

**IN VITRO INVESTIGATION OF ANTIBACTERIAL AND ANTILEISHMANIAL ACTIVITIES OF GUM-RESIN OF *BOSWELLIA SERRATA* USING (0.5%) DMSO SOLUTION AS AN ALTERNATIVE SOLVENT FOR EXTRACTION**

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**ABSTRACT**

*Boswellia serrata* belongs to the family Burseraceae, is a plant that produces frankincense. The plant is native to India and extends to Pakistan. The tree yields oleo-gum- resin which has been used for a variety of therapeutic purposes such as cancer, inflammation, arthritis, asthma, psoriasis, colitis, Crohn's Disease, and hyperlipidemia, antibacterial and antifungal activities. The current study investigated the antibacterial (*S. aureus* and *E. coli*) and antileishmanial (*L.tropica* and *L. donovani*) activities of *B. serrata* aqueous crude extract using (0.5%) DMSO solvent for extraction. The phytochemical analysis of

crude extract was positive for saponin only. The extract failed to inhibit bacterial growth on Muller- Hinton agar plate. Lower concentrations (5%, 2.5% and 1.25%) of extract showed antileishmanial activity against two species of *Leishmania*. The (10%) concentration lacked antileishmanial activity. The study concludes that *B. serrate* gum- resin has a potential antileishmanial activity against two species of *Leishmania* using a safe concentration of (0.5%) DMSO as an alternative aqueous solvent for methanol extracting gum- resins.

**KEYWORDS:** *Boswellia Serrata*, *Staphylococcus Aureus*, *Escherichia Coli*, *Leishmania Tropica*, *Leishmania Donovanii*.

## INTRODUCTION

The scientists' approach for the development of novel drugs from plants' secondary metabolites, researchers around the world are interested in investigating the antimicrobial, anticancer and antioxidant activities in addition to many effects.

*Boswellia serrata* belongs to the family Burseraceae, is a plant that produces frankincense. The plant is native to India and extends to Pakistan.<sup>[1]</sup> This tree yields oleo-gum- resin which has been used for a variety of therapeutic purposes<sup>[2]</sup> such as cancer<sup>[3]</sup>, inflammation<sup>[4]</sup>, arthritis<sup>[5]</sup>, asthma<sup>[6]</sup>, psoriasis<sup>[7]</sup>, colitis<sup>[8]</sup>, Crohn's Disease<sup>[9]</sup> and hyperlipidemia<sup>[10]</sup>, antibacterial and antifungal activities.<sup>[11]</sup>

Resin portion mainly composed of pentacyclic triterpine acid of which boswellic acid is the active moiety. The composition of oleo-gum-resin of *Boswellia serrata* is moisture 10-11%, volatile oil 8-9%, resin 55-57%, gum 20-23%, insoluble matter 4-5%.<sup>[12]</sup>

Dimethyl sulfoxide (DMSO) is an organosulfur compound with the formula (CH)<sub>2</sub> SO. This colorless liquid is an important polar aprotic solvent that dissolves both polar and non- polar compounds and is miscible in a wide range of organic solvents as well as water.<sup>[13]</sup>

*Staphylococcus aureus* is a gram- positive, round- shaped bacterium and that is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need of oxygen.<sup>[14]</sup>

*Escherichia coli* is a gram- negative, facultative anaerobe, rod- shaped bacteria commonly found in lower intestine.<sup>[15]</sup>

*Leishmania tropica* is a species of flagellate parasites that infects humans and cause cutaneous leishmaniasis.<sup>[16]</sup>

*Leishmania donovani* is a species of intracellular parasites belonging to the genus *Leishmania*, a group of haemoflagellate that cause visceral leishmaniasis or kalazar.<sup>[17]</sup>

The objectives of this study is to investigate the *in vitro* *Boswellia serrata* aqueous crude extract antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli* bacteria and antileishmanial activity against promastigotes of *Leishmania tropica* and *Leishmania donovani* using(0.05%) DMSO aqueous solution.

## MATERIAL AND METHODS

The gum-resin of *Boswellia serrata* was purchased from local market in Baghdad, Iraq. The gum was grinded by an electrical grinder. Ten grams of the powder macerated in 100 milliliters of 0.5% dimethyl sulfoxide (DMSO) solution for 72 hours with occasional stirring. The macerate was filtered using three layers of guaze and then filtered by Whatman No. 1 filter paper. Different concentrations of extract 10%, 5%, 2.5% and 1.25% were prepared which contains 0.5%, 0.25%, 0.125% and 0.0625% of DMSO, respectively.

### Phytochemical Investigation

Phytochemical screening of *B. serrata* extract carried out as follows<sup>[18, 19]</sup>

- 1- Detection of saponins: Foam test
- 2- Detection of glycosides: Benidect's test
- 3- Detection of flavonoids: Alkaline potassium hydroxide test
- 4- Detection of alkaloids: Dragendroff's test
- 5- Detection of polyphenols and tannins: Ferric chloride test

### Antimicrobial activity of *B. serrata* extract

Eighteen to 24 hours single colonies on agar plates were used to prepare the bacterial suspension with turbidity of 0.05 McFarland (equal to  $1.5 \times 10^3$ ) cells/ml. Aqueous extracts were evaluated using well diffusion method on Muller- Hinton agar.<sup>[20]</sup> The bacteria used for antimicrobial assay of extract were *Staphylococcus aureus* (Gram + ve) and *Escherichia coli* (Gram-ve) supplied by Research and Production for Drugs and Medical Supplies, Baghdad, Iraq. Positive control was Amoxicillin (30 µg) for both bacteria and DMSO as a negative control. The extract and DMSO solution sterilized by 0.22µm Millipore filter.

The Muller-Hinton agar plates inoculated with bacterial strains under aseptic condition. A 6 millimeter diameter wells were made using a sterile cork borer and 50µl of each concentration of extract (100mg/ml, 50mg/ ml, 25mg/ml and 12.5 mg/ ml) and DMSO solution of different concentrations as a negative control (0.005mg/ml, 0.0025mg/ ml, 0.00125mg/ml and 0.000625mg/ml) respectively introduced into the well. The agar plates of bacterial strains incubated at 37°C for 24 hours. After the incubation period, the diameters of inhibition zones were measured.<sup>[21]</sup> The experiment repeated three times and the average values recorded.

***In vitro* antileishmanial activity against *L. tropica* and *L. donovani* promastigotes**

*Leishmania tropica* and *L. donovani* promastigotes supplied by Biotechnology Research Center, Al-Nahrain University. The strains were isolated from patients in Iraqi hospitals. The promastigotes used to evaluate the effect of the antileishmanial activity of the aqueous extracts of *B.serrata*. *Leishmania* promastigotes in late log phase were incubated in RPMI-1640 (Roswell Park Institute Park Memorial) medium enriched by 10% fetal calf serum, at an average of  $10^6$  parasites/ml.

For the antileishmanial activity assays, 100  $\mu$ l/well of culture containing  $10^6$  cells/ml, concentrations of extracts added to triplicate wells. The plates incubated for 24 hours at  $25 \pm 1^\circ\text{C}$ . The first well of 96 wells is a blank well which only contains 100  $\mu$ l of culture medium without any plant extract, drug or parasite. Negative control well contained only medium and parasite. Positive control wells contain different concentrations of DMSO. At the end of incubation, 10  $\mu$ l of MTT (3-(4,5- dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide), to assess cell metabolic activity, was added to each well and plates were incubated for 4 hours at  $25 \pm 1^\circ\text{C}$ . Dimethyl sulfoxide (DMSO), as a solubilizing solution added and incubated for 30 minutes. Relative optical density (OD) measured at a wavelength of 620 nm using a multi-well scanning spectrophotometer (ELISA reader). The absorbance of the formazan produced by the action of mitochondrial dehydrogenases of metabolically active cells is shown to correlate with the number of viable cells.<sup>[22,23,24]</sup> The live cells, percentage of viability and inhibition ratio calculated according to the formula:

Inhibition (%) =  $(A_c - A_s / A_c) \times 100$   $A_c$  and  $A_s$  are the optical density for medium and extract samples, respectively.

**RESULTS****Phytochemical Investigation**

Phytochemical investigation revealed negative results for alkaloids, glycosides, flavonoids and polyphenols and tannins but positive result for saponin as shown in (Table 1).

**Table. (1): Phytochemical screening tests of *Boswellia serrata* gum- resin extract.**

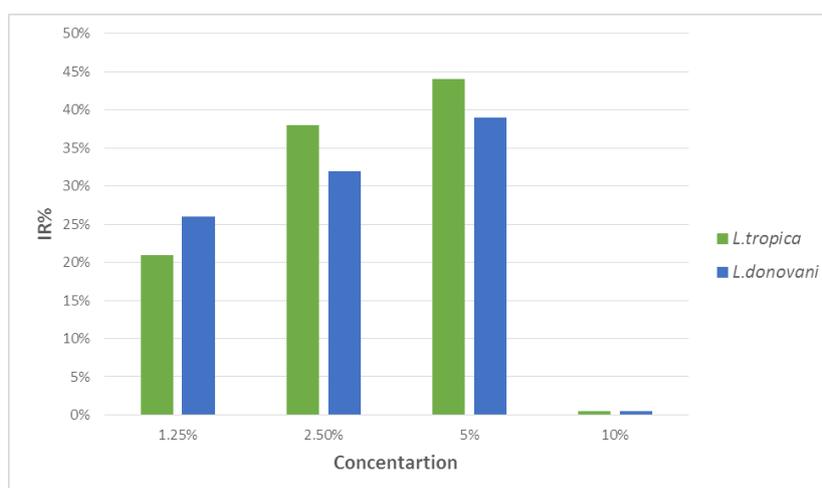
Phytochemicals	Result
Alkaloids	Negative
Flavonoids	Negative
Glycosides	Negative
Polyphenols & Tannins	Negative
Saponins	Positive



**Table. 3: *In vitro* inhibitory rate (IR%) of *B. serrata* aqueous extracts against promastigotes of *L. tropica* and *L. donovani*.**

Concentrations of <i>B.serrata</i> Aqueous Extracts	IR (%) <i>L. tropica</i>	IR (%) <i>L.donovani</i>
Extract 10%	NA	NA
Extract 5%	44%	39%
Extract 2.5%	38%	32%
Extract 1.25%	21%	26%

NA= No Activity.



**Figure. 2: *In vitro* antileishmanial activity of aqueous extract of *Boswellia serrate* against *L. tropica* and *L. donovani* promastigotes.**

## DISCUSSION

In the present study, DMSO (0.5%) solution was used to test its power in extracting the active constituents from *B. serrata* gum- resin as an alternative aqueous solvent for methanol and to investigate the inhibitory activity of the crude extract against selected strains of bacteria and protozoa. In the current study, phytochemical screening of the secondary metabolites of *Boswellia serrata* showed positive result for saponin only and negative result for others as in (Table 1) for aqueous extract. A study conducted by Muniraj *et al.*<sup>[25]</sup>, a methanol extract showed trace amounts of tannins, saponins, glycosides and phenolic compounds, while flavonoids and alkaloids were absent. Another study showed positive results for phenolic compounds, alkaloids and saponin but negative results for tannins and flavonoids in 70% methanol extract.<sup>[26]</sup>

Antimicrobial screening of the extract against *S.aureus* and *E.coli* did not show inhibitory zone compared to Amoxicillin (Positive control). The result is consistent with a previous

study done by Mohan *et.al.*<sup>[27]</sup> that showed no inhibitory activity of a methanol crude extract of *Boswellia serrata* against *S.aureus* and *E.coli*, but showed activity against *Bacillus cereus* and *Proteus vulgaris*. The results of the current study did not agree with a study conducted by Mishra *et.al.*<sup>[28]</sup> that showed an antimicrobial activity of a methanol extract of gum-resin of *Boswellia serrata* against many strains including *S.aureus* and *E coli*. A study by Muniraj *et.al.*<sup>[25]</sup> showed activity against *S.aureus* and *P.aureginosa* using alcoholic resin extract of *Boswellia serrata*. The oils of *Boswellia* species were investigated for their antimicrobial efficacy by Vuuren *et.al.*<sup>[29]</sup>, the study showed a poor activity against reference *S. aureus* (ATCC 12600).

A study carried out by Mandal *et. al.*<sup>[30]</sup>, showed *in vitro* antileishmanial activity against *L. donovani* by saponin isolated from *Careya arborea*. The current study revealed antileishmanial activity at the 5%, 2.5% and 1.25% concentrations against both species of *Lieshmania* promastigotes, but the 10% concentration of extract failed to show inhibitory activity.

Previous studies used methanol for extraction of resins from *B. serrata*. Converting alcoholic extract to aqueous extract to be used for different cell viability assays is time consuming, in addition to a decreased solubility in water that requires the addition of DMSO to increase the solubility. In the current study, the crude extract of *B. serrata* lacked antibacterial activity against *S. aureus* and *E. coli* but showed antileishmanial activity against *L. tropica* and *L. donovani* promastigotes. This variation in the inhibitory activities of cells (bacteria and promastigotes) may be due to low concentration of saponin extracted from the resin or due to the low concentration of DMSO used in the extraction or may be due to poor permeability of saponin inside the cells due to low solubility.<sup>[31]</sup> It is not preferred to increase the concentration of DMSO above (0.5%) which may affect the assays. In a study conducted by Timm *et. al.*, showed that 1% DMSO is a critical concentration, because lower concentrations will result in increased cellular activation while higher concentrations will exert inhibitory effects.<sup>[31]</sup> This was the case of antibacterial assay in the present study, the negative control (DMSO) did not show any inhibitory activity. To the best of our knowledge, the aqueous crude extract of *B. serrate* antileishmanial activity has not been studied before. Studies are limited or absent concerning the antileishmanial assay of the resin of *B. serrata*. Further studies are needed for investigating different concentrations of DMSO as an alternative solvent for methanol in extracting gum- resins.

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