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Preparation and Evaluation of Olibanum Extracts and Determine their Biocompatibility.

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ABSTRACT

In modern science, there are new approaches based on the use of plants. The use of plants is very practical and useful and has advantages such as availability, bio-degradability, non-toxicity and chemical neutrality. Recently, it is believed that these materials are a substitute for synthetic materials, because in addition to the above properties, they have the potential for chemical optimization, do not cause environmental pollution, and their important feature is their biocompatibility.Olibanum is an oleo-gum resin that consists of three parts: gum, resin and essential oil. The purification of Olibanum was carried out in two methods: water and alcohol, and compared to FTIR, MTT and cellular cultures and cell adhesion. The results show that this natural polymer is biocompatible and suitable for use as a natural polymer in biomedical applications.

Keywords: Olibanum, Extract, Chemical Composition

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INTRODUCTION

Natural gums or plant exudates have adhesion properties, smoothness and elastic nature, which are compose mainly carbohydrates and sometimes a small amounts of proteins and minerals. One of this natural gum is Olibanum, which is taken from various types of Burseraceaetrees [1-3]. Olibanum is a Oleogum resin that has different types[4].The use of natural polymers (gums) for pharmaceutical applications is attractive, the reason for this is economics, availability, non-toxic and the possibility of chemical modifications, potentially biodegradability and biocompatibility [5].

The therapeutic benefits of Olibanum are anti-arthritic, anti-diarrhoeal, anti-hyperlipidemic, anti-inflammatory, anti-asthmatic, analgesic activity, anti-rheumatic anti-microbial and anticancer. In addition, it also possesses immune modulatory activity and hepatoprotective[1, 2, 5-14].

Olibanum consists of chiefly gum (30-36 %), an acid resin (50-60 %), and volatile oil (3-8 %). The resin consists mainly a resin (olibanoresin) and a resin acid (boswellic acid) in equal proportions [3, 13, 15-20].

The gum fraction is about one-third of the Olibanum. This part is soluble in water and contains polysaccharide and polymeric parts. There are four different types of proteoglycans and glycoproteins in the structure of these proteoglycans: there are D-galactose units in the main chain and there are glucuronic acid, uronic acids, 4-O-methel-glucuronic acid and arabinose in the side chains[6].

The Resin part is soluble in alcohol and includes monoterpenes, diterpenes, triterpenes, tetracyclic triterpenic acids and pentacyclictriterpenic acids[5].

Four of the main penta cyclictriterpenic acids are[6]:

- $\beta - \text{Boswellic acid}$
- $\text{acetyl} - \beta - \text{Boswellic acid}$
- $11 - \text{keto} - \beta - \text{Boswellic acid}$
- $\text{acetyl} - 11 - \text{keto} - \beta - \text{Boswellic acid}$

Approximately %10 of the structure of the Olibanum is its essential oil. Drying the juice from the tree and performing the hydrolysis, and it is obtained bydistilling an essential oil component [1, 8, 10, 11, 21].

Gas chromatography analysis of 99 materials in the composition of this extract showed that n-octylacetate and NerolidolIso buty rate were most existent [1, 8, 11, 12, 21]. Generally, this extract consists of monoterpenes, diterpenes, alcohols and esters, the most important monoterpenes in it can be E-Bocimene and Limonene [1, 6, 8, 11, 22].

The extract was studied on pancreatic, breast and intestinal cancer cells, and the results indicate the anti-cancer effect and the results indicate that the anti-cancer activity of this extract depends on the duration and temperature of the hydrolysis[1, 10]. Anti-microbial and antioxidant properties of this extract have been investigated and confirmed the results of these properties[10, 11]. Essential oil of Olibanum is practically used in aromatherapy and cosmetic applications[1].

In this research, the purification and characterization of olibanum was studied. In different papers, two different methods of purification with water and alcohol have been reported, each having its own results.

The question of which method is more appropriate and what are the differences between these two methods is not investigated in any research. Definitely the choice of a better purification method for olibanum can be effective in research results on this substance. In this research work, we have tried to investigate this issue.

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MATERIALS AND METHODS

Materials

Methanol and MTT powder were purchased from Merck Co. Ltd (Germany).
Deionized water were used for all aqueous solutions. Indian Olibanum was used.

Preparation of olibanum extract

Below, two methods of purifying with water and alcohol are discussed.

Preparation of olibanum extract by water

At first, Olibanum was carefully powdered. The powder (PK) (25 g) was mixed with 200 ml of deionized water and stirred with 800 rpm overnight at room temperature. This mixture was centrifuged at 1,500 rpm for 10 min and the supernatant was collected. Thereafter, the supernatant was again centrifuged at 2,500 rpm for 10 min and successively at 10,000 rpm for 20 min, and then was filtered. The filtrates were stored at -20^0 C and then freeze-dried with -58^0 C and 0.5 Torr for 24 h to yield 4.02 gr of water soluble extract (PKW). At the next step, resulted PKW powder was dissolved at 100ml methanol and stirred with 500 rpm for 12 hr. at room temperature. Then solation was staid for 1 hr. without any movements. After this time precipitate phase was collected and dried at oven with50^0 C (PKWA).

Again PKWA was dissolved at 80 ml deionized water. Three above centrifuge steps was again performed for this solution and then was filtered. The filtrates were stored at -20^0 C and then freeze-dried with -58^0 C and 0.5 Torr for 24 h (PKWAW)[7].

Preparation of olibanum extract by alcohol

In this method, 100 gr of Olibanum powder with 400 ml of methanol is mixed. This mixture was placed on a stirrer with a round of 650 rpm for 24 hours. The resulting mixture is made up of two phases, the upper phase is alcoholic and yellow, and contains substances that are soluble in alcohol. The material was placed in an oven at 50^0 C to dry (SA).

The bottom phase has a sedimentary and white state, which is set to 50^0 C in the in the oven until dry. The resulting powder in the water is well dissolved and the obtained solutionwas centrifuged at 1,500 rpm for 10 min and the supernatant was collected. Thereafter, the supernatant was again centrifuged at 2,500 rpm for 10 min and successively at 10,000 rpm for 20 min, and then was filtered. The filtrates were stored at -20^0 C and then freeze-dried with -58^0 C and 0.5 Torr for 24 h (PKAW).

Characterization of olibanum

The below analysis was performed for characterization of Olibanum samples.

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectrophotometer (FTIR, Thermo Nicolet-Nexus 870-USA) was used for evaluation of Chemical characteristics of the Olibanum samples. For FTIR analysis 1 mg of the powder samples was carefully mixed with 300 mg of KBr and were analyzed between 400 and 4000 cm^{-1} with a resolution of 4.0 cm^{-1}[23-25].

X-ray diffraction analysis

To evaluate the characteristic of olibanum, X-ray diffraction analysis measurement was used by 3003 PTS-SEIFERT(GE-USA) with Cu-Kα radiation (400 kV, 30 mA, and scan speed 1˚/min).

pH determination

0.4 gr of PK, PKW,PKAW and PKWAW was mixed with 10 ml of deionized water and stirred overnight at room temperature. Then pH of samples were measured by pH meter (Crison, Basic 20, Spanish).

Cell culture and MTT test

For evaluating the percentage of cell vitality and biocompatibility PKWAW and SA with concentration of 1, 10 and 100 µg/ml at 1 day, 3 day and 5 day after treatment, cells were evaluated by MTT assay and cell culture.

To measure the effects of olibanum on cell proliferation in the G292 fibroblasts, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used. Briefly, 10^4 cells/well were evenly distributed and incubated on 96-well plates overnight. The cells were then treated with olibanum at concentrations of 1, 10 and 100 µg/ml, and incubated for 1, 3 and 5 day. Subsequently, the medium in each well was replaced with 20 µl MTT and incubated at 37°C for 4 h. The purple-blue formazan precipitate was dissolved in 100 µl dimethyl sulfoxide and the optical density was measured at a wavelength of 570 nm on a 96-well plate reader.

Cell culture studies the adhesion of cells. The cells were maintained in monolayer tissue culture Petri dishes prior to examination. RPMI-1640 medium was supplemented with 10% fetal bovine serum (both Sigma-Aldrich, St. Louis, MO, USA), 100 IU/ml penicillin, 100 µg/ml streptomycin and 2 mM/l glutamine and cultures were maintained in a humidified atmosphere at 37°C in 5% CO2[26].

RESULT

Characterization of olibanum

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR is one of the best method for chemical groups determination. The FTIR spectra of PK, PKW, PKWA and PKWAW are shown in Fig.1 and the FTIR spectra of PK, SA are shown in Fig.2. In the PK sample, the O-H stretching band is observed at 3445.88 cm^{-1}. C=O peak 1652.13 cm^{-1} is detected. The peak at 1731.31 cm^{-1} corresponds to CH_3O. The peaks at 1460.68 cm^{-1} and 1247.05 indicate the CH_3CO group. Peak 1033.75 cm^{-1} indicates the existence of an ester group in the olibanum[5, 27-29].

After preparation of olibanum extract, it is observed in the PKWAW that the O-H stretching band and C=O band have been shifted to 3422.04 cm^{-1} and 1609.25 cm^{-1} respectively and methoxy peak has been removed.

Comparison of the peaks of PK and SA samples shows that peaks 1456.06 cm^{-1} and 1246.29 cm^{-1} represent CH_3CO, which is extracted by alcohol and is well evident in SA. The peak of 1376.82 cm^{-1} is also related to CH_2, which is seen in SA. C=O peak has shifted to 1645.91 cm^{-1}.

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Figure 1: FTIR for PK, PKW, PKWA and PKWAW.

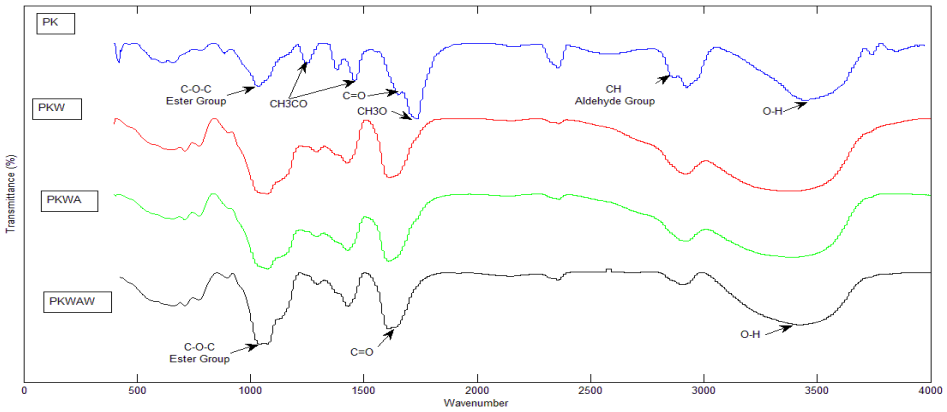
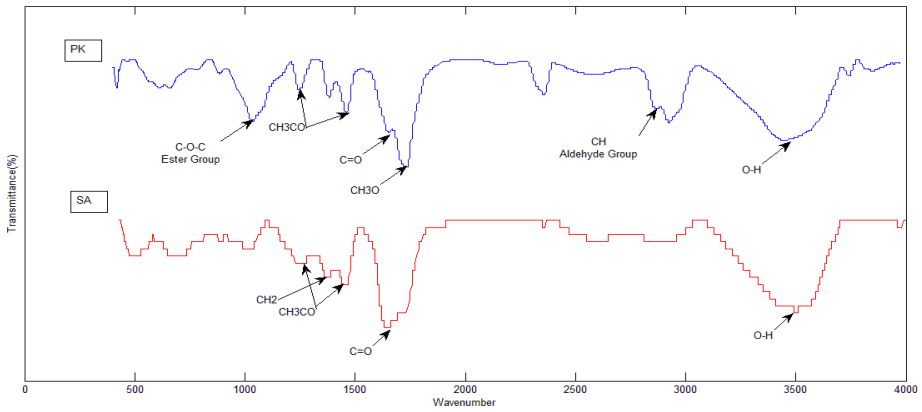


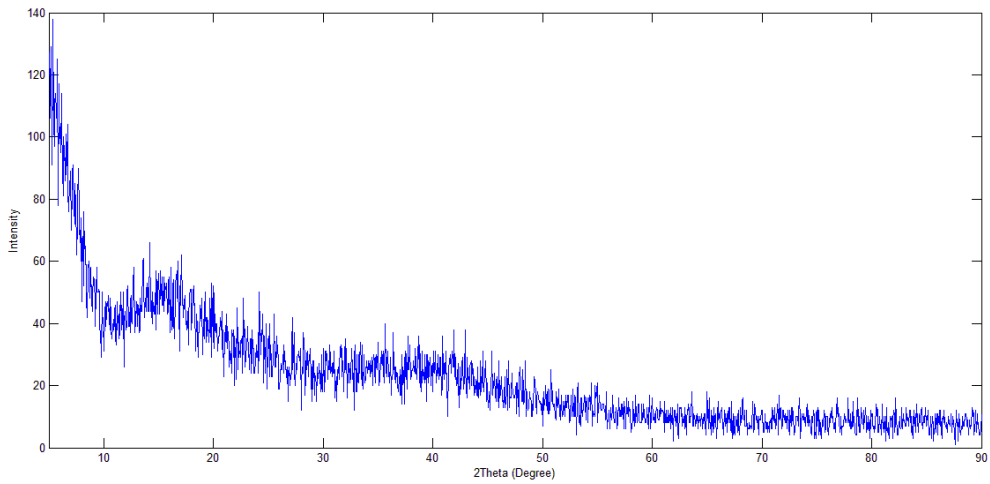
Figure 2: FTIR for PK, SA.



X-ray diffraction analysis

The X-Ray pattern of PK shows the peak at $2\theta = 15$, Fig.3 , and this pattern results the crystalline nature of olibanum as mentioned by previous studies[5].

Figure 3: XRD of Olibanumsample.



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pH determination

pH of PK, SA and PKWAW samples were determined and the results were 4.68, 5.90 and 6.90 respectively. It was concluded that when the PK sample was subjected to two-stage extraction, It reached a neutral range and pH is close to 7.

Cell culture and MTT test

MTT assay was performed on fibroblast cells for study the cytotoxicity of PKWAW and SA. Formazan is indirectly indicator for cell viability because it is only formed by viable cells. Fig. 4and Fig.5 shows the cell viability data of the samples at different concentrations of 1, 10 and 100 $\mu g/ml$ which took place at 1, 3 and 5 day. The results of PKWAW sample show that in the concentrations of 1 and 10 $\mu g/ml$, the survival of cells is %100 and at a concentration of 100 $\mu g/ml$ is equal to %60. On the third day in the concentrations of 1 and 10 $\mu g/ml$, the survival of cells is %80 that is a good amount but at a concentration of 100 is reached to %20.
On day 5, concentration of 1 $\mu g/ml$ showed a good result and equal to %95 survival, and at concentrations of 10 and 100 $\mu g/ml$, the survival was %80 and %15 respectively.

Results for SA sample on the 1 day at each of three concentrations indicate %100 cell survival. On day 3, the survival of the concentrations is about %90 and on day 5 at concentrations of 1 and 10$\mu g/ml$, survival is %80 and at a concentration of 100 $\mu g/ml$, the survival of the cells is %60.

Figure 4: The results of cell survival for PKWAW

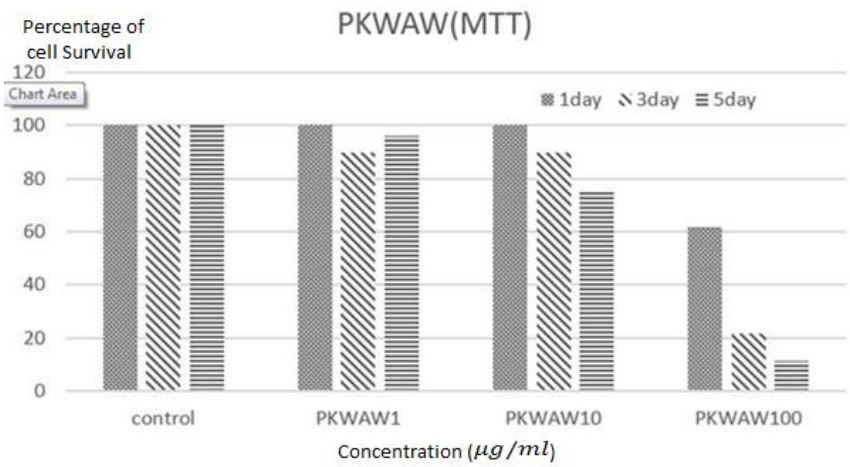
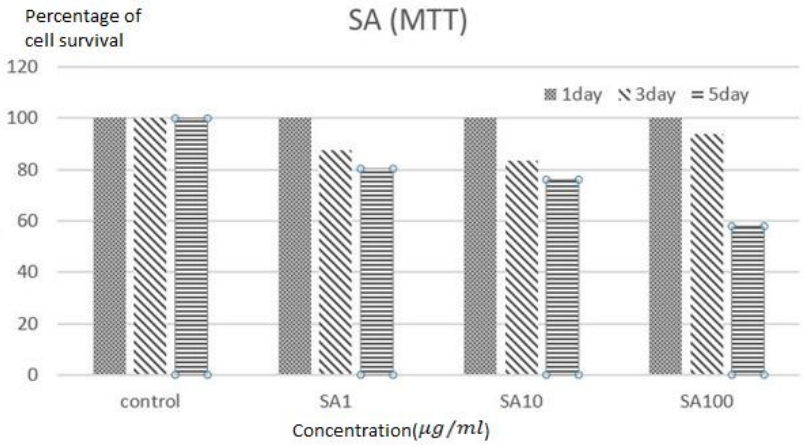


Figure 5: The results of cell survival for SA



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Samples were cultured at concentrations of 1, 10 and 100 at 1, 3 and 5 day.

Figure 6: From top to bottom: concentration 1, 10 and 100$\mu g/ml$ (a) control, (b) 1 day, (c) 3 day and (d) 5 day for PKWAW.

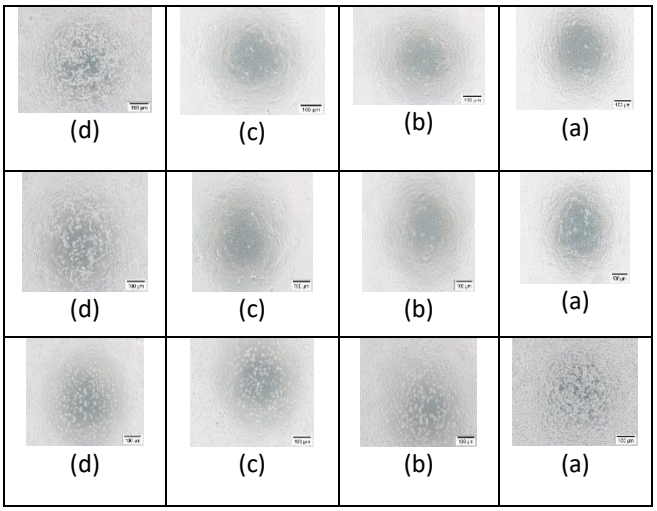
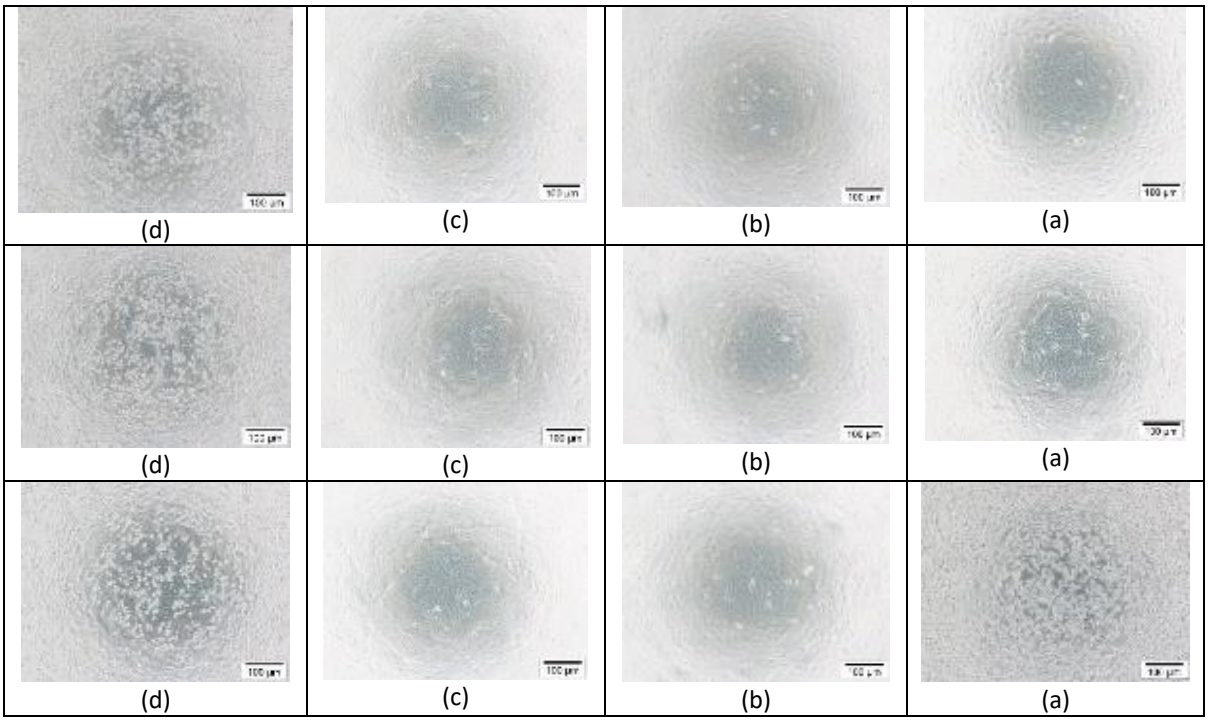


Figure 7: From top to bottom: concentration 1, 10 and 100$\mu g/ml$ (a) control, (b) 1 day, (c) 3 day and (d) 5 day for SA.



As shown in the pictures, in the compare with control sample the PKWAW sample is perfectly suitable for 1 day and 3 day at concentrations of 1 and 10$\mu g/ml$, and the cells have good adhesion, but at a concentration of 100$\mu g/ml$, a number of cells are rounded and their adhesion on the surface is reduced. On the 5 day, adhesion has decreased for all three concentrations (Fig. 6).

For sample SA at concentrations of 1, 10 and 100 $\mu g/ml$ in 1 day, in comparison with the control sample, all three cell adhesion concentrations were appropriate. On day 3, in each of the three concentrations, the cells showed good adhesion. On day 5, they had good adhesion at concentrations of 1 and 10 $\mu g/ml$, and at 100 $\mu g/ml$ concentration a number of cells were rounded and not completely spread over the surface.

According to these testes, it can be concluded that the olibanum is a natural biocompatible polymer and can be used in biomedical applications.

CONCLUSION

Natural gums and secretions of plants are sticky, soft and elastic. Most of these polymers are hydrophilic and they are mainly composed of monosaccharide units, absorbing water, and insoluble in oil and capable of hydrolyzing.

In this research, a natural polymer of olibanum was used and the purification and characterization of olibanum was studied. In different papers, two different methods of purification with water and alcohol have been reported, each having its own results. The question of which method is more appropriate and what are the differences between these two methods is not investigated in any research. Definitely the choice of a better purification method for olibanum can be effective in research results on this substance. With the XRD test, this plant gum has a crystalline structure. By measuring the pH, it was found that the purification would cause the pH to go to neutral. FTIR analysis showed OH and C=O Groups in both extracts. The peak of the CH_3O group has been removed in aqueous extract. The peak in the aqueous extract of the steric group is quite evident, but it is less evident in the alcoholic extract.

The aqueous extracts and alcoholic extract were evaluated by MTT assay. For cell adhesion, cell culture was performed on fibroblast cells. MTT and cell adhesion show that the alcoholic extract has better results in this regard, but the aqueous extract also has acceptable and useable results.This research provides a framework that each researcher based on his purpose and method of research with the help of the results of this research can choose the optimal method for olibanum purification. Also according to the results of these tests, it can be said that the Olibanum has a suitable biocompatibility and is an appropriate alternative for use in medical and pharmaceutical applications, and it can be used as drug carriers and for the synthesis of microspheres and microcapsules.

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