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Research Article

Study on Antimicrobial Properties of U.V. Treated Shilajit

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ABSTRACT

To investigate the antimicrobial activity (antibacterial & antifungal) of U.V. treated shilajit (US) and normal shilajit (NS) and to determine the minimum inhibitory concentration (MIC) value.**Methods:**The antibacterial activity of NS & US was evaluated at different concentrations by agar well diffusion method against selected gram positive and gram negative organisms, MIC was determined by prescribed method and antifungal activity was also checked by the well diffusion method. Phyto chemical analysis was done by Kokate et al., 2009; Evans et al., 2002; Khandelwal et al., 1995 method.**Results**:NSdid not show any antimicrobial activity. However, after U.V. treatment shilajit showed the potentially good antimicrobial activity against all available bacterial strains and antifungal activity only against *Penicillium chrysogenum.* MICwas 2mg/ml for *Bacillus subtilis, Bacillus cereus, Escherichia coli, Proteus vulgaris*, 5mg/ml for *Pseudomonas aeruginosa* and *Klebsiella pneumonia*and 3mg/ml for *Staphylococcus aureus.*Phyto chemical analysis showed presence of carbohydrates, triterpenes, flavonoids and cardiac glycosydes.**Discussion:**The results in the present study suggest that the shilajit change its antimicrobial properties by U.V. treatment, which showed good antibacterial activities. This studycould be useful to formulate the new antimicrobial bioactive compounds from U.V. Treated shilajit.

Key words: Shilajit, Humic matter, U.V treated, Antibacterial, Antifungal, MIC

INTRODUCTION

Nature has provided us the plenty of bioactive components, medicines, nutraceuticals, and chemical entities for drug synthesis. Natural and herbal products are great source of economically important and less or nontoxicmedicines all over the world, also referred as the "Green medicine"[1].WHO has estimated that, a large part of the word (80%) relies on the ethnopharmacology and medicinal plants for their primary health care needs.Therefore, natural products have immense potential to produce the new synthetic drugs for great benefit to the humanity. Gradual failure of chemotherapy and increasing administration of antibiotics against several clinically important bacterial strains, leads to the development of new multidrug resistance pathogens[2]. In addition, the synthetic drugs could be expensive, adulterated,inadequatein action andmay have side effects[1].

Shilajit is dark brown or black coloured humic matter, exudates from the layers of Rocky Mountains, especially from Himalayan regions of Nepal to Kashmir. Shilajit also known as Salajit, Shilajatu, Mumie or Mummiyo[3].It is formed by the long term humification process of *Euphorbia* and *Trifolium* (clover) plants in the mountains[4,5]. Shilajit is a venerable medicine of the ancient Indian medicinal practice (*Ayurveda).*In Sanskrit, it is classified as "Rasayana" meaning rejuvenator and immunomodulator. It is believed that ithas several medically beneficial properties *viz*; anti-inflammatory, antidiabitic, anticancer, it is an aphrodisiac, anti-ageing agent and activate the immunity[3,10,14].Shilajit is mainly composed of humic substances (60-80%) (humins, humic acid (HA), and fulvic acid (FA)) and minerals. HA strongly absorb U.V. radiation and photochemically altertheir structure[6].Inthe presence of U.V. radiation HA generatesfree radicals such as singlet oxygen (O), peroxy radicals ($R00^-$), superoxide anion (0^{2-}) and its structure is excited to its triplet state. The free radicals formed in this U.V. treatment are very reactive and very short-lived[7].Therefore,inour study, we used U.V. treated shilajit to check the antimicrobial effects of photochemically altered humic matter (shilajit)as compare to the normal shilajit (NS).

MATERIAL AND METHOD

Collection of sample, test organism and stock preparation: Shilajit was collected from town Jhulaghat(29°34′16″N 80°22′30″E),Baitadi district, of far-western Nepal and transported to the laboratory for further study.Different concentration of shilajit 10 mg/ml, 5 mg/ml, 3 mg/ml, 2 mg/ml and 1mg/ml were prepared in distilled water. This shilajit was separated in to two parts, one part (US) was kept in UV reaction chamber at 280 nm for 15 minutes and other part was used normally (NS). Following microorganisms, collected from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Bacteria cultures of *Staphylococcus aureus* (MTCC No 6908), *Bacillus subtilis* (MTCC No 9878), *Bacillus cereus* (MTCC No 8714), *Escherichia coli* (MTCC No 1698), *Klebsiella pneumonia* (MTCC No 7028), *Pseudomonas aeruginosa* (MTCC No 4673) *and Proteus vulgaris*(MTCC No 744). For antifungal activity *Penicillium chrysogenum* (MTCC No 161), *Aspergillus fumigates* (MTCC No 870), *Penicillum citrinium* (MTCC No 8628) *and Neurosporacrassa*(MTCC No 1876), were selected.Bacterial stock cultures were sub-cultured on

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nutrient agar slants and maintained at 4˚C.Fungus cultures were grown in Potato Dextrose agar and maintained at 4˚C[8].

Anti-bacterial Activity: Antibacterial activity of Shilajit was tested by well diffusion method[9].Bacterial cultures were standardisedto the 1-2x10^6 colony-forming unit(CFU)/ml) and 100 µl this was introduced on the surface of Nutrient Agar plate, and speeded uniformly with the help of a spreader. Same procedure was followed for all the bacterial cultures. Different concentrations of shilajit US and NS was added into the appropriate well and zone of inhibition was measured after incubation at 37°C for 18-24 hour.Tetracyclinewas used as positive control and doubled distilled water as negative control.

Determination of Minimal Inhibitory Concentration (MIC): To measure the MIC value, various concentrations of stock solution, 10mg/ml, 5mg/ml, 3mg/ml, 2mg/ml and 1mg/ml were prepared to assay bacteria cultures. MIC was defined as the lowest concentration at which there is no visible bacterial growthwas observed[11].

Table: 1 Showing the zones of inhibition in mille meter (mm) for different concentrations of U.V. treated shilajit (US). Tetracycline was taken as positive control and distilled water as negative control.

Bacteria	Concentrations → / Species ↓	Zone of inhibition in mm for different concentrations (mg/ml) 10 mg/ml	5 mg/ml	3 mg/ml	2 mg/ml	1 mg/ml	Control 10mg/ml
Gram positive	*S. aureus*	14	13	8	-	-	21
	B. subtilis	15	13	13	12	11	21
	B. cereus	17	16	13	10	-	22
Gram negative	*P. aeruginosa*	14	12	10	-	-	23
	P. vulgaris	16	13	12	12	-	25
	K. pneumonia	13	10	-	-	-	18
	E. coli	19	17	15	15	14	20

Table: 2 Minimal Inhibitory Concentration (MIC) of U.V. treated shilajit (US).

Bacteria	Species	MIC
Gram positive	*S. aureus*	3mg/ml
	B. subtilis	2mg/ml
	B. cereus	2mg/ml
Gram negative	*P. aeruginosa*	5mg/ml
	P. vulgaris	2mg/ml
	K. pneumonia	5mg/ml
	E. coli	2mg/ml

Table 3:Showen Phytochemical analysis (+) indicate presence; (-) indicate absence.

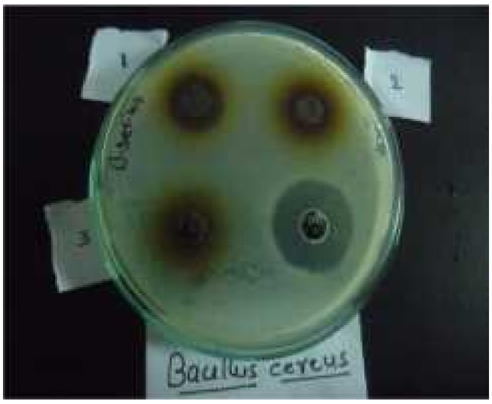
Phytochemicals	Tests	Shilajit
Alkaloids	Mayers	-
	Hager	-
Carbohydrate	Molish	+
	Fehling	+
	Benedict	+
	Comnelisation	+
Protein	Ninhydrin	-
Sterol and triterpins	Salkoeski	-/+
Tennins	Ferric chloride	-
	Alkaline reagent	-
Glycosides	Bromine water	-
Flavonoids	Zinc hydrochloride	-
Cardiac glycosides	Keller killiani test	+

Anti-fungal activity: Antifungal activity of Shilajit was tested by well diffusion method[9].100µl standardised fungal suspensions(1-2x10^6 CFU), were seededinto the potato dextrose agar plate and spreaduniformly. Four wells were made on solid agar plate and Different concentrations of both US and NSshilajit added into the appropriate well. Zone of inhibition was measured after incubation at 25°C for 24-60 hour.

Phytochemical analysis: All the test was done by qualitative method[15].

1) Alkaloids: Mayers and Hager tests
2) carbohydrate: Molish, Fehling, Benedict and Comnelisation
3) Protein: Ninhydrin test
4) Sterol and triterpenes: Salkoeski test
5) Tannins: Ferric chloride and Alkaline reagent test
6) Glycosides: Bromine water
7) Flavonoids: Zinc hydrochloride

8) Flavonoids: Zinc hydrochloride reduction and Alkaline reagent test



In addition to several medicinal properties, shilajitis an immune-modulating agent and an excellent tonic[3,10,14].

Zone of inhibition in B.subtilis and B.cereusat different concentration of US.

9) Cardiac glycosides: Keller killiani test

RESULTS

Antibacterial properties of NS and US was examined against several human pathogens and results are gives in Table no 1 and the antifungal property also checked. Antibacterial property was found absent in the normal shilajit. However, shilajit showed potentially good antibacterial property against all bacteria, after U.V treatment(Table 1). *Bacillus subtilis* and *Escherichia coli* was found to be susceptible for all concentrations US, zone of inhibition increased with increasing concentration of the US. Maximum zones of inhibition was found 19, 17, 16 and 15 mm for *Escherichia coli, Bacillus cereus, Proteus vulgaris* and *Bacillus cereus* respectively at 10 mg/ml of US. Antifungal property of US was found only against *Penicillium chrysogenum*. MIC value of US are shown in Table 2, for *Bacillus subtilis, Bacillus cereus, Escherichia coli, Proteus vulgaris* was found to be 2mg/ml. For *Pseudomonas aeruginosa* and *Klebsiella pneumonia* MICwas5mg/ml and for*Staphylococcus aureus* MICwas 3mg/ml. No antibacterial property was observed in normalshilajit. phytochemical analysis showed that Shilajit contains carbohydrate, reducing sugar and triterpens, cardiac glycosides which are summarised in table 3.

DISCUSSION

In present study NS did not show antibacterial property and in another study of El-Sayed *et al.*, 2012[12] on antibacterial properties ofIndian shilajit from Kumaunalso had no antibacterial property. However, treatment of shilajit with U.V. radiation drastically changes the antibacterial properties of the shilajit, indicates the photochemical changes in the shilajit due to the U.V. treatment.Shilajit contain the 60-80% Humic matters (HA & FA)responsible for antimicrobial activity.It,increases the permeability of cell membrane, affects intracellular homeostasis, disturbs electrochemical gradient and cell osmolarityeventually leads to cell lyses [13]. US showed antifungal activity only against the particular types of the fungus, indicates that shilajit also could be used as an antifungal agents.

However, with all beneficial properties, there is no reported antibacterial property of Indian shilajit form Kumaun region. However, U.V. treated shilajit shows good antibacterial property. Therefore, present studysuggested that, humic matters of shilajit changes photo-chemically and alters normal shilajit to an antibacterialagent against several pathogenic bacteria. It is anticipated that this study may guide to the establishment of some bioactive components of U.V. treated shilajit that could be used to formulate the new, potent, cost effective and less or non toxic natural antimicrobial drugs.

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