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Alcoholic Extract of Shilajit as Anti Protein Denaturation, Anti Blood Hemolysis, and Anti Microbial

Saba Hadi[1], Sundus Hameed Ahmed[1], Noor Talib[1], Hassan Abdul Hussein[1] and Isam Hussain T. Al-Karkhi[2]

[1]Mustansiriyah University/ College of Science/ Biology Department,
[2]Former Associate Professor, University of Baghdad, Iraq

Abstract

Shilajit is a natural material found mostly in the Himalayas (India), formed by the slow decomposition of certain plants by the action of microorganisms. It is an effective and extremely safe dietary supplement, and potentially able to prevent several diseases. Shilajit alcoholic extract was prepared by dissolving 10 grams of it in 200 mL of 95% ethanol. FTIR and other chemical procedures were used to detect the presence of Phenols, Alkaloids, Terpenoids, Tannins, Proteins Carbohydrates and, Steroids. Different concentrations of the extract were prepared and antimicrobial activity against several positive and negative gram stains, anti-protein denaturation and anti-blood cell hemolysis. Investigation results show that the nanoparticles were effective in inhibiting protein denaturation of albumin. Proteins denaturation refers the cause of inflammation, while the maximum inhibition of albumin was 87% observed at 300 µg/ml of Shilajit alcohol extract compared with aspirin at the same concentration. The results of inhibiting hemolysis at 300µg/ml in comparing with standard drug Diclofenac sodium 300µg/ml resulted in good protection against the damaging effect of heat solution

Keywords: *Shilajit, anti blood hemolysis, phenol, alkaloid, anti protein.*

Introduction

Shilajit is a brownish-black powder from high mountain rocks, particularly in the Himalayans Mountains (India), Russia, Tibet (China), Afghanistan, and recently in South America (Chile). Shilajit has been known and used for centuries as medicine, and as anti-aging compound. Lately, additional properties was found in Shilajit that is, to increase physical ability and to support human health [1-3]. It is also used to treat chest problems, diabetes mellitus, nervous disorders, immune disorders, obesity, kidney disorders, asthma, gall stones, painful and bleeding piles, liver, fermentative dyspepsia, worms, renal and bladder calculi, nervous debility, sexual neurasthenia, hysteria, fainting, female infertility, joint pains, wounds, ulcers and skin diseases [4, 5].

Shilajit has demonstrated good inhibition against viral enzymes and anti HIV activity it is available in tablet form in medicines such as Abana, Cystone and Diabecon. It is also available in syrup form as Evecare and Geriforte. Variation in the quality of shilajit humus (both chemical and biological) and the factors that cause variations in shilajit humus are: (i) altitude and the nature of shilajit-bearing rocks; (ii) atmospheric conditions (e.g. alternate wetting and drying); (iii) pH and moisture content of the rock source; and (iv) activity of the rhizospheric microorganisms and their exo-enzymes [7].

Current knowledge on the phytochemical screening and antimicrobial activity of shilajit is sparse and thus there remains a wide gap in our knowledge of it and thus it needs to be explored [8].

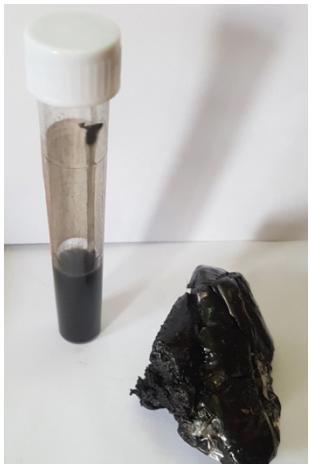
In this article researchers Shilajit alcoholic extract was prepared. FTIR and other chemical technique were used to detect the presence of Phenols, Alkaloids, Terpenoids, Tannins, Proteins Carbohydrates and, Steroids. Different concentrations of the extract were prepared and antimicrobial activity against several positive and negative gram stains, anti-protein denaturation and anti-blood cell hemolysis. Investigations were carried out.

Corresponding Author :
Sundus Hameed Ahmed
email: drsundusahmed@uomustansiriyah.edu.iq

Materials and Method

Alcoholic extract

10 grams of the shilajit powder were add to 200 ml of 95% ethanol; stirred well on hot plate for 15 min, and kept for ten day at 28 °C, then filtered, dried and was kept at 4 °C [8].



shilajit extract

Total Phenols

The total phenolic compounds were detected by taking 150 µL of the extract dilute with 2 mg/ml distilled water, reagent of Folin–Ciocalteu was added and mixed well for five minutes, after that 2 ml of 20% sodium carbonate were added . The mixture was put in the dark place for 60 minutes. Absorbance was measured at 650 nm., the total phenols were quantified from calibration curve obtained by measuring the absorbance of known concentration of Gallic acid [9].

Detection of Flavonoids

The total flavonoids were measured as explained by Ohkawa and his co-workers [10]. Peel extract 0.1 g were added to 5 ml distilled water, then 5 ml ammonia solution was added, stirred well then mixed with 1 ml sulfuric acid. Yellow color refers flavonoids component.

Detection of Alkaloids

Peel extract 0.5 g. was added to 3 ml of hexane, mixed well, and then 5 ml of 1% of HCl was added, the mixture was heated till boiling, 1-3 drops of picric acid were added. Yellow- colored precipitate appeared indicated alkaloids component.

Detection of Terpenoids

Terpenoids content was determined as was described by Oyagi and his co-workers [11]. Peel extract powder (0.5 g) was mixed with 10 ml 90% methanol then 2 ml of chloroform and 3 ml of sulphuric acid were added and mixed well. Reddish brown color indicates the presence of terpenoids.

Detection of Tannins

Tannins were measured according to procedure mention by Oyagi and his co-workers [11] by adding (0.5 g) of peel extract to 10 mL distilled water then 2% of $FeCl_3$. A blue-green color appeared indicated tannins.

Detection of Proteins

Protein content was measured by an assay as described by Oyagi and his co-workers [11] Violet color appearance suggests the presence of amino acids and proteins.

FTIR spectroscopy

The spectra of Fourier transform infrared generated by the radiation of electromagnetic absorption in the frequency range 500 to 4000 cm^{-1}. The absorption and intensity of different active functional group indicate geometry features of these groups. FTIR spectra were taken using Shimadzu model.

Antibacterial activity assay

The antibacterial activity was determined by agar disc diffusion Oyagi and his co-workers [12]. Agar plates were inoculated with 0.1 ml broth culture of tested organisms and was spread with sterile an L-shaped rod glass spreader. Whatman No. 1 filter paper of 5 mm diameter were impregnated with different concentration of crude extracts and dried in a hot air oven at 60 ºC for 5 min. The disc in the center of agar plate which impregnated with sterile distal water was used as control.

Denaturation inhibition of albumin

Human albumin (1%) was incubated at 37 °C for 20 minutes, heated at 51 °C and then the albumin was cooled. The turbidity was measured at 660 nm by UV Visible Spectrophotometer, Denaturation percent was determined according to the following equation:

Detection of membrane RBCs stabilization

Human blood cells centrifuged by 3000 rpm for

(10) min, washed the precipitate with normal saline then re-suspended to (10%) v/v in normal saline, the suspension was incubated at 56 °C by water bath for 30 min, centrifuged at 3000 rpm for five minutes, and the absorbance was measured at 560 nm. The Percentage of haemolysis inhibition calculated using the following formula:

Statistical Analysis

Data were expressed as a mean values ± SD by the statistical software package SPSS (version 16).

Results and Discussion

Shilajit consist of different groups of active components, Table 1 shows Shilajit components.

Table 1: Shilajit active component

No.	Component	Test
1	Total phenol	+++++
2	Alkaloids	+++
3	Terpenoids	+++
4	Tannin	+++++
5	Protein	+++++
6	Carbohydrates	++++
7	Steroids	+++

Flavonoids are secondary metabolites, it protect cell from degradation, stress, and act as anti-cancer and anti-viral molecule. Phytoalexins, detoxifying agents, reduce toxic effects and stimulants. Recent research indicated that active components can be nutritionally helpful by triggering the production of natural enzymes that fight disease, such as cancers, heart disease, and age-related degenerative diseases [13].

FTIR

The band at 1181 cm^{-1} can be attributed to C=O stretching indicating the presence of polysaccharide or polysaccharide like compounds, at wavenumber 1411 cm^{-1} (O-H) bending indicating the presence of alcohols or carboxylic acid. A peak at 2930 cm-1 stretch is referred to aliphatic (C-H). The peak at region of 1613 cm^{-1} (aromatic C=C double bond) figure 1.

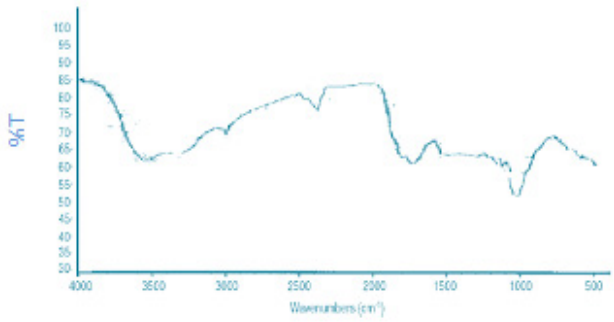


Figure 1: FTIR spectra

Table 2 shows that different bacterial species exhibited different sensitivities towards the extract of shilajit. The sensitivities of bacterial species against phenolic compounds showed more activity against gram positive bacteria compared to gram negative bacteria under this study. These variations in inhibition may be because of differences in the composition and structure surface between Gram positive and Gram negative bacteria [14].

Table 2: The mean of inhibition zone of the aqueous extract of shilajit against certain bacterial strains

Strain	Concentration		
	100	200	300
Streptococcus pyogenes	10	14	22
Proteus vulgaris	-	8	24
Staphylococcus aureus	12	18	23
Klebsiella pneumonia	8	16	20
Pseudomonas euroginosa	-	11	26

Maximum inhibition of albumin was 87% observed at 300 µg/ml of shilajit alcohol extract compared with aspirin (which exhibit 91%) at the same concentration figure 2. Each value represents the mean ± SD. All values showed significant results when it compare with control $p<0.01$. This result investigated that nanoparticle was effective in inhibiting protein denaturation of albumin. Proteins denaturation refers to cause of inflammation [15].

In this study, protein denaturation was used for detection mechanism of the anti-inflammatory activity.

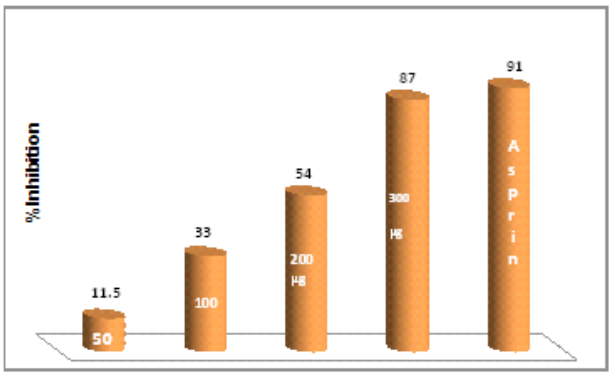


Figure 2: Results of albumin denaturation inhibition of shilajit extract

Heat Induced Haemolysis

Figure 3 show the results activity of inhibiting haemolysis at different concentrations of shilajit. The effective concentration was 300µg/ml in comparing with standard drug Diclofenac sodium 300µg/ml resulted in good protection against damaging effect of heat solution.

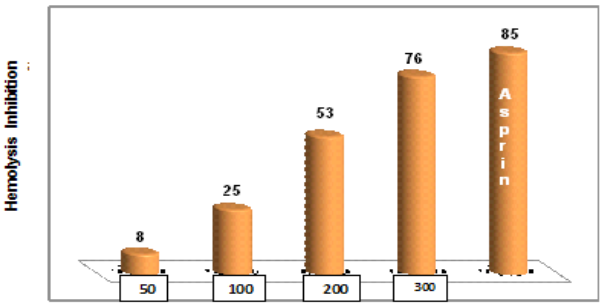


Figure 3: Result of anti hemolysis effect of shilajit extract

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Ethical Clearance: The researchers already have ethical clearance from College of Science, Mustansiriyah University, Iraq

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