

Phytochemical analysis and anti-microbial investigation of some medicinal plants used by tribals of Ranchi District

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Received : 18th November, 2024 ; Accepted : 20th December, 2024

DOI:- <https://doi.org/10.5281/zenodo.15068742>

ABSTRACT

This report describes the phytochemical relation of the antibacterial, antioxidant activity of leaves extracts of *Saraca asoca* (Roxb.) De Wilde, *Boswellia serrata* and *Azadirachta indica*. In the present study preliminary phytochemical analysis of leaf extracts was conducted, which revealed the presence of Alkaloids, Flavonoids, Glycosides, Saponins, Phenols, Steroids, Tannins and Triterpenoids. Quantitative estimation of Flavonoids and Phenols was conducted using ethanolic and aqueous extracts. Screening for antibacterial activity of extracts of *Saraca asoca*, *Boswellia serrata* and *Azadirachta indica* explants were performed against gram positive and gram-negative bacteria by the disk diffusion method. The present study confirms of phytochemical, antibacterial activity of all plant extract of *S. asoca*, *B.serrata* and *A.indica*.

Key Words - Ethnomedicinal plants, phytochemical and anti-microbial investigation

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INTRODUCTION

Neem (*Azadirachta indica*, A. Juss.) is a multi-purpose tree that is highly popular in India, where it provides food and insecticide, and is used for its great number of ethnomedicinal properties. *Azadirachta indica*, A. Juss (neem) is one of these plants and has been used for more than 2000 years in India and neighboring countries. As proven scientifically, it has a wide spectrum of biological activity and classified in one of the most versatile plants. (Mona *et al.*, 2015; Beena and Radha krishnan, 2002; Siddiqui, 2011). All parts of the tree, from seeds, flowers, twigs, barks, roots and leaves have their medicinal potential to humans. Part of it, the leaves of the neem tree are traditionally used in medicinal preparations purposely for anti-inflammatory, antibacterial, antiviral, antioxidant hepatoprotective and others. Neem seed has the most oil content among the parts of the neem tree. (Shah *et al.*, 2009; Braga *et al.*, 2021).

Besides, the neem flower also shows a medicinal effect. Neem flower contains aromatics, fatty acids, steroids, hydrocarbon and sesquiterpenes. These active ingredients can be used in treating intestinal worm, removal of phlegm and bile suspension. Neem seed and neem flower are seasonal, while, the neem leaves are obtained for the whole year. Thus, neem leaves will be further studied in this research. (Kanagasanthosh *et al.*, 2015; Ashafa *et al.* 2012) Ashok or Ashoka (which is a Sanskrit term meaning one 'without sorrow or grief') also called 'Ashok briksh' and botanically known as *Saraca asoca* (Roxb.) W. J. de Wilde or *Saraca indica* L. is among the most ancient medicinal plants known in India. It belongs to the family Caesalpiniaceae. Known by many local names in different languages, this plant has been regarded as a universal panacea in old Indian Ayurvedic texts and has been reported to be thus used since ancient times. It is also widely

distributed across the Western Ghats (both South and Central), the Sahyadri region and throughout the Himalayas. Almost all plant parts such as bark, flowers, leaves and seeds are considered therapeutically valuable due to the presence of secondary metabolites such as alkaloids, terpenoids, flavonoids, steroids, glycosides, anthraquinones, phenolics, tannins, saponins and other phytochemicals (vide infra Phytochemistry) which are generally considered the biologically active ingredients in most natural products and herbal formulations. (Bandyopadhyay *et al.*, 2004; Arsene *et al.*, 2021; Boeke *et al.*, 2004)

Boswellia serrata, also known as the frankincense tree, is a medium to large-sized deciduous tree that is native to India, the Middle East, and North Africa. The tree is known for its fragrant resin, which is used in incense and has anti-inflammatory properties. *Boswellia serrata* (also called as Salai/Salai-guggul) belongs to the family 'Burseraceae'. Many studies on *B. serrata* for osteoarthritis have been shown to be effective in treating pain and joint inflammation. Various *B. serrata* extracts and its gum resins have been shown to possess pharmacologically important phytocompounds. *Boswellia serrata*, it is widely distributed in Western Himalayas, Madhya Pradesh, Gujarat, Orissa, Rajasthan, Bihar, and Maharashtra. It is tapped from the incision made on the trunk of the tree, which is then stored in specially made bamboo

basket. The semi-solid gum-resin is allowed to remain in the. The fresh gum obtained from the tree is hot with pleasant flavour and slightly bitter in taste. It had been the 'frankincense' of ancient Egyptians, Greeks and Romans who used it as prized incense, fumigant as well as a multipurpose aromatic. The leaf extracts and bark are used in treatment of RA. (Airaodion *et al.*, 2019; Septyani & Wibowo, 2019).

METHODOLOGY

- The samples were collected from various regions of Ranchi District and was identified. They were thoroughly washed and dried and after grinding they were stored in air tight container for further use.
- For Confirmation of various phytochemicals, qualitative and quantitative test were performed.
- Phytochemical tests were carried out by ethanol solvent.
- For Antimicrobial investigation two gram positive (*Bacillus subtilis* & *Staphylococcus aureus*) and two gram-negative (*E. coli* & *Pseudomonas aeruginosa*) were selected and investigation is performed by disc diffusion method.

RESULTS

Antimicrobial results: Minimum inhibition concentration

Table 1. Showing level of inhibition concentration

Name of bacteria	Plant extract	MIC of extracts
<i>P.aeruginosa</i> (Gram-ve)	Neem	0.5mg/ml
<i>S.aureus</i> (Gram+ve)	Neem	0.2 mg/ml (Partial but no zone of inhibition)
<i>E.coli</i> (Gram-ve)	Neem	1 mg/ml
<i>B.subtilis</i> (Gram+ve)	Neem	No zone of inhibition
<i>P.aeruginosa</i> (Gram-ve)	Ashoka	0.2 mg/ml (No inhibition)
<i>S.aureus</i> (Gram+ve)	Ashoka	0.2 mg/ml
<i>E.coli</i> (Gram-ve)	Ashoka	3 mg/ml
<i>B.subtilis</i> (Gram+ve)	Ashoka	5mg/ml
<i>P.aeruginosa</i> (Gram-ve)	Shallaki	0.2 mg/ml
<i>S.aureus</i> (Gram+ve)	Shallaki	0.2 mg/ml
<i>E.coli</i> (Gram-ve)	Shallaki	2 mg/ml (Partial inhibition)
<i>B.subtilis</i> (Gram+ve)	Shallaki	Partial inhibition of 8 mm

Note: partial inhibition (reduction of growth but not complete inhibition)

TLC RESULTS:

Ashoka yields 7 components

Neem yields 6 components

Shalaki yield 5 components

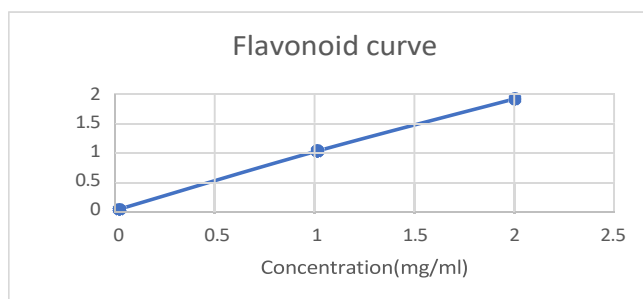
Table 2- Qualitative Phytochemical Test Results

Phytochemical test name	Name of phytochemicals	Plants name		
		Neem	Ashoka	Shallaki
a) Saponification test	Fixed oils and fats	+	+	+
b) Biuret test	Phytosterols (Phenols)	+	-	+
c) Gum and mucilage test	Phytosterols (Gums and mucilage)	-	+	-
d) Ferric chloride test	Phenolic compounds and tannins	-	-	Not shown
e) Winger's test	Alkaloides	+	+	+
f) Alkaline reagent test	Phenolic compounds and tannins (Flavonoides)	+	+	+

QUANTITATIVE ANALYSIS OF KNOWN STANDARD AGAINST UNKNOWN SAMPLE**Table 3- Flavonoid test**

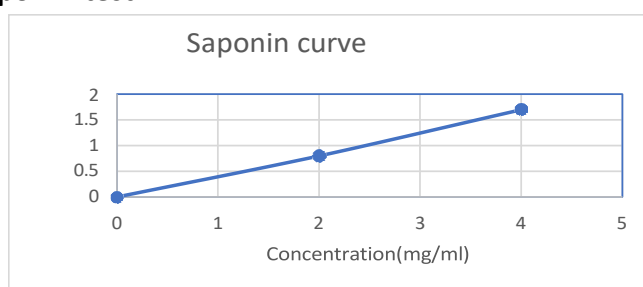
Standard mg/ml	OD at 600 nm
1 mg	1.0
2 mg	1.9
4 mg	6.9
Unknown (Neem)	0.2
Unknown (Shalaki)	0.3
Unknown (Ashoka)	0.4

● Neem	0.25 mg/ml
● Shalaki	0.3 mg/ml
● Ashoka	0.4 mg/ml

**Table 4- Saponin test**

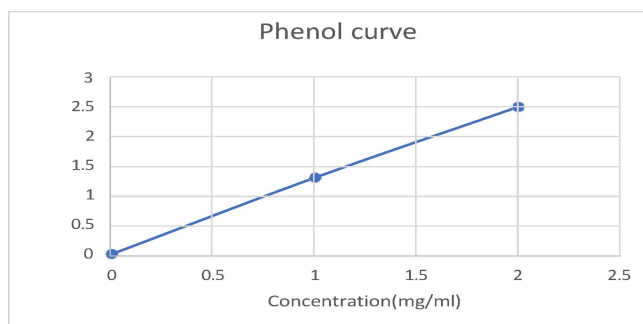
Standard mg/ml	OD at 600 nm
1 mg	1.3
2 mg	0.8
4 mg	1.7
Unknown (Ashoka)	0.4
Unknown (Neem)	0.5
Unknown (Shalaki)	0.7

● Ashoka	1.0 mg/ml
● Neem	1.25 mg/ml
● Shalaki	1.7 mg/ml

**Table 5- Phenol test**

Standard mg/ml	OD at 600 nm
1 mg	1.3
2 mg	2.5
4 mg	2.9
Unknown (Shalaki)	0.2
Unknown (Neem)	0.3
Unknown (Ashoka)	0.4

● Shalaki	0.2 mg/ml
● Neem	0.35 mg/ml
● Ashoka	0.45 mg/ml



DISCUSSION

From the above results of Antimicrobial activities it was concluded that Minimum inhibitory conc. 0.2 mg, 2 mg, 3 mg and 5mg conc. of shalaki show partial and full inhibition against *S. aureus*, *E.coli*, *P.aeruginosa* and *B.subtilis*. 3mg and 5 mg of Asoka shows full inhibition against the *E.coli* and *B.subtilis* only. 0.5 mg and 0.2 mg, 1 mg conc. of neem able to inhibit *E.coli*, *S.aureus* and *P.aeruginosa*. From the TLC chromatogram we had found that Qualitative analysis of Ashoka showed seven phytochemicals, Neem showed six phytochemicals and Shallaki shows five phytochemicals. Qualitative analysis of neem shows the presence of flavonoids, phenols, saponins, alkaloids, oils and fats and Ashoka shows the presence of oils and fats, phenols, saponins, flavonoids, alkaloids only and shallaki shows the presence of flavonoids, phenols, saponins, alkaloids, oils and fats. Quantitative analysis of Phenols, saponins and flavonoids were performed against the standard to quantify the unknown conc. present in Ashoka, Shallaki and Neem. These data are helpful in understanding the importance of phytochemicals which are helpful in treating rheumatism and their antimicrobial activities against the microbes (Arindam *et al.*, 2015; Gayathri & Jeyanthi, 2013). After that we had isolated each of phytochemicals from TLC plates and each component was tested against *E.coli* to ensure that which phytochemical was able to inhibit the bacterium. By matching the Rf values of phytochemicals with previous work we can conclude that flavonoid is the main components which was found to fall in near Rf values references and it was found in all 3 samples (Adithya *et al.*, 2021; Al Akeel *et al.*, 2017).

ACKNOWLEDGEMENT

I would like to express my gratitude to my esteemed faculties of Department of Botany, DSPMU, Ranchi for all the support, instructions, and encouragement I received throughout this topic. Last but not least, I would like to express gratitude to my friends, mentors and colleagues whose encouragement kept me focused and helped me overcome numerous challenges during my research journey.

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