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To determine antimicrobial and phytochemical properties of *Boswellia serrata* leaf extracts

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Abstract

Various parts of *Boswellia serrata* is used as medicine in treating various diseases by human beings from the ancient times. In this article the study is carried out on the antimicrobial activity of *Boswellia serrata* leaf extracts. It shows the antimicrobial activity of ethanolic leaf extracts against actinomycetes bacteria. The phytochemical analysis of leaf extracts showed the presence of secondary metabolites like flavanoides, reducing sugars, terpenoids, quinones, tannins, saponins, and glycosides exhibiting antimicrobial activity. It is concluded that leaf extract of *Boswellia serrata* exhibit highest antimicrobial activity at 0.4g/ml concentration.

Keywords: Antimicrobial, *Boswellia serrata*, Phytochemical analysis, DMSO, well diffusion method.

1. Introduction

The medicinal plants have been used as medicines from the ancient times by humans to treat various diseases and it had a great effect. *Boswellia serrata* is also known as Indian Frankincense tree. It is a deciduous tree of medium size mostly found in India, Asia and Africa (Chaurasia A, Gharia A, 2017) [2]. Leaves of *Boswellia serrata* are alternate and imparipinnate. The trees usually have papery and thin bark. Flowers are small and white with sepals and petals. Fruits are trifid splitting into three valves. Seeds are heart shaped and attached to the inner angle of fruits (Siddiqui MZ 2011). It is used in the treatment of various diseases like cough, asthma and bronchitis. The gum resins are mostly used in medicines (Aman and Balu 2009) [1]. This article contains the study of antimicrobial activity of leaf extracts of *Boswellia serrata* against actinomycetes bacteria.

2. Materials and Methods

2.1 Collection and extraction of plant material:

The *Boswellia serrata* plant was identified by Prof Piyush J Godhani Botany Department, Harivandana College, Rajkot. The fresh and matured leaves of *Boswellia serrata* was collected from a farm from Munjka village, Rajkot. The leaves were given a wash of distilled water followed by vim then again washed with distilled water in order to remove dust and impurities on the leaves. The leaves were oven dried at 45°C for 72hrs and finely powered then stored at 4°C for further use.

2.2 Preparation of plant extract for Phytochemical Analysis:

The powered leaves were extracted with distilled and sterile water. 25g powder mixed with 25ml sterile water and boiled at 50-60°C for 30min in water bath then the extract was filtered with Whatman No.1 filter paper and the filtrate was centrifuged at 2500rpm for 15min and the supernatant is stored at 5°C and used for Phytochemical analysis (Gowdhami. M 2014) [5].

2.2.1 Phytochemical Analysis

The prepared plant extract is used for the analysis of flavonoids, alkaloids, reducing sugars, terpenoids, quinines, tannins, saponins and glycosides.

1. **Test for alkaloids:** To 3ml plant extract 1ml 1% Hcl is added and heated gently for 20min. Then it is cooled and filtered. Add 2 drops of Mayer's reagent to 1ml filtrate. Turbidity or creamy precipitates indicate presence of alkaloids (Narasimhan. R 2012) [7].
2. **Test for flavonoids:**
 - a. **Alkaline reagent test:** To 1ml plant extract 1ml 10% NaOH is added to get intense yellow color.
 - b. **NH₄OH test:** To 3ml extract 10% NH₄OH is added to get yellow fluorescence.

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- c. **Zn test:** To 2ml extract Zn dust is added followed by concentrated HCl to get red color (Sawant RS 2013)^[10].
3. **Test for reducing sugars:** 2-3ml Fehling solution A and B are gently heated in a test tube and cooled and then 1ml extract is added and boiled for 5-10min. Brownish red precipitates are observed (Paul. R 2016)^[8].
4. **Test for terpenoids:** To 0.8g sample 10ml methanol is added, shaken well and filtered. To 5ml of this filtrate 2ml chloroform is added and 3ml H₂SO₄ is added. Reddish brown color indicates the presence of terpenoids (Kakad S.L 2017)^[6].
5. **Test for Quinones:** Small amount of extract is taken in a test tube and concentrated HCl is added in it, which shows yellow ppt. indicating the presence of quinones^[6].
6. **Test for tannins:** To 0.5 ml extract 1ml of water and 1-2 drops of Ferric chloride solution is added. Then blue or green black color is seen which indicates the presence of tannins.
Blue color – Gallic tannins
Green color – Catechol tannins (Dutta BK 2010)^[4].
7. **Test for Glycosides:** To the extract in a test tube glacial acetic acid is added and then few drops of ferric chloride is added followed by concentrated H₂SO₄ by the side of the tube. Now reddish brown color is seen at junction of two layers and bluish green color appears in the upper layer (Chhetri HP 2008)^[3].
8. **Test for Saponins:** 2ml extract is taken in a test tube and shaken vigorously for froth formation. The froth formation indicates the presence of saponins (Paul. R 2016)^[8].

2.3 Preparation of plant extract for Antimicrobial analysis

The dried and finely ground leaf powder of *Boswellia serrata* (25g) is extracted with methanol (250ml) by using Soxhlet apparatus at 63°C for 24hrs. The obtained methanolic extract is oven dried at 35°C for 48hrs then scraped with scalpel and stored at 4°C for further use. For antimicrobial analysis solution of different concentrations from 0.1g/ml – 0.6g/ml are used. For stock solution DMSO is used as solvent, then the working solution is prepared using stock solution and 10% DMSO solution. The concentration of working solution is 1mg/ml (Ranjit *et al.* 2014)^[9]. The prepared working solution is used for antimicrobial analysis. Likewise working solutions are prepared for all concentrations from 0.1g/ml – 0.6g/ml. The prepared solutions are stored at 4°C for further use.

2.4 Preparation of Agar plates

For the preparation of nutrient agar plates 3% Nutrient Agar is used and autoclaved at 15psi for 20min, then the agar plates are prepared and kept undisturbed until the agar gets solidified.

2.5 Preparation and spreading of inoculum

The bacterial culture was isolated from soil by mixing it in water and then diluting the mixture. Sucrose added as the nutrient and the flask is sealed with cotton knob and the flask is stirred at regular time interval. After one week the supernatant is collected and kept undisturbed for 2 days, then again the supernatant is collected and then inoculated on a petri plate from it the bacterial colonies were isolated and were morphologically identified by the microbiologists as

actinomycetes. From this culture inoculum is prepared and used for inoculation by spread plate method using L-shaped spreader.

2.6 Agar well diffusion method

To determine antibacterial property Agar well diffusion method is used. The wells of about 5mm diameter were made in the agar plate by using sterile cork borer. Then by using micro pipette 50microliter of working solution prepared from different concentrations i.e, 0.1mg/ml – 0.6mg/ml are poured in 6 different agar plates. These petri plates are incubated in the incubator at 37°C for 24hrs and then the zones of inhibition for all of the 6 petri plates are noted.

3. Result and discussion

3.1 Phytochemical Analysis

The phytochemical analysis of the leaf extracts of *Boswellia serrata* shows the following results given in the table.

Table 1: Phytochemical analysis of *Boswellia serrata* leaf extracts

Sr. no.	Phytochemicals	Result
1	Flavanoids	+
2	Alkaloids	-
3	Reducing sugars	+
4	Terpenoids	+
5	Quinones	+
6	Tannins	+
7	Saponins	+
8	Glycosides	+

Present: + Absent: -

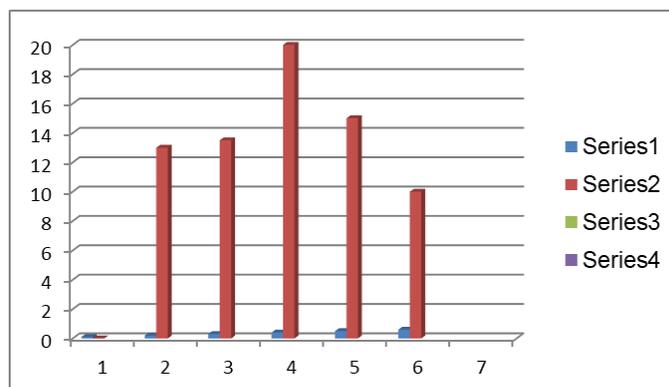
The preliminary phytochemical analysis revealed the presence of flavonoids, reducing sugars, terpenoids, quinones, tannins, saponins and glycosides in *Boswellia serrata* and alkaloids are absent when the extract is prepared with water.

3.2 Result of anti-bacterial activity

Table 2: The anti-bacterial activity of leaf extracts of *Boswellia serrata* shows the following result given in the table below

Sr. No	Concentration(g/ml)	Inhibition Zones(mm)
1	0.1	nill
2	0.2	13
3	0.3	13.5
4	0.4	20
5	0.5	15
6	0.6	10

From this anti-bacterial activity it is known that the highest inhibition zone is observed in the concentration of 0.4g/ml of the leaf extract.



Graph 1: graph of Zone of Inhibition v/s concentration of *Boswellia serrata* leaf extract.

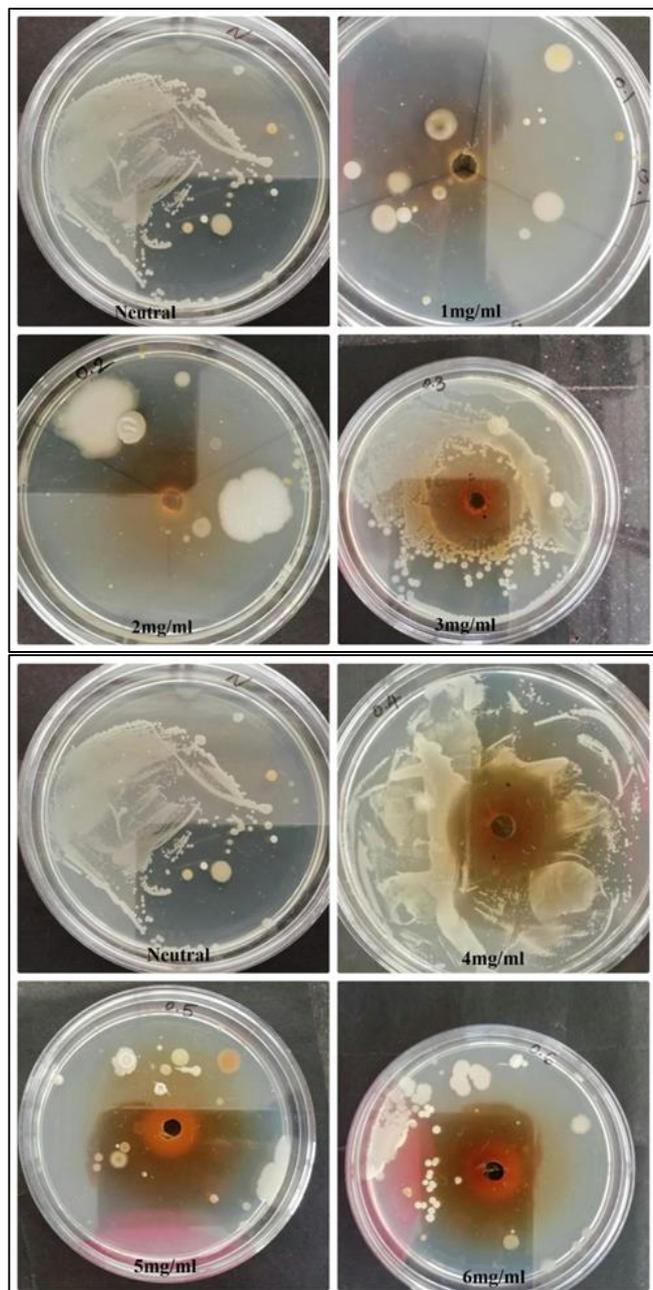


Fig 1: Anti-microbial activity of leaf extracts of *B. serrata* by Agar well diffusion method

From the Fig.1 it is revealed that the anti-microbial activity of leaf extracts of *Boswellia serrata* is highest in the petri plate with concentration 4mg/ml.

4. Conclusion

From the antimicrobial analysis of leaf extracts of *Boswellia serrata* it can be concluded that leaves of *Boswellia serrata* show antimicrobial effect against Actinomycetes bacteriand the phytochemical analysis shows the presence of secondary metabolites like flavonoids, reducing sugars, terpenoids, quinones, tannins, saponins and glycosides in *Boswellia serrata* which are responsible for antimicrobial effect.

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