Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 6
Open Access

Antibacterial and Phytochemical profiling of *Pterocarpus marsupium* bark extracts from Barnawapara, (C.G), India

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Cite this paper as: Garima Moitra, Snehalata Das (2024) Antibacterial and Phytochemical profiling of Pterocarpus marsupium bark extracts from Barnawapara, (C.G), India. Frontiers in Health *Informatics*, *13(6)* 4630-4636

Abstract-
Pterocarpus marsupium a medicinally significant tree, traditionally used in Ayurvedic medicine and was investigated for its phytochemical composition and antibacterial activity during July,2024. The phytochemical analysis of *Pterocarpus marsupium*bark extracts from three sites was likely quantifieddifferent solvents like alkaloids, flavonoids, tannins, phenols and saponins andreported percentage ranged from3.76 to 20.51%,with the methanolic and ethyl acetate extracts showing higher concentrations of these compounds.The tested substance exhibited significant inhibitory effects on the antimicrobial activity andzones of inhibition against *S. aureus*ranged from 15.86 to 22.50 mm*, E. coli*from 21.61 to 25.11 mm*, St. mitis*from 12.10 to 18 mm*, P. aeruginosa*from 20.50 to 27.89 mm*, E. faecalis*from 12.20 to 20 .80 mm, *M. luteus*from 10.00 to 17.45 mm, *S. typhimurium*from 8.67 to 20.10 mm*,* and *B. subtilis*from 11.00 mm to 19.80 mm respectively.The minimum inhibitory concentration (MIC) against different pathogenic bacteria ranged from 45 to 66 µg/ml.Ethyl acetate and methanol extracts were found to be more active towards the organisms tested than hexane extract.These findings demonstrate that methanolic and ethyl acetate extracts of *Pterocarpus marsupium* bark possess potent antibacterial activity,can be used as a potential source for developing of novel antimicrobial agents.

Key words:Antibacterial, phytochemical,bark,profile,extract

Introduction

Over the last decade, there has been an increasing worldwide fascination with traditional herbal medicines, particularly in developing countries, where they are integral to health management initiatives (Verma, 2012).Plants are regarded as the pharmaceutical factories of natural origin as a plant medicine for most of the drugs used byhuman beings. As India is the largest producer andconsumer of the medicinal drugs and is rightly called the botanical garden of the world (Ncube et al., 2008).Most of the plantsare major sources of natural products used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients,food additives and pesticides. Plants used for traditional medicine contain a wide range of substances that can beused to treat chronic as well as infectious diseases (Kadam et al., 2012). Large number of primary metabolites acts as precursorsof pharmacologically active metabolites in pharmaceutical compounds for the synthesis of drugs (Gairola et al.,2010).Traditionally, bark products have been particularly prominent as sources of medicines and raw materials (Patil et al.,2011).Different chemical compounds isolated from the bark exhibit wide pharmacological activities and plays role intreating the various disorders related to human health (Bigoniya et al., 2011).*Pterocarpusmarsupium*Roxb., commonly known as the Red Kino plant native to India, historically, its various parts have been used in traditional remedies for headaches, inflammations, fever, worms, mental issues, ulcers and digestive problems (Katiyar et al., 2016). The bark, known as 'Gum Kino' when injured, is

prominent in Indian deciduous forests, while the heartwood is valued for its astringent, bitter and cooling properties, serving purposes like anti-inflammatory and haemostatic effects (Prusti et al., 2007).This study aimed to conduct the importance of this species, phytochemical screening and evaluate its antimicrobial activity, contributing thorough assessment.

Materials and Methods

Phytochemical Analysis

Collection of Plant Material

Thebark of*P. marsupium* was collected from three sites of Barnawapara,(C.G) during July'2024(Fig.1a&b) used for identification and authentication of the plants. Fresh bark samples from mature *P. marsupium* trees collected and washed thoroughly in runningtap water, rinsed in distilled water and shade dried in open air and grounded into powder form.

Figure 1: (a) Tree of *P. marsupium* (b) Bark of *P. marsupium*

Preparation of Phytochemical Extracts

The bark samples from three sites with triplicate underwent a series of steps: First air-dried, powdered, and then subjected to sequential extraction using solvents with increasing polarity namely from hexane, followed by ethyl acetate, methanol, aqueous, and finally chloroform for 72hemploying a Soxhlet extractor. The resulting extracts were concentrated under reduced pressure using a rotary flashevaporator to yield crude extractsand stored at 4° until assay.

Phytochemical screening and quantitative analysis

The crude extracts underwent screening to detect the presence of diverse phytochemicals, such as alkaloids, flavonoids, tannins, phenols, and saponins can be carried outusing accepted techniques. The quantitative estimation of phytochemical constituents was conducted(Raghuramuluetal.,2003)through well-established spectrophotometric techniques. Alkaloids were quantified employing the Dragendorff's reagent method, flavonoids assessed via the aluminium chloride colorimetric assay, tannins determined using the Folin-Ciocalteu method, phenols measured through the Folin-Denis method, and saponins evaluated through the foam test.

Antimicrobial activity of bark extracts

Test Microorganisms

Four standard strains of Gram-positive bacteria-*Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*,*Streptococcus pyrogens* and four strains of Gram-negative bacteria - *Escherichia coli, Salmonella typii, Serratiamarcescens* and *Pseudomonas aeruginosa* were used for antibacterial activity (Table-1) cultivated them on suitable media for maintenance.

Antimicrobial Assays

The agar well diffusion method was employed to assess the antimicrobial activity of *P. marsupium* bark extracts against the test microorganisms. Mueller-Hinton agar was used for bacteria, supplemented with chloramphenicol (Verma et al., 2012)andZone of inhibition is measured in mm.

Minimum Inhibitory Concentration (MIC) Assay

The Minimum Inhibitory Concentration (MIC) assay is a quantitative method used to determine the lowest concentration of an antimicrobial agent (such as a plant extract, antibiotic) that inhibits visible growth of a microorganism after a specified incubation period. The MIC method was employed on extracts demonstrating significant efficacy against microorganisms as determined by the agar well diffusionmethod(Kirby et al.,1966). In a 96-well titre plate, 20 µl of individual microorganisms and 20 µl of selected plant extract were dispensed and incubated at 37°C for 24 hours. The highest dilution of the plant bark extract that maintained its inhibitory effect, resulting in no microbial growth, was recorded as the MIC value. A parallel control experiment was conducted to assess the impact of the solvent alone means without plant extracts on the growth of the test organisms (Dharshan,2014).

Results

The results of phytochemical analysis (values in %) such as tannins, phenols saponins,flavonoids and alkaloids of the bark extract of *P. marsupium* from three different sites of the present study was represented in(Table-2). The highestflavonoids have observed 20.51±0.05%,16.40±0.04%,18.01±0.42% and lowest percentages ofalkaloid 5.51±0.12%,3.76±0.11,4.22±0.56% were observed in favour of site-1, site-2, site-3 respectively. Saponinand alkaloid content are present in smaller amounts compared to tannins and phenols. Flavonoids and phenolics are notably high and also abundant can contribute to antioxidant and antidiabetic activity.Alkaloids are present in moderate amounts and also contribute to bioactivity butphenols have strong antioxidant activity; varies with solvent. Site-1 has highest levels of all measured phytochemicals then site-2 and site-3.The order of phytochemical content of bark extracts from three different study sites observed as flavonoid > Phenol>Tannins>Saponins>Alkaloids.

Table:1. Bacterial strains used in the present study.

Sl.No	Bacterial strain	Gram (+/-)
1	*Staphylococcus aureus*	+
2	*Escherichia coli*	-
3	*Streptococcus*	+
4	*Pseudomonas aeruginosa*	-
5	*Enterococcus facalis*	+
6	*Micrococcus luteus*	+
7	*Salmonella typhimurium*	-
8	*Bacillus subtilis*	+

Table 2: Phytochemicalanalysis of *P. marsupium*bark extracts from different sites of Barnawapara with (mean and ±SD)

Phytochemicals	Site-1	Site-2	Site-3
Alkaloids(%)	5.51±0.12	3.76±0.11	4.22±0.56
Flavonoids (%)	20.51±0.05	16.40±0.04	18.01±0.42
Tannins (%)	8.75±0.11	7.23±0.89	6.33±0.43
Saponins (%)	7.25±0.13	5.32±0.67	5.01±0.53
Phenols (%)	19.37±0.03	14.17±0.76	17.41±0.78

The different solvents such as Hexane, Ethyl acetate, Methanol, Aqueous and Chloroform were used for phytochemical analysisof*P. marsupium*extracts and tested against the pathogenic bacteria *S. aureus, E. coli, St.*

mitis,P. aeruginosa,E. faecalis, M. luteus,S. typhimurium and *B. subtilis*data represented as (Table-3)and observed that Maximum zone of inhibition recorded against *P. aeruginosa*extraction by the highest order of chloroform,aqueous,methanol,ethyl acetateand thenhexane respectively. The minimum zone of inhibition observed against *S.typhimurium* extractionbyEthyl acetate extraction. Similarly, extraction by Chloroform showed maximum zone of inhibition against *P. aeruginosa* and *E. coli.*The tested substance exhibited significant inhibitory effects on the antimicrobial activity andzones of inhibition against *S. aureus*ranged from 15.86 to 22.50 mm, *E. coli*from 21.61 to 25.11 mm, *St. mitis*from 12.10 to 18 mm, *P. aeruginosa*from 20.50 to 27.89 mm, *E. faecalis*from 12.20 to 20 .80 mm, *M. luteus*from 10 to 17.45 mm, *S. typhimurium*from 8.67 to 20.10 mm, and *B. subtilis*from 11 to 19.80 mm respectively.Antimicrobial activity by agar well diffusion method indicated the zone of inhibition which ranges from 8-27 mm for bark extracts.*Pterocarpus marsupium* exhibited antimicrobial activity with different extracts showed varying degrees of effectiveness against different bacteria.

Table 3.: Antimicrobial Activity of *P. marsupium* in terms of diameter of zone of inhibition in mm (Mean±S.D.)

	Zone of Inhibition in mm							
Solvents	*S. aureus*	*E. coli*	*St. mitis*	*P. aeruginosa*	*E. faecalis*	*M. luteus*	*S.typhimurium*	*B. subtilis*
Hexane	7.67±0.52	22.18±0.81	17.66±0.20	20.50±0.58	20.80±1.15	17.26±0.30	20.10±0.14	19.80±0.28
Ethyl acetate	2.50±0.546	21.61±0.34	12.10±0.14	25.82±0.85	12.20±0.18	17.45±0.63	8.00±0.45	11.00±0.08
Methanol	5.86±0.710	22.58±0.67	16.26±0.30	25.83 ± 0.21	15.06±0.34	11.70±0.42	13.10±0.14	12.43±0.56
Aqueous	8.50±0.233	24.64±0.01	13.36±0.46	26.77 ± 0.50	19.50±0.74	10.00±0.09	15.95±0.70	17.66±0.20
Chloroform	8.60±0.111	25.11 ± 0.6	18.00±0.58	27.00± 0.50	12.64±0.28	14.33±0.20	18.00±0.14	12.10±0.14

The data of minimum inhibitory concentration (MIC) of hexane, ethyl acetate and chloroform weredeterminedbyusingDMSOasnegativecontrolandampicillinas positive control represented as(Table-4). The minimum inhibitory concentration (MIC) against different pathogenic bacteria ranged from 45 to 66 µg/ml against hexane and chloroform but ethylacetate extracts are also found to be more active towards the organisms tested than hexane extract.Further evaluation of test samples for minimum inhibitory concentration (MIC) showed that the chloroform solvent had exhibited significant activity by inhibiting *S.typhi*, *E. coli, S mitis,P.aeruginosa*at 62 µg/ml,64µg/ml,66µg/ml,63µg/ml, respectively.The bark extract showed theleast minimum inhibitory concentration of 63 µg/ml against *S. aureus* and *P. aeruginosa*, 64 µg/ml against *E. coli and M. luteus,*64µg/ml, against *E. faecalis*45 µg/ml, against *S. typhimurium* 62 µg/mland59 µg/ml against *B. subtilis* describes the MIC of test bacteria by agar well diffusionmethod (Table-4).

Table 4: Antibacterialactivityand MIC of *P. marsupium* bark extract wasexpressed intermsofdiameter ofzoneofinhibition(Mean±S.D)

S. No.	**Bacteria isolates**	**Solvent**	**Zone of inhibition**	**MIC**	
				Bark extract (µg/ml)	**Ampicillin (µg/ml)**
1	*S. aureus*	Ethyl acetate	29.18±0.16	63	10
2	*E. coli*	Chloroform	22.19±0.76	64	25

3	*St. mitis*	Chloroform	27.01±0.21	66	35
4	*P. aeruginosa*	Chloroform	28.33±0.84	63	25
5	*E. faecalis*	Hexane	24.70±0.92	45	15
6	*M. luteus*	Ethyl acetate	26.33±0.24	64	20
7	*S. typhimurium*	Chloroform	25.25±0.20	62	15
8	*B. subtilis*	Hexane	28.46±0.14	59	25

Discussion

Phytochemical Analysis

The results of phytochemical analysis of *P. marsupium*bark extracts,revealed that the presence of various constituents differs from one site to other. Alkaloids ranged from 3.76±0.11 to 5.51± 0.12 % with standard deviation. While these phytochemicals are present in *P. marsupium*, their concentrations vary based on factors like plant part, extraction method, and environmental conditions. This finding is consistent with the work of Gupta et al.,(2017), who also reported the presence of alkaloids in *P. marsupium* bark. flavonoids and saponins were found to be present in concentrations ranged from 16.40±0.04 to 20.51±0.05% and 5.01±0.53 to 7.25 ±0.13% with standard deviation fromsite-1 to site-3.These phytochemical compounds, which are associated with beneficial health effects and supported by (Sharma et al.,2017 and Grover et al., 2002).Antioxidants are molecules that inhibit the oxidation of other molecules in living organisms.Tannins and phenols were observed in the range of 6.33±0.43%of site-3 to 8.75±0.011% of site-1and.14.17±0.76% of site-2 to 19.37±0.03% with standard deviation of site -1. Similarly, the presence of percentage of flavonoids, alkaloidsand phenolsin *P. marsupium* bark extract reportedranged from 6.32-20%.25,0.04-18.70%,12.80-19.26% has been elucidated by various workers (Tiwari and Rao,2002;Sudharameshwari, 2007 and Ramya et al.,2008).

Antimicrobial Activity

In this study, ethanolic extract of *P. marsupium*possesses antibacterial activity against various bacterial strains and shows a significant difference in results observed between the gram-positive and gram-negative bacteria. The antimicrobial activity of *P. marsupium*bark extracts was assessed against eight pathogens: *Staphylococcus aureus*, *Escherichia coli,St.mitis, P aeruginosa,E faecalis,M luteus,S typhimurium* and *B. subtilis* andthe zone of inhibition against these bacteria ranged from 15.85 to 22.50 mm,21,61 to 25.11mm,12.10 to 18.00 mm,20.5 to 27.89mm,12.20 to 20.80 mm,10.00 to 17.45 mm ,8.67 to 20.10mm,11.00 to 19.80 mm respectively.Similar to our previous study qualitative antioxidant activity has been done to identify the extract which is having the best antioxidant activity among all the five extracts (Nithya et al., 2016).Similarly methanol extract of *P. marsupium* (bark)showed maximum activity against *Pseudomonas aeruginosa.*Similar observations have been reported by (Nair et al., 2005; Sambathkumar et al., 2006) where it has been shownthat ethanol extracts of *P. marsupium* exhibited significant anti-ulcer and antioxidant properties
Results parallel to our study have also been reported by (Londonkarand Hugar2017;Padhi et al.,2011).

Ethyl acetate and methanol extracts were more sensitive to the bacteria than extracts made out of hexane.Both the extracts exhibited concentration dependent variation in their anti-bacterial activity. Hexane moderatelyinhibited growth of the bacterial strains tested. All fractions showed a promising activity towards Gram negativebacteria however low inhibition was observed in hexane fractions of *P. marsupium* towards *faecalis*species. Our plant extract was observed with more activity against positive strains than negative strains and the same kind of results were previously reported (Bhatiaet al., 2017 and Pant et al.,2017). The highest zone of inhibitory was observed inGram-positiveand lowest was in the gram-negative tested group with the concentration of 10 mg/ml of bark extract.The results zone of inhibition corroborated byPushpangadan et al., (1997). The minimum inhibitory concentration (MIC) against *P. marsupium* bark extract ranged from 45 to 66 µg/ml and similar results reported by Pradhan et al., (2011).

Conclusion

In conclusion, the findings suggest that, the bark extracts of *Pterocarpus marsupium* exhibits notable antimicrobial properties alongside a range of beneficial phytochemicals. The presence of alkaloids, flavonoids, tannins, phenols, and saponins may contribute to its therapeutic potential. These results support the traditional use of *P. marsupium* in herbal medicine and highlight its potential as a source of natural antimicrobial agents. These attributes underscore its potential as a natural source of therapeutic agents, particularly in combating microbial infections. The study suggests that *Pterocarpus marsupium* contains a rich array of phytochemicals with promising antibacterial properties and encourage further investigation into its bioactive components and mechanisms of action.

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