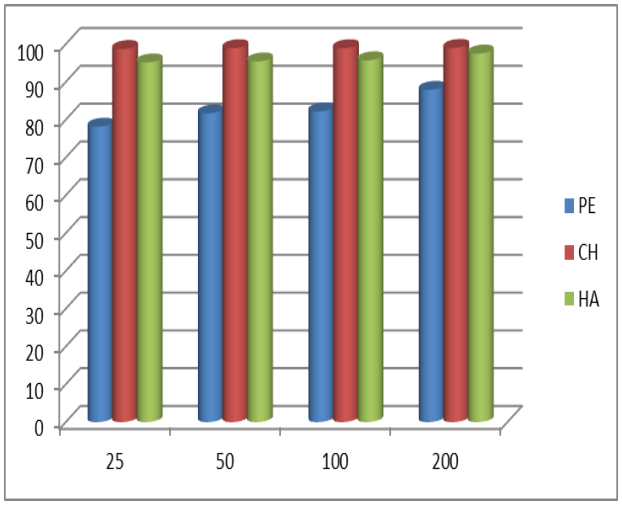

Fig 2: Results of antioxidant activity of *Olea europaea* fruits.

In this study, we have examined free radical scavenging activity by DPPH assay of petroleum ether, chloroform and hydro alcoholic extracts of *Olea europaea* fruits collected from Al dakhliya region, Sultanate of Oman. The extracts showed different levels of efficacy in a dose-dependent manner.All the three extracts of *Olea europaea* fruits showed very good antioxidant activity. Chloroform extracts showed maximum activity followed by ethanolic and petroleum ether. Chloroform extracts showed 98.98**%** inhibition at dose of 200µg/ml; hydro-alcoholic extracts showed 97.5**%** and petroleum ether 87.9**%.** Olives and olive oil have been known for centuries. Their strong antioxidants in the fruits have shown interesting anti-ageing effects in skin.

These findings are useful for further research to identify, isolate and characterize the specific compound which is responsible for the higher antioxidant activity of Omani species. According to the results of present study, *Olea europaea* fruits were found to serve as a potential source of natural antioxidants.

CONCLUSION

Phytochemical screening indicates the presence of primary and secondary metabolites. Biological assay of *Olea europaea* fruits extracts of petroleum ether showed cytotoxic activity against brine shrimp larvae. The antibacterial activity of fruits of *Olea europaea* against gram positive and gram negative bacteria was performed. Plant extracts were found active against the gram negative bacteria. According to the results of anti-oxidant activuity, *Olea europaea* fruits were found to serve as a potential source of natural antioxidants.

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