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Original article

Phytochemical, Antioxidant, hair growth and wound healing property of *Juniperus excelsa*, *Olea oleaster* and *Olea europaea*

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ABSTRACT

To study phytochemical screening, Antioxidant property, and wound healing and hair growth of Olives tree (wild and cultivars) and *Juniperus excelsa* are the most important natural sources used to treat many diseases around the sultanate of Oman. Preliminary phytochemical analysis and DPPH method of antioxidant assay was carried out *invitro*. The experimental plant materials were also used to test their effect on hair growth and wound healing on Swiss albino mice(n = 3). Results of phytochemical study showed that tannins, alkaloid, saponin, terpenes, phenolic were found at high concentration (+++), while the rest of the constituents were found at moderate (++) to low concentrations (+). DPPH free radical scavenging potential of water extract of juniperus excelsa was (50 µg/ml = 83.30 %) found to be more effective, at 200 µg/ml concentration be 64.62 % free radical scavenging percentage was noticed. In the wound healing study, it was observed that juniperus extract showed significant result on the recovery of linear wound. Against the hair growth study, it was observed that the wild and cultivar olive water extracts have the best effect on hair growth. From these findings it is clear that these traditional medicinal plant parts can be effectively used for pharmacological preparations.

1. Introduction

From the history many medicinal plants are used for the treatment of various diseases in Traditional usage as herbal medicine. Usage of natural traditional medicine is increasing in modern era since they are safer without side effects and more effective. The herbal plant has therapeutic activity was proven to prevent and to treat different physical and mental diseases like ''malaria, epilepsy, infantile convulsion, diarrhea, and dysentery, fungal and bacterial infections'' (Amin et al., 2015). Alkaloids, glycosides, saponins, resins, oleoresins and oils are non-nutritive phytoconstituents have protective or disease preventive properties (Sofowora,1996). Most of the medicinal plant that have antioxidant property found to show significant health benefits in disease control, repairing damaged cells etc (Bjelakovic et al.,2013). The demand for the medicinal plants are increasing day to day in developing and developed countries to be used as antimicrobial agents, antioxidants, and for many more applications since its easy availability, less side effects and sometimes as source of primary health care where there no proper facilities available. Traditionally, in Sultanate of Oman essential oils are used rather than extracts in the treatment of infectious diseases, hair growth, and wound healing (Rios and Recio 2005). During wound recovery the invasion of pathogens into the damaged tissue must be prevented by using antimicrobial agents till partial or complete reformation of damaged tissue (Gupta et al.,2006). The healing process includes ''inflammation, granulation, fibrogenesis, neovascularization, wound contraction and epithelialization'' (Clark, 1996). It is known that the plant secondary metabolites namely ''flavonoids, triterpenes, alkaloids'' and many other constituents triggers the wound healing process at some stages.

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Olea oleaster (wild olive) and *Olea europaea* (Cultivar) are used widely as traditional herbs against high blood pressure, high levels of LDL, diuresis, antibacterial, antiviral, anticancerous, Fever, Cystitis, Hyperuricemia, Diabetes treatment, and myocarditis(Besnard et al.,2001). Olive products have been employed since ancient time as food, natural preservatives and in folk medicine in continuation during 19th century boiled olive leaf extracts are used to treat malaria. Olive leaf extracts contain active phytochemicals which shows olive as effective anti-microbial substance against bacterial infections, fungal infections, and mycoplasma. The significant amount of important constituents of olive leaf extracts are commercialized to treat many common diseases without being evaluated scientifically only based on their traditional efficacy.

Juniperus excelsa is one of the flowering plant belonging to the family *Cupressaceae*. It is a wild plant in the Al Hajar Mountains in Sultanate of Oman, as well it specifically grows only in highlands like ''Al Jebel Al-Akhdar, Jebel Qubal and Jebel Al-Khawr''(Gardner and fisher, 1996). *J excelsa* is traditionally used in rural areas as a primary health care agent in Oman for the treatment of diseases like ''diuresis, stomachic, and digestive disorders, flatulence, and diseases of the kidney and bladder. The essential oil of *J excelsa* is widely used as aromatherapy for ''mood scents, scent masks, soaps, candles, cosmetics and fragrances, lotions and remedies'' (Nabi et al., 2012a, 2012b). In Oman, it is also used traditionally for bronchitis, the common cold, jaundice and tuberculosis (Mmohammed et al., 1992; Tumen et al., 2012; Khan et al., 2012; Mossa et al., 1992; Fisher and Gardner, 1995; Lesjak et al., 2011). Due to various medicinal values, the research was aimed to explore comparative phytochemical, wound healing and hair growth of these selected Omani plants.

2. Material and methods

2.1. Plant material collection and extraction

The leaves of. *Juniperus excelsa* sample,*Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) samples were collected from Al Jabel AL Akhadar, sultanate of Oman during January 2017. The collected leaves were cleaned, dried under the shade and made fine powder by using heavy blender. 20 g of each plant sample was weighed and dissolved in 200 ml of 80 % methanol and subjected for extraction through maceration (Reddy et al., 2018). The samples were placed in rotary shaker under RT at 200 rpm for 24 h. The filtrate was collected and to the residue the process of maceration was repeated and the final filtrates were evaporated under constant pressure and temperature through Rota evaporator. While the aqueous extract was evaporated in boiling water bath at 100^0c. The final crude extracts were collected and stored in refrigerator till further use.

2.2. Phytochemical screening

The prepared extracts were subjected to standard method (Trease and Evans, 1989) of phytochemical analysis, viz.tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenol, carbohydrate and protein. 1 % of individual extract from crude extracts were dissolved in 10 ml DMSO and final volume was adjusted with distilled water for phytochemical analysis.

2.3. DPPH assay

The antioxidant activity of each samples was determined through free radical scavenging by DPPH method(Goggi and Malpathak, 2017). Four different concentrations 50, 100,150,200 μg/ml were tested for each extract (water, methanol and oil). 0.1 mM DPPH was prepared in 100 ml pure Methanol. Each concentration of individual extract was placed in a reaction mixture containing DPPH solution (2.050, 2.00,1.950, 1.900 ml) at a final concentration to become 50, 100,150,200 μg/ml, and shaken vigorously for 1 min. The reaction mixtures were incubated at room temperature under dark condition for 10 min, later incubation the samples were measured at 517 nm in UV spectrophotometer. 100 % Methanol was used as the blank. The scavenging activity of each extract was estimated based on the percentage of inhibition of DPPH using the following formula,

$$\%\text{inhibition} = \frac{A\,control - A\,extract}{A\,control} \text{x}\,100$$

2.4. Effect of various extract on wound healing and hair growth

Twelve healthy male mice from animal house facility, University of technology and Applied sciences- Higher College of Technology, prior to experiment all the ethical approval was taken and were segregated into 4 groups and each group consists of three mice as follows.

Group1: treated with aqueous extract of *J. excelsa.*
Group 2: treated with wild olive extract.
Group 3: cultivar olive extract and.
Group 4: positive control.

Briefly, the wound was made longitudinal measuring 2 cm on the dorsal side of the mouse was made by using sterile scissor and razor. The experimental groups received the respective treatment using swaps around the wound continuously for 2 weeks.

To study the hair growth, the hair on each mice measuring a distance of 2 cm length and 2 cm width at abdomen side was removed using sterile blade. For both plant material different extracts were applied on all the experimental animals respectively. While for control cage, mice were separated, two mice were treated as positive control by applying commercial medicine (Betadine-wound care), whereas coconut oil was used as positive control for hair growth study. The treatment was continued for 10 days and the observations were recorded by measuring the distance recovered (±cm) /density of hair growth.

3. Result and discussion

3.1. Phytochemical screening

The water, methanol and oil extracts of *Juniperus excelsa*, *Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) showed the presence of saponin, cardiac glycosides, terpenes, phenol and carbohydrates. The water extract of wild /cultivar olives leaves junipers leaves revealed the presence of all the tested phytochemicals except tannins and alkaloids in methanol extract, terpenes and flavonoids in *J excelsa* aqueous extract represented in Table 1.

The methanol extract of tested plant materials revealed the presence of all above mentioned phytochemical except the tannins (-), protein and flavonoids (-). All the oil extract showed the presence of all tested phytochemicals (+++) except flavonoids (-) and alkaloids (-). In addition, the methanol and oil extract of both the tested plant samples do not show any positive result for flavonoids (-). Saponins (+++) and cardiac glycosides (+++) are found abundant in all tested plant materials with respect to the solvents. While phenols also found abundant (+++) in all the tested plant material except oil extract of *J excelsa* (-) and cultivar olive (-). Terpenes are also showed the presence at high quantity (+++) in all the tested plant materials except *J excelsa* leaf aqueous extract (-). Alkaloid are found at high concentration (++++) in aqueous and methanol extract of both the plant materials except oil extract. Tannins

Table 1
Phytochemical screening of selected plant materials.

Test	water extract			methanol extract			oil extract		
	Wild olive extract	cultivar olive extract	juniper leaves extract	Wild olive extract	cultivar olive extract	juniper leaves extract	Wild olive extract	cultivar olive extract	juniper leaves extract
Tannins	+ + +	+ + +	+ + +	–	–	–	+ + +	+ + +	+ + +
Alkaloids	+ + +	+ + +	+ + +	+ + +	+ + +	–	–	–	–
Saponins	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Cardiac glycosides	+ + +	+ + +	+ + +	+ + +	+ + +	- + +	+ + +	–	+ + +
Terpenes	+ + +	+ + +	–	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Flavonoids	+ - -	+ - -	–	–	–	–	–	–	–
Phenols	+ + +	+ + +	+ + +	+ + +	++ +	+ + +	+ + +	–	–
Carbohydrates	+ + +	+ + +	+ + +	+ + +	+ + +	- + +	+ + +	+ + +	+ + +
Proteins	–	–	–	–	–	–	+ + +	–	–

Note: Here +++: high, ++: moderate, +: low, -: Absence of the tested phytochemicals in three replicates. All the values expressed in the table are the mean average of three replicates.

showed (+++) in all the plant extracts with respect to solvents except methanolic extract.

Several reports revealed that phytochemicals are responsible for protection from several chronic diseases like polygenic disorder, cancer, cardiovascular disease and Alzheimer's. Phytochemicals are able to scale back the aerophilic injury to our cells that causes varied diseases like cancer because they tend to inhibit cell proliferation and angiogenesis (i.e. the growth of new blood vessels) which are both trademarks of cancer. In addition, phytochemicals also play a role in regulation of nitric oxide, important in relaxing blood vessels and therefore increasing blood flow (Oliveira 2015). Differences in the ingredients in leaves grown in different part of world results the composition of individual plant may vary widely due to the climate, geographical location and time of collection. Nabi et al., 2012a, 2012b Reported that alkaloids, flavonoids, saponins, phenols and terpenes are present in the leaves of crude extracts whereas terpenoids and cardiac glycoside were absent in the leaves of crude extract of *Junipers excelsa*. The exploration of various phytoconstituents and their importance is an interesting and valuable field in the modern era to treat many human ailments with less side effects. Among the search of plants for saponins has been stimulated by the need for readily accessible sources for saponins, which can be converted in the laboratory to animal sterols of therapeutic importance (Reddy et al., 2012). Presence of some of the phytochemicals like terpenoids, alkaloids, phenolic, flavonoids, saponins and cardiac glycosides are involved in hypoglycemic activity (Reddy et al., 2016).

3.2. DPPH assay

Free radical scavenging activity of *Juniperus excelsa*,*Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) extracts by DPPH method and the results are presented in Graph 1A,B&C.

The antioxidant Property is depending on the interaction of active substances with oxidative free radicals. The assumption of DPPH method is that the antioxidants react with the stable free radical and gradually leads discoloration. The degree of discoloration indicates the scavenging potential of samples. In this study, the three extract (water, methanol and oil) from *Juniperus excelsa, Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) were capable to decolorize the DPPH at varied levels. DPPH free radical scavenging potential of water extract of junipers (50 µg/ml = 83. 30 %) found to be more effective while at 200 µg/ml the scavenging percentage was found to be 64.62 %. whereas the water extract of cultivar olives found to be 22.92 % at 150 µg/ml. Since the junipers and cultivar extract does not show the presence of flavonoids and phenols therefore, it showed the lowest percentage of antioxidant compared with wild olives that has rich content of flavonoids and phenols. The presence of flavonoids and phenols in the sample extract convert to reactive non-stable DPPH free radical into stable nonreactive stable DPPH from by donating electron or hydrogen radical.

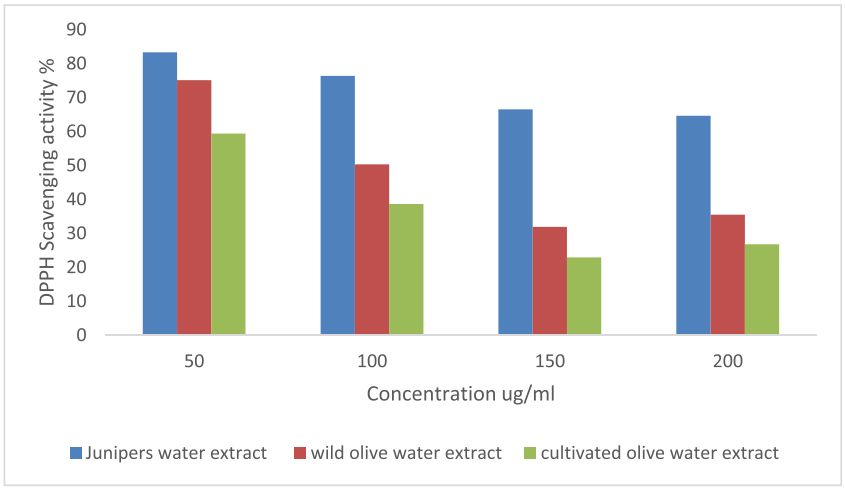
The antioxidant property of the respective plant extracts is relevant the presence of phenols and flavonoids. The strong free radical scavenging activity is directly proportional with the different extract that contain high quantity of phenols and flavonoids. This indicates that the tested plant extracts possess a good source for natural antioxidant to prevent free radical oxidative damage. The antioxidants of plant act as inhibitor of oxidative damage even at minute concentration, which have diverse physiological role in the body (Weli et al., 2013). During oxidative stress, this property helps the plants to survive through such conditions. Especially in Mediterranean diet rich in natural antioxidants, limit the incidence of cardio and cerebrovascular diseases (Sudhir et al.,1986). The specific antioxidants belonging to several phytochemical components like phenols, flavonoids, and carotenoids are responsible to scavenge free radicals such as O_2, OH, or lipid proxy radicals in plasma (Banerjee et al., 1993).

3.3. Wound healing study

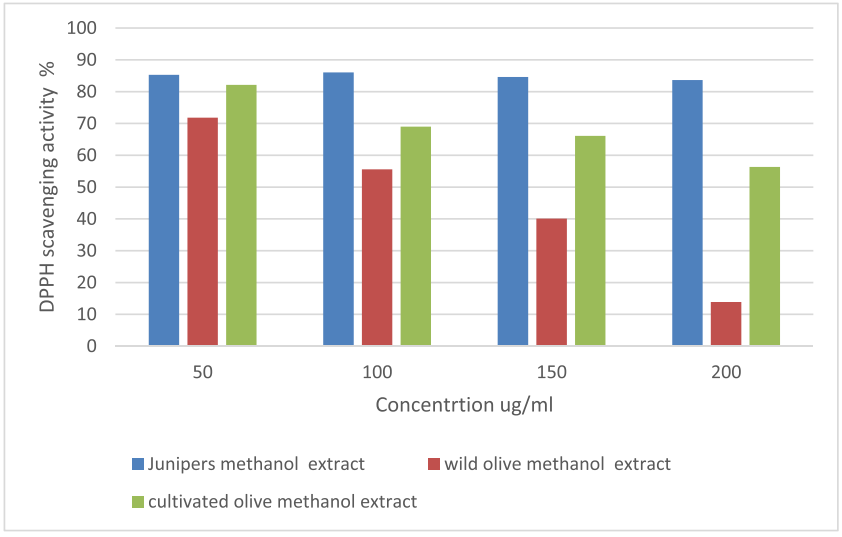
Wound healing effects of water extract obtained from *Junipers excelsa*,*Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) by maceration method of extract was studied on mice, the results are placed in Table 2 and Fig. 1. It was noticed that the *J excelsa* extract showed the best effect on the recovery of linear wound which is 1.6 cm followed by cultivar (1.467 cm) and wild olive (1.433 cm) respectively, the initial size of the wound was 2.0 cm. Several reports suggested that the *J excelsa* extract is enriched with natural bioactive substances which provides antimicrobial activity, moisturizing activity and building of the skin to help in healing the wound and cuts, also reduction of inflammation at the wound site.

3.4. Hair growth

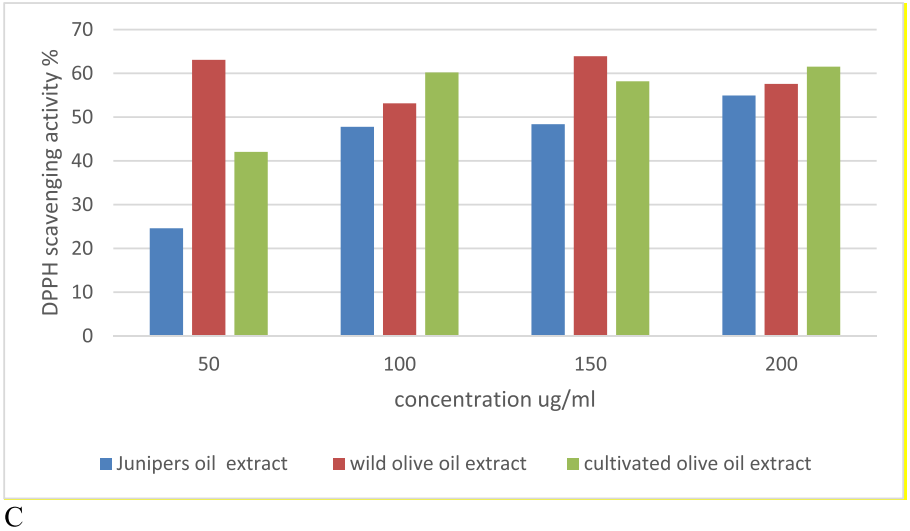
On the hair growth study, we found that the wild and cultivar olive water extract have the best effect on hair growth which are 1 cm and 1.05 cm respectively, the results are placed in Table 3 and Fig. 2. The *J excelsa* has the least distance covered than the other extract (wild, cultivar olive and control (coconut oil) which is 0.5 cm. *J excelsa* and olive extract will simply penetrate the scalp and its cleansing result helps in detoxifying the hair follicles and promotes blood circulation within the scalp. It will increase nutrients to the hair follicles that facilitate in up hair growth. Continuous use of *J excelsa* oil and olive oil can restrict the assembly of DTH that successively forestall hair fall.



A



B



C

Graph 1. DPPH assay A. Water extract, B. Methanolic extract and C. Oil. All the values are the mean of triplicates.

Most of the living organisms specifically plants and animals keep huge amounts of antioxidants on it, due to their role in counteracting cell damage, as well as their ability to repair damaged cells. Many experts revealed that increased antioxidants in the body can also help slow the process of ageing, and may even increase longevity (Edward 2009). Many factors can adversely affect this process and lead to improper and impaired wound healing such as improper diet, infection at the wound site with

Table 2
Effect of *Juniperus excelsa*,*Olea oleaster* (wild olive) and *Olea europaea* (Cultiver olive) on linear wound.

Extracts and dosage	Initial distance in cm	Distance left uncovered after 10 days of treatment in cm	Distance of wound recovered after 10 days of treatment in cm
Control	2	0.66	1.33
Juniperus excelsa	2	0.4	1.6
Wild olives	2	0.56	1.43
Cultivar olives	2	0.53	1.46

Note: All the values expressed in the table are the mean average of triplicate.

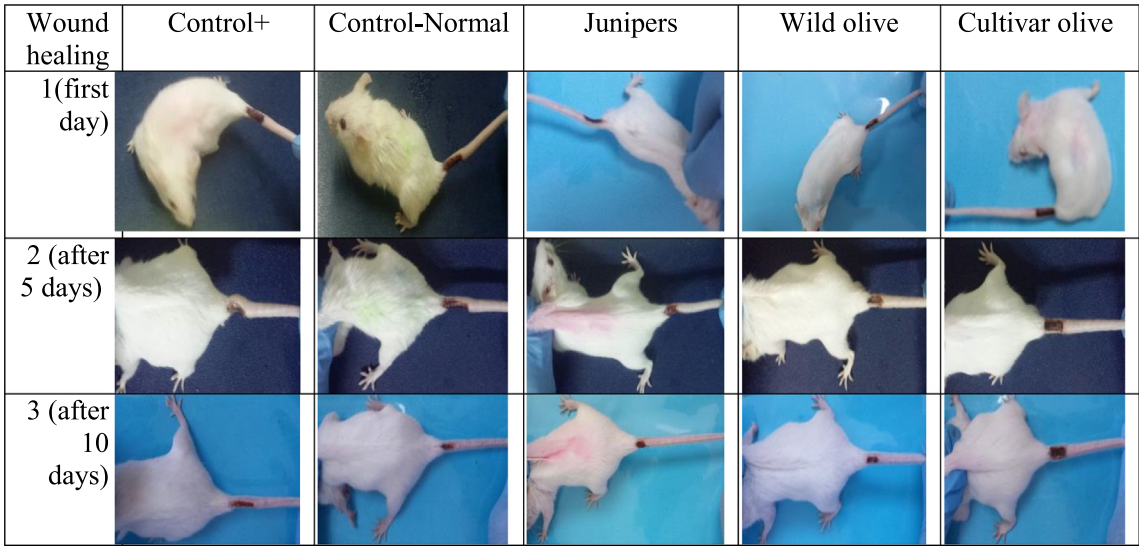


Fig. 1. Effect of *Juniperus excelsa*, *Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) on wound healing.

Table 3
Effect of *Junipers excelsa*, *Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) on hair growth.

Samples (Types of water extract)	Initial distance in cm	Distance left uncovered after 10 days of treatment in cm	Distance of Hair growth recovered after 10 days of treatment in cm
Control	2 × 2	1.16	0.83
junipers	2 × 2	1.5	0.5
Wild olives	2 × 2	1	1
Cultivar olives	2 × 2	0.95	1.05

Note: Here 2 × 2 cm = 2 cm horizontal; 2 cm = vertical, All the extract placed in the table are mean of 3 replicates.

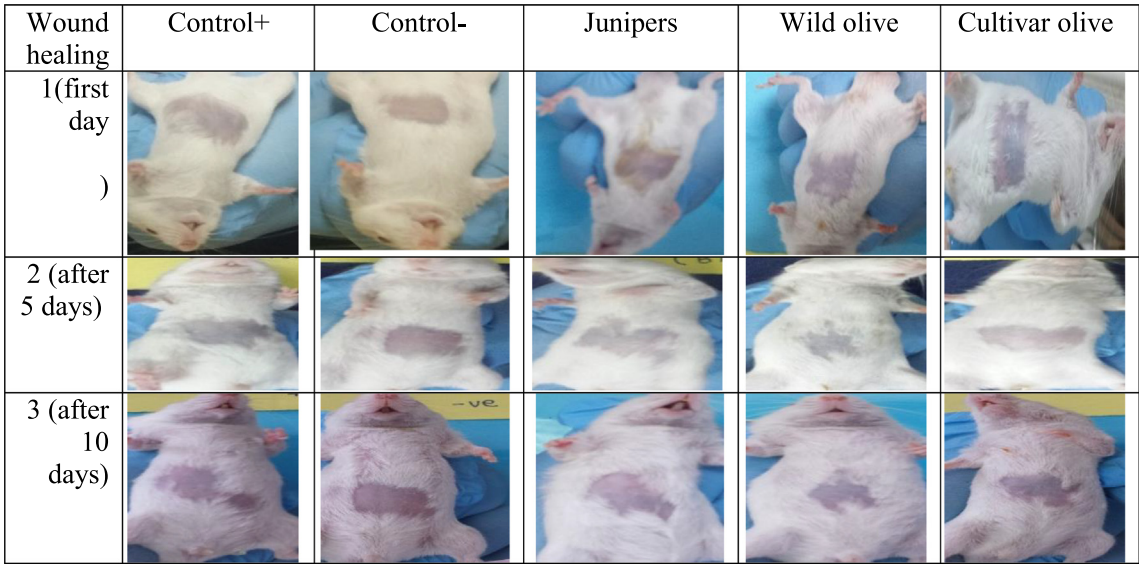


Fig. 2. Effect of *Juniperus excelsa*, *Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) on hair growth.

microorganism, drugs, elderly age, diabetes and other disease conditions (Kerstein 2007). In addition, oil could be a natural source of antioxidants that fight with free radicals.

4. Conclusion

In conclusion, from the study the leaf extracts of *Juniperus excelsa*,*Olea oleaster* (wild olive) and *Olea europaea* (Cultivar olive) shown the significant amount of saponin(+++), cardiac glycosides (+++), terpenes (+++), phenol(+++) and carbohydrates. The present study revealed that the tested plants displayed strong antioxidant activity based on DPPH assay. Also the tested plants displayed the best effect on wound healing and hair growth based on preliminary studies conducted. These preliminary studies helps to explore medicinal values and therapeutic applications from the studied plant material.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102446.

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