

Original Research Article

***Pterocarpus indicus* Willd : A lesser known tree species of medicinal importance**

ABSTRACT

Barks of *Pterocarpus indicus* were extracted with methanol and acetone and obtained the yield of 35.5 mg/g in methanol and 27 mg/g in acetone. The terpenoids content was found to be maximum of 1.78 mg/g and the steroids are the second major compound in the bark with 1.404 mg/gm in methanol extract. GC-MS analysis was on the identification of the phytochemicals present in the methanol extract of *P. indicus* which showed secondary metabolites such as Campesterol, Cyclopropane, 2,6-bis(1,1-dimethylethyl)-4-methyl with promising antibacterial and antifungal activities, the other secondary metabolites like Halfordinol and Butylated hydroxytoluene with high antioxidant property. The antibacterial activity of the methanol and acetone extracts of *P.indicus* were tested against various human pathogens 2 gram positive bacteria *Bacillus subtilus* (Bs) *Staphylococcus aureus* (Sa) and 1 gram negative bacteria *Escherichia coli* (Ec). The methanol extract gave positive result than the acetone extract. The bark extract was used against the fungus *Nigrospora oryzae* which showed positive result by inhibiting the growth of the organism. Therefore it is evident from the present study that commercial utilisation of *P. indicus* could be a potential source of medicinal tree for the treatment of various infectious diseases.

Key word: *Pterocarpus indicus*, medicinal tree, infectious diseases

1. INTRODUCTION

Various therapeutic studies on sub species of *Pterocarpus* plants that reviewed for the studies as *Pterocarpus marsupium* showed that plant have various potential of medicinal values. *P. marsupium* extracts was used to analysis the activity of antidiabetic property that showed high level response against diabetic problem (Grover *et al.*, 2002a, b). The phenolic constituents of the heartwood of *P. marsupium* activity of antidiabetic that demonstrated by Manickam *et al.* (1997) in rat model induced by streptozotocin and significantly lowered the blood glucose level of hyperglycemic rats. The medicinal property of *Pterocarpus* species to treat the hyperglycaemic with the extract of leaf, stem and bark that tested to antihyperglycemic activity that showed in high level biological properties of anti-hyperinsulinaemic in *P. marsupium* (Jahromi & Ray, 1993). *Pterocarpus indicus* Willd is also one of the medicinally important tree species found in India. It is common in Philippines, decoctions of the various parts of the tree find applications in common diseases like boils, ulcers, prickly heat, stone in the bladder, diarrhoea, dysentery, thrush and syphilitic sores (Quisumbing, 1978). The root extract has been used to treat syphilitic sores and mouth ulcers. The young leaves have been used as treatment for boils, ulcers and prickly heat rashes (Thomson, 2006). Since there is paucity of information on the species the present study has been made to extract and characterize bio active compounds from bark of *P. indicus* in order to ascertain its medicinal properties.

2. Materials and methods

Comment [R1]: The literature review of the manuscript is out of date. I suggest that the authors look for more recent articles on the proposed theme.

2.1 Sample collection

The bark samples of *P. indicus* Willd were collected from Saibaba colony, Coimbatore, Tamil Nadu, India situated between latitude 11° 1' 25.01" N and longitude 76° 56' 30.96"E. The collected plant materials were authenticated by a taxonomist at Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore. The collected samples were brought to the phytochemistry laboratory, IFGTB, Coimbatore (Fig.1). The barks were shade dried, cleaned thoroughly with a brush to remove dust and debris, followed by stored in tightly closed containers till extraction at room temperature.

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2.2 Extraction

The dried barks were making into coarse powder using mechanical grinder. About 20 grams of dry powder was extracted with the organic solvents such as methanol and acetone (50- 60°C) by hot continuous percolation using soxhlet apparatus. The extractions were continued for 48 hours. The extracts have been recovered from the solvents by evaporation process by using rotary evaporator; the crude extracts thus obtained were stored in sterilized amber coloured bottles maintained at 4°C in a refrigerator till further analysis. The recovered extracts were weighed after the complete evaporation of the solvents to calculate extract yield in percentage.

Comment [R3]: What was the volume of each solvent used for extraction?

Calculation:

$$\text{Percentage of extract (\%)} = \{(Y_g - X_g) / W_{gs}\} \times 10 \text{ Where,}$$

Yg = Weight of extract with beaker, Xg = Empty weight of beaker,
Wg = Weight of Bark.

2.3 Phytochemical Screening

Phytochemical constituents such as alkaloids, flavanoids, tannin, saponin, quinone, sterols and phenols etc., of bark extract of *P. indicus* using methanol and acetone was made standard procedure. The separation and characterization of active compounds in *P.indicus* extract was also carry out using GC-MS analysis.

Comment [R4]: Describe the methodology used to analyse each group of secondary metabolites.

2.4 Gas chromatography and Mass Spectroscopy analysis of methanol and acetone extract of *P. indicus*.

The samples were dissolved in the respective organic solvents (methanol and acetone), till dissolved completely and analyzed by GC-MS (Thermo GC- Trace Ultra Version 5.0). For GC-MS analysis, a 30 m×0.25 m MS capillary standard non polar column with a film thickness of 0.25 µm was used. The carrier gas was helium maintained at a column flow of 1 ml/min. A 1.0 µl sample of the extract was injected and the column temperature was maintained at 70°C /min to 260°C for 6 min. This was raised to 260 °C at a rate of 6°C min for x min, and finally to 300°C at a rate of 35°C /min for 2 min. The individual constituents showed by GC were identified by comparing their MS with standard compound of NIST library.

2.5 Antimicrobial activity: Agar well diffusion method

The effect of extracts on the several microbial strains was assayed by agar well diffusion method. The

extracts were allowed to diffuse out into the medium already seeded with test organisms. The diameter of the zone of inhibition was measured in millimetres (mm), which showed the activity of extracts against the human pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Nigrospora oryzae* (Ahmed *et al.*, 2015). The pure bacterial cultures were maintained on nutrient agar medium and stored at 4 °C for further use.

2.6 Media preparation

The growth media employed in the present study were nutrient agar and Muller Hinton agar. Nutrient broth was composed of Beef extract 3.0 g, Peptone 5.0 g and Distilled water 1000 ml (pH 7.4). Muller Hinton agar was composed of beef, infusion 300 g, Casein acid hydrolysate 17.50 g, Starch 1.50 g, Agar 17 g and distilled water 1000 ml (pH 7.3), sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. All glassware was sterilized in hot air oven at 160°C for 1 hour prior to use. The growth media for the fungal culture was used with Potato Dextrose Agar.

2.7 Procedure

Autoclaved molten Muller Hinton agar medium were poured in sterilized petri plates and allowed to solidify. Test organisms were inoculated in sterilized nutrient broth and incubated at 37°C for 24 hrs. Petri plates containing Muller Hinton medium were swabbed with 24 hrs microbial culture using sterile cotton swabs.

Wells of 5 mm diameter were made on Muller Hinton Agar plates using cork borer. Using a micro pipette, 20 µl of each plant extract (separately for methanol and acetone extract) was added on to each well on all plates. Amoxicillin solution 1mg/ml used as the positive control. Kept for incubation at 37°C for 24 hrs. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

3. Results and discussion

P. indicus Willd is commonly known as Rose wood plant (Narra) in English. It is one of the most promising multipurpose tree species in the Pacific islands for reforestation, village-level woodlots, living fencing, and large amenity trees. Traditionally is one of the most important multipurpose trees for timber and medicine (Thomson, 2006). The useful parts of the tree are its leaf, stem, bark and root. Folklore states that it promotes digestive power, improve health condition, and useful for alteration of fever, diarrhoea, dysentery and heavy menstruation. Thus the effective utilization of *P. indicus* with respect to medicinal values is warranted; hence the present study has been conducted to study the active compounds present in the bark extract and their biological activities of the extracts.



Comment [R5]: What is the concentration in mg of each plant extract used for antimicrobial activity?

Comment [R6]: This is introduction, it does not fit the results and discussion.

Fig. 1 Barks of *Pterocarpus indicus*

Plant materials contain various solute molecules with more than one functional group. Therefore, it is difficult to predict the solubility of solutes in a particular solvent. An alternative way of considering solubility is to use the concept of polarity. The yield of extracts for sample materials using different polar organic solvents is given in (Table 1). The bark of *P. indicus* extracted with two different polar (methanol and acetone) solvents showed higher yield in methanol with 0.710 mg and in acetone 0.540 mg. The data search in relation to extract yield are similar with earlier observation made in a study on high yield of extract obtained by using methanol as solvent for extraction (Noufou *et al.*, 2017). Similarly, previous study reported that the yield of hot extract was high in dried bark material of *P. soyauxii* (Tchamadeu *et al.*, 2011).

Comment [R7]: Insert quotes from other authors to support these affirmations.

Table 1: Methanol and Acetone extracts of *P. indicus* (Willd) using Soxhlet apparatus

Plant sample	Weight of extract (mg/20g)	
	Methanol	Acetone
<i>Pterocarpus indicus</i> (Willd) bark sample	0.710±0.026	0.540±0.022

3.1 Phytochemical analysis

Phytochemicals are secondary metabolites produced by all plants. The preliminary phytochemical screening of the extracts of *P. indicus* revealed the presence of various chemical substances such as alkaloids (Kesari *et al.*, 2004); flavonoids, tannins, phenols, terpenoids, sterols quinines, protein, steroids, anthocyanin, carbohydrate (Manickam *et al.*, 1997) and stigma sterol (Noufou *et al.*, 2017). Phytochemical constituents such as flavonoids, tannins, saponins, phenols, steroids, and terpenoids were present in both methanol and acetone bark extracts (Table 2) and alkaloids, quinines, anthocyanin, protein, sigma sterol and carbohydrates were found absent. To determine the chemical constituents, qualitative phytochemical screening of the aqueous stem bark extract of *P. soyauxii* was carried out with various standard procedures routinely used in the laboratory (Tchamadeu *et al.*, 2010); the usefulness of plant materials medicinally is due to the presence of bioactive constituents such as saponins, tannins, flavonoids, steroids, terpenoids and phenolic compounds. The presence of wide range of phytochemical constituent indicated that the plant could be used in a multiple of ways which may useful to the medicinal purpose (Kesari *et al.*, 2004).

3.2 Quantitative analysis of secondary metabolites

Chemical investigation of the genus, *Pterocarpus* woods started more than 100 years ago, yet new compounds are still being discovered. There are a variety of compounds with different carbon skeletons, some of which have been considered unique to the genus. A broad classification of these components is given below along with the special features of each group (Seshadri and Vydeeswaran 1971). The plant *Pterocarpus* (Fabaceae) is important plant that contains various phytoconstituents and used traditionally for medicinal purpose such as protein, pterostilbene, epicatechin, pterosupin, maruspsin and five new flavonoids (Gairolaseema *et al.*, 2010).

The results showed the presence of high quantity of terpenoids and steroids and the other

phytoconstituents such as the phenols, flavonoids, tannins and saponins were present in low quantity in both extracts (Fig 2.). A red, gum-like resin from the bark is used in folk remedies for tumours and the leaf for cancers, especially the mouth cancer and the leaves significantly inhibited the growth of Ehrlich as cites carcinoma cell in mice (Orwa *et al.*, 2009). Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plants and the structural analysis shows that the crystal is a macromolecular compound of tannic condensation and glucoside (Wang *et al.*, 1997). A mixture of loliolide (> 85%) and paniculatadiol (< 15%) was obtained from the ethyl acetate leaf extract of *P. indicus*. Isoflavonoids were the earliest compounds to be studied and are major components in the genus. They can be subdivided into three sub-groups; pterocarpan, isoflavones and deoxybenzoins (Seshadri and Vydeeswaran 1971).

Table 2: Phytochemical screening of secondary metabolites in the bark extracts of *P. indicus*

S. No	Phytochemical test	Methanol extract	Acetone extract
01.	Test for Alkaloids (Wagner's test)	-	-
02.	Test for Flavonoids (Sulphuric acid (H ₂ SO ₄) test)	+	+
03.	Test for Tannins (Braymer's test)	+	+
04.	Test for Saponins	+	+
05.	Test for Quinines	-	-
06.	Test for Sterols (Sulphuric acid (H ₂ SO ₄) test)	-	-
07.	Test for Phenols (Ferric chloride test)	+	+
08.	Test for proteins (Ninhydrin(acetone))	-	-
09.	Test for Carbohydrates (Fehling's test)	-	-
10.	Test for Terpenoids	+	+
11.	Test for Steroids	+	+
12	Test for Anthocyanin Sodium hydroxide (NaOH) test	-	-

(+): Present, (-): Absent

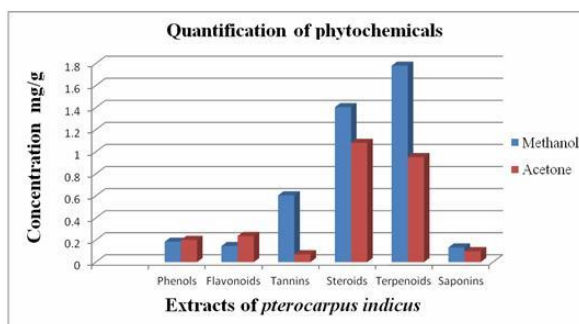


Fig. 2 Estimation of phytochemicals in the two different extracts of *P. indicus*

3.3 GC-MS analysis of *P. indicus* (Willd)

The Gas chromatography and mass spectrometry analysis of methanol and acetone extract of bark of *P. indicus* elicited 60 individual compounds (Fig. 3) and (Fig. 4). All identified individual compounds were assessed for their biological properties using physico-chemical property calculations according to Tice Rules. As per Tice rule compounds are more likely to have properties of antibacterial, antifungal, anti-inflammatory, anti-cancerous, antitumor, antioxidants, antiprotozoal, anti-diabetic, antiallergenic, antiviral, insecticide, germicide and anti-toxic activities. If molecular weight is within ≥ 150 and ≤ 500 ; theoretical logarithm of the octanol/water partition coefficient (log P), is less than or equal to 5.0; hydrogen bond acceptor is within 1-8; hydrogen bond donor is less than or equal to 2 and the number of rotatable bond is less than or equal to 12 (Table 3) and (Table 4). Noufou *et al.*, (2017) reported that DPPH and CUPRAC methods were used to determine the antioxidant capacity of MeOH extract, ethyl acetate and butanol fractions from *Pterocarpus erinaceus* roots. The results of above methods, Methanol extract and fractions had the same antioxidant activity; however the reference compounds (trolox, rutin, chlorogenic acid) had higher activity. Free radical production is necessary during body aggression by pathogens, because free radicals are involved in defensive system against pathogens aggression; but their excessive production can cause cell damages and oxidative stress. Free radical-mediated oxidative stress in inflammatory diseases including cancer, diabetes, arthritis, infections, alzheimer and atherosclerosis, has been well documented (Oliveira *et al.*, 2014) and the antioxidant power of extract shows that extract and fractions may inhibit free radical production. The structure was elucidated based on spectroscopy data of UV, LC-MS and FT-IR. Antioxidant was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. The isolation and identification led a stigma sterol as Compound and a new flavonol - glycoside [(2R)-7-hydroxy-3-(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy]-2-(3,4,5-trihydroxyphenyl) chroman-4-one] or ptevon-3-*D*- glycoside as Compound Antioxidant activity of Compound showed IC₅₀ for 18.53 μ ml and blank of quercetin was 7.94 μ ml and Vitamin C was 40.25 μ ml. These compounds and antioxidant activities are the first time reported from this plant (Sri Hartati *et al.*, 2016). Aqueous extract of the heartwood of *Pterocarpus marsupium* contains flavonoids C- glucosides namely 6 - hydroxyl - 2, 4 - hydroxybenzyl - benzofuran - 7C - β - D - glucopyranoside, 3 α - methoxy - 4 - hydroxybenzylidene - 6 - hydroxybenzo - 2(3H) - furanone - 7C - β - D - glucopyranoside, 2 glucopyranoside, 8 C - β - D - glucopyranosyl - 7, 3, 4-trihydroxyflavone and 1, 2 - bis (2, 4 - dihydroxy, 3 - C glucopyranosyl) - ethanedione and two known compounds C- β -D-glucopyranosyl-2,6-dihydroxyl benzene and sesquiterpene were isolated reported (Shah Alam *et al.*, 2015).

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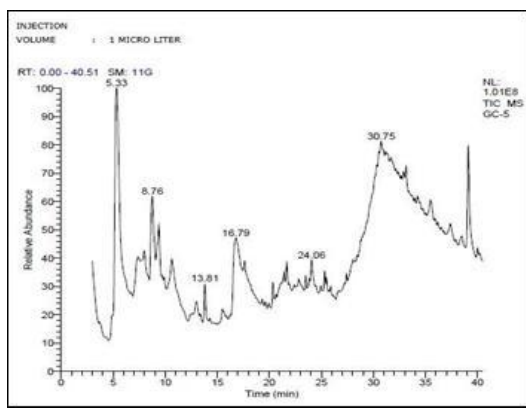


Fig. 3 GC-MS Chromatogram of methanol extract of *P. indicus*

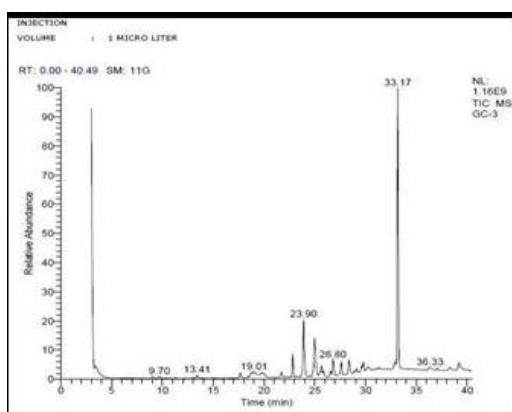


Fig.4 GC-MS Chromatogram of acetone extract of *P. indicus*

Table 3: GC-MS analysis of methanol extract of bark of *P. indicus*

S. No	RT (min)	Compound Name	Molecular Formula	Trivial Name	Group	Area (%)	Biological Importance
01	6.76	Dodecane	C ₁₂ H ₂₆	Dihexyl	Alkane	0.62	Antifungal activity
02	13.55	17-Pentatriacontene	C ₃₅ H ₇₀		Alkane	0.23	No activity
03	17.8	1-Tricosanol	C ₂₃ H ₄₈ O	Campesterol	Alcohol	0.9	Antibacterial fungalactivity
04	19.27	1-[Bis(methylthio)methylene]acetyl]-2-(4-(4-methoxyphenyl)-1,3-butadienyl)cyclopropane	C ₁₉ H ₂₂ O ₂ S ₂	Cyclopropan e	Alkane	0.31	Antimicrobial activity
05	21.85	14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	Isopropyl myristate	Ester	0.5	Antioxidant, antimicrobial
06	22.98	1-ethoxycarbonyl-4,5-di(hydroxydimethylsilyl)-1H-azepine	C ₁₃ H ₂₅ NO ₄ S ₂		Amine	0.34	No activity

07	23.86	2,6-bis(1,1-dimethylethyl)-4-methyl	C15H24O	Butylated hydroxytoluene	Phenol	0.69	Antioxidant, Antimicrobial
08	25.59	methyl 9,9-dideutero-octadecanoate	C19H36D2O2		Ethyl ester	2.41	Antibacterial , Antifungal activity
09	26.65	4-Normethyl-9,19-cyclolanoststan-7-one,3-acetoxy	C31H50O3	Momordicin	polyphenol	0.33	No activity
10	27.22	4-Normethyl-9,19-cyclolanoststan-7-one, 3s-acetoxy	C31H50O3	Momordicin	Polyphenol	0.27	No activity
11	27.67	4-Normethyl-9,19-cyclolanoststan-7-one,3-acetoxy	C31H50O3	Momordicin	Polyphenol	0.39	No activity
12	28.1	4-Normethyl-9,19-cyclolanoststan-7-one, 3-acetoxy-	C31H50O3	Momordicin	polyphenol	0.34	No activity
13	28.45	Cholest-2-eno[2,3-b]naphthalene	C35H50		Ketone	0.36	No activity
14	28.77	Cholestano[7,8-a]cyclobutane,3- methoxy-6-oxo-2'-methylene	C31H50O2	Sigmasterol acetate	Ketone	0.32	Antimicrobial, Antitumour, Antiinflammatory
15	29.06	Cholestano[7,8-a]cyclobutane,3- methoxy-6-oxo-2'-methylene	C31H50O2	Sigmasterol acetate	Ketone	0.32	Antimicrobial, Antitumour, Antiinflammatory
16	30.1	Cholestano[7,8-a]cyclobutane,3- methoxy-6-oxo-2'-methylene	C31H50O2	Sigmasterol acetate	Ketone	0.31	Antimicrobial, Antitumour, Antiinflammatory
17	30.32	Cholestano[7,8-a]cyclobutane,3- methoxy-6-oxo-2'-methylene	C31H50O2	Sigmasterol acetate	Ketone	0.2	Antimicrobial, Antitumour, Antiinflammatory
18	30.75	17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17,-dodecahydro-1H-cyclopenta(a)phenanthren-3-ol	C29H50O	Prostasal	Alcohol	7.77	Antimicrobial, Antitumour, Antiinflammatory
19	31.2	Cholestano[7,8-a]cyclobutane,3- methoxy-6-oxo-2'-methylene	C31H50O2	Sigmasterol acetate	Hydroxyl	0.24	Antimicrobial, Antitumour, Antiinflammatory
20	31.62	5Alpha-cyano-3alpha-formyl-3beta-methylcholestane	C30H49NO		Sterol, Alkane	1.5	Antiinflammatory Antidiabetic
21	32.42	Phenol, 4-[2-(3-pyridinyl)-5-oxazolyl]-	C14H10N2O2	Halfordinol	Pyridine heterocyclic	2.5	Antioxidant Antimicrobial
22	33.15	1,2-Benzenedi carboxylic acid, bis (2-ethylhexyl) ester	C24H38O4	Dioclyltetra phthalate	Carboxylic acid, Ester	2.85	Oral toxicity during pragnancy and suckling in the long - evans rats
23	33.72	Cholestano[7,8-a]cyclobutane,3- methoxy-	C31H50O2	Sigmasterol	Ketone	0.22	Anticancer, Anti protozoal,

		6-oxo-2'-methylene		acetate			antimicrobial, antiinflammatory
24	33.99	Cholestano[7,8-a]cyclobutane,3-methoxy-6-oxo-2'-methylene	C ₃₁ H ₅₀ O ₂	Sigmasterol acetate	Ketone	0.21	Anticancer, Anti protozoal, Antimicrobial, Antiinflammatory
25	34.56	Cholestano[7,8-a]cyclobutane,3-methoxy-6-oxo-2'-methylene	C ₃₁ H ₅₀ O ₂	Sigmasterol acetate	Ketone		Antimicrobial, Antitumor
26	35.58	Cholestano[7,8-a]cyclobutane,3-Methoxy-6-oxo-2'-methylene	C ₃₁ H ₅₀ O ₂	Sigmasterol acetate	Ketone	2.53	Anticancer, Antiprotozoal, Antimicrobial, Antiinflammatory
27	37.15	1-Phenanthrene carboxaldehyde,7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl-,[1R-(1a,4aa,4ba,7a,10aa)]-	C ₂₀ H ₃₀ O	Ferruginol	Anthracene	60.3	
28	38.41	(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17,-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	C ₂₉ H ₄₈ O	Sigmasterol	Alcohol	5.37	Antimicrobial, Antioxidant
29	39.33	Methyl hexacosanoate	C ₂₇ H ₅₄ O ₂	Cerotic acid	Ester	1.86	
30	40.02	(3S,10S,13R,14R,17R)-17-[(E,2R,5R)-5,6-Dimethylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,12,14,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-3-ol	C ₂₈ H ₄₂ O	Dehydroergosterol	Cholesterol	0.3	Antiallergy, Antiinflammatory

Table 4: GC-MS analysis of acetone extract of bark of *P. indicus*

S. No	RT (min)	Compound Name	Molecular Formula	Trivial Name	Group	Area (%)	Biological Importance
01	3.41	2-Pentanone,4-hydroxy-4-methyl	C ₆ H ₁₂ O ₂	Butyl acetate	Ester	1.34	Antibacterial activity
02	9.02	Azulene	C ₁₀ H ₈	Naphthalene	Benzene derivative	0.47	Antibacterial activity
03	9.7	1-tetradecene	C ₁₄ H ₂₈	Tetradecene	Alkene	0.41	Antimicrobial, antioxidant
04	11.22	7-Methoxychromone-2-carbonitrile	C ₁₁ H ₇ NO ₃	Benzonitrile	Cyanide	0.21	Antibacterial, antiviral activity
05	13.06	2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethylethyl)	C ₁₄ H ₂₀ O ₂	Butibufen	Ester	0.27	Antioxidant, Antibacterial activity

6	13.41	Hexadecane	C16H32	Cetene	Alkene	0.95	Antibacterial activity
7	14.9	2,3-dihydro-1H-cyclopent[e]azulene	C13H12	Diphenylmethane	Alkane	0.21	Antimicrobial, Antiinflammatory activity
8	17.66	(E)-heptadec-15-enal	C17H32O	E-15-Heptadecenal	Aldehyde	1.2	Antioxidant, Antibacterial
9	18.8	1-(4-Methoxyphenyl)-3-methylazetidin-2-one	C11H13NO2	Fenmetramide	Amide	2.27	Antibacterial, Antiinflammatory, Antifungal activity
10	19.84	(4-(2,4-dimethylheptan-3yl)phenol)	C15H24O	Butylatedhydroxytoluene	Phenol	1.45	Antioxidant activity
11	21.73	Cycloicosane	C20H40	Cetyl ethylene	Alkene	1	
12	22.52	N,N-Diethyl-3,4-methylenedioxybenzamide	C12H15NO3	Beta-keto-Methylbenzodioxolobutanamine	Amine	0.22	Anticancer
13	22.83	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl) ester	C16H22O4	Cholesterol	Sterol	3.41	Anticancer, Antimicrobial Antiinflammatory
14	23.21	Phthalic acid,isobutylpropyl ester	C15H20O4	phthalic acid	Ester	0.24	Antimicrobial, Antimicrobial
15	23.9	1,2-Benzene dicarboxylic acid,bis(2-methylpropyl) ester	C16H22O4		Ester	11.56	Antibacterial
16	24.96	Dibutyl phthalate	C16H22O4	n-butyl phthalate	Ester	9.3	Antimicrobial
17	25.67	9,9-Dimethyl-8,10-dioxapentacyclo[5.3.0.0(2,5).0(3,5).0(3,6)]decane	C10H14O2		Alkane	3.17	
18	26.8	Phthalic acid, butyl 3-methylbutyl ester	C17H24O4	3-methylbutyl benzoate	Ester	4.23	Anticancer
19	27.61	Phthalic acid,3-methylbutyl pentyl ester	C18H26O4	Isopentyl phthalate	Ester	2.53	Anticancer, antioxidant
20	28.36	Phthalic acid, 3-methylbutyl pentyl ester	C18H26O4	Isopentyl phthalate	Ester	2.98	Anticancer
21	29.12	Phthalic acid, di(2-methylbutyl) ester	C18H26O4	Diisopentyl phthalate	Ester	1.02	Anticancer
22	29.77	Phthalic acid, bis(2-pentyl) ester	C18H26O4	Diisopentyl phthalate	Ester	2.38	Anticancer
23	30.26	1,30-Triacontanediol	C30H62O2	Tricontane- 130-diol	Alcohol	1.3	Anticancer
24	31.36	Phthalic acid, 2-cyclohexylethyl isobutyl ester	C20H28O4	Carnosic acid	Ester	0.53	Antimicrobial, antiviral, Antioxidant
25	33.17	Di-(2-ethylhexyl) phthalate	C24H38O4	Dicoctyleterephthalate	Ester	43.2	Antitoxic activity
26	34.5	Synaptogenin b	C30H46O4	Glycyrrhetic acid	Carboxylic acid	0.22	Antiallergic, antibacterial, antiviral activity
27	36.33	1,3-Dithiane, 2-phenyl	C10H12S2	Acetophenone ethane	Ester	0.82	

28	37.06	Decanedioic acid, bis(2-ethylhexyl) ester	C ₂₆ H ₅₀ O ₄	di-(2-ethylhexyl)sebacate	Ester	0.34	
29	38.27	Cyclooctacosane	C ₂₈ H ₅₆	1-Octacosene	Alkene	0.96	
30	39.18	13-Docosenamide	C ₂₂ H ₄₃ NO	Erucylamide	Amide	1.83	Germicide, insecticide activity

In this present study the compounds identified are 1-Tricosanol, Dodecane, 1-[Bis(methylthio)methylene]acetyl]-2-(4-(4-methoxyphenyl)-1,3-butadienyl) (Cyclopropane), 14-methyl-, methyl ester, 2,6-bis(1,1-dimethylethyl)-4-methyl, methyl 9,9-dideutero-octadecanoate have antimicrobial and antifungal properties and the other compounds like Cholestano [7,8-*a*]cyclobutane,3-methoxy-6-oxo-2'-methylene- (Sigma sterol acetate), 3-methylbutyl benzoate (Phthalic acid), Isopentyl phthalate are contain the property of anticancer and the antitumor activity the compounds such as Halfordinols and Butylatedhydroxytoluene have contain strong antioxidant property. GC-MS analysis of *P. indicus* of bark extract revealed the presence of secondary metabolites of anticancerous, antibacterial, antifungal, antidiabetic, anti-inflammatory, antitoxic, immunomodulatory, antioxidants and insecticidal properties and hence it can be used in pharmacology industry as an efficient novel drug. As rich source of phytochemicals *P. indicus* bark could be a potential source of useful drugs.

Comment [R9]: Authors should write the results of the work based on the research data, as only antimicrobial activity was evaluated. To suggest that the plant can be used as a new efficient drug is rash information.

3.4 Antibacterial activity of *P.indicus* (Willd)

The antibacterial activity of plant products such as various plant extracts have been reported by different researchers and gaining due attention as they are environmentally safe a non-toxic dye as an antibacterial component in shampoos (Hatinguais *et al.*, 1981), and also the compound loliolide with potential antimicrobial activity was observed from. (Hofilena and Ragasa, 2002). The related plants from the same genus was studied for antibacterial activity against pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* showed the inhibition of bacterial growth and consist of great antibacterial activity. The Phyto compounds Lupeol 3 and Phytol esters were identified from the air-dried flowers of the *P. Indicus* plant extract that antimicrobial activity with test high percentage (Ragasa, *et al.*, 2014). The antimicrobial activity demonstrated by Jain *et. al.*, (2011) on ethanolic heartwood extract of *P. marsupium* (EPPM) was evaluated for an anti-diarrheal activity using castor oil and charcoal induced gastrointestinal motility test in rats. EPPM at a dose of 250 and 500 mg/ kg significantly reduced the frequency and severity of diarrhea. At the same doses, the extract significantly delayed the intestinal transit of charcoal meal in the test animals as compared to the control. The results of the study confirm anti-diarrhoeal potential of the heartwood of *P. marsupium*, thus provide the scientific basis for the traditional use of this plant as a remedy for diarrhea. The antimicrobial activity of in vitro from the aqueous extract of *P. marsupium* inhibited growth of bacteria with the minimal inhibitory concentration ranging from 0.04 mg to 0.08 mg concentration (Gairola *et al.*, 2010). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, flavonoids, phenols, etc., which have been found *in vitro* to have antimicrobial properties (Kachhawa *et al.*, 2012). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. In the

Comment [R10]: Please, discuss your results related to antimicrobial activity.

present study the antibacterial activity of the methanol and acetone extracts of *Pterocarpus indicus* were tested against two gram positive bacteria *Bacillus subtilis* (Bs) *Staphylococcus aureus* (Sa) and one gram negative bacteria *Escherichia coli* (Ec) showed in (Fig. 5) and the zone of inhibition given in (Table 5). The worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus* and many other β -lactamase producers are reported to cause various pathological conditions in humans. Methanol and acetone extracts were showed positive antibacterial activity against these four strains. *P. indicus* has been demonstrated to be a good antibacterial agent and such a skin protective agent from pathogenic bacteria (Kachhawa *et al.*, 2001).



Fig. 5 Antibacterial activity of bark extract of *P. indicus*.

Table 5: Antibacterial activity of bark extract of *P. indicus*

S. No	Bacterial Strain	Zone of inhibition	
		Methanol Extract (mm)	Acetone Extract (mm)
01.	<i>E.coli</i>	18	15
02.	<i>Bacillus substilis</i>	15	14
03.	<i>Staphylococcus aureus</i>	16	15

4 Conclusion

Pterocarpus indicus (Willd) belongs to the family Fabaceae (legume) Pontederiaceae is commonly known as Narra or Rose wood tree. It is native to South Asia and East Indian regions. It is reported as very important plant in the forestry and culturing for wood and multiple uses in traditional medicine. The tree parts such as leaf, stem, and bark have various traditional medicinal uses. In several regions the shredded bark is boiled and the fluid is taken orally for treatment of dysentery, diarrhea, tuberculosis, headaches, sore , heavy menstruation, and gonorrhoea , cuts and wounds, stomach ache, leprosy, menstrual pain, flu, rheumatoid arthritis, and diabetes and as a progate. A large number of biologically active compounds have been extracted from *P. indicus* since it is endowed with many potent phytochemicals like alkaloids, flavonoids, tannins, terpenoids, saponins many others. The GC-MS analysis of the methanol and acetone extracts of barks of *P. indicus* revealed the presence of numerous biologically active compounds with potential medicinal properties, hence this species may be considered as a potential medicinal tree for treatment of various infectious diseases.

Comment [R11]: Authors must write the conclusion of the work based on the results of the research.

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