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Research Article

EVALUATION OF ANTIMICROBIAL AND SYNERGISTIC ANTIMICROBIAL PROPERTIES OF *PTEROCARPUS SANTALINUS*

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ABSTRACT

Objectives: The aim of the present study was to evaluate antibacterial and synergistic antimicrobial properties of leaf, stem, and bark of *Pterocarpus santalinus.*

Methods: The extraction was done by decoction method. The antimicrobial activity was evaluated by agar well diffusion assay, and the synergistic antimicrobial activity was evaluated by agar disc diffusion assay.

Results: The synergistic activity was studied with plant extracts plus antibiotics, namely, ampicillin, polymyxin-B, clotrimazole, and fluconazole.

Conclusions: Among the three parts, the best antimicrobial activity was shown by bark extract. All the three parts showed synergistic antimicrobial activity with antibiotics, but their levels varied. The results suggest that all the three parts enhance the antimicrobial efficacy of the antibiotics against some microorganisms and hence can be developed as a new therapeutic weapon against infectious diseases.

Keywords: *Pterocarpus santalinus*, Synergistic antimicrobial activity, Antibiotics, Bark, Fluconazole.

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INTRODUCTION

Asian countries are famous for traditional medical practices such as Ayurveda, Unani, and Siddha [1]. Medicinal plants have been a valuable source of natural active constituents that products used for maintaining human health and treatment of many human diseases [2]. Many of the plant materials used in traditional medicine are readily available in rural areas. According to the World Health Organization, 80% of developed countries use traditional medicine [3]. Plants are easily available, renewable in nature, not expensive, and have fewer side effects and better patient tolerance. Herbal medicine is a traditional or folk medicinal practice based on the use of plants' seeds, berries, roots, leaves, bark, and flowers extracts as medicine [4]. It is cheaper than modern medicine. Medicinal plants show various activities like antioxidant, antimicrobial, antitumor, antimutagenic, anticarcinogenic, anticancer, anti-ulcer, antiurolithiatic, anti-inflammation, antidiabetic, diuretic, etc.

Plants are a good source of many kinds of economically important compounds such as phenolic compounds, nitrogen containing compounds, vitamins, and minerals. Phytochemical constituents are the chemical compounds formed during the plants normal metabolic growth, and these are potential bioactive compounds which are precursors for the synthesis of useful drugs [5]. Plants are rich in a wide variety of secondary metabolites such as phenolics, tannins, terpenoids, alkaloids, saponins, glycosides, and flavonoids [6,7].

Microbial infections constitute a major public health problem in developing countries. In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of antibiotics. Antibiotics have been called miracle drugs, but more than 60 years of use, the efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug-resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials [8,9].

Therefore, the demand for new and effective antimicrobial agents with broad-spectrum activities from natural sources is increasing day by day. In view of this, the search for new antimicrobial agents from medicinal plants is even more urgent in the countries like India. Infectious diseases of bacterial origin are not only rampant but also the causative agents are also developing an increasing resistance against many of the commonly used antibiotics. Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified. The plant-based antibacterial compounds would be ideal to combat this problem. Some of the promising plants with antimicrobial properties are given in Table 1.

Antibiotics that work today may not work tomorrow. As resistance to old antibiotics spreads, new antimicrobial agents have to be discovered if the problem is to be contained. Unlike synthetic drugs, antimicrobials of plant origin are not associated with side effects and have a great therapeutic potential to heal many infectious diseases. Sometimes, the use of single antibiotic does not produce the desired inhibitory effects, and to overcome this, a combination of drugs often exercises their synergistic effect. Synergism is defined as a positive interaction created when two agents are combined and together they exert an inhibitory effect that is greater than the sum of their individual effects. The synergism is a new concept in developing agents for antibacterial, antioxidant, and also for anticancer activity. The new approach is combination therapy or synergistic therapy against resistant microorganisms which may lead to new ways of treating infectious diseases.

The aim of the present work was to evaluate antimicrobial and synergistic antimicrobial properties of *Pterocarpus santalinus* leaf, stem, and bark extracts.

Plant description [10]

P. santalinus Linn. f.

- Scientific name: *P. santalinus* Linn. f.
- Family: *Fabaceae*
- Description: *P. santalinus* is a light-demanding small tree, growing to 8 m (26 ft) tall with a trunk 50-150 cm diameter. It is fast-growing

Table 1: List of some medicinal plants, their family, and microorganism used for antimicrobial activity

S. No.	Botanical name	Family	Microorganisms	References
1	*Aloe vera* L. *Coleus aromaticus* Benth.	*Xanthorrhoeaceae* *Lamiaceae*	EC, PA, KP, BS, SA	[11]
2	*Alcea pallid* L., *Alcea apterocarpa* Fenzl.	*Malvaceae Malvaceae*	SA, PA, EC, SP, CA	[12]
3	*Artemisia santonica* L.	Succory	SA, PA, EC, BC	[13]
4	*Asplenium nidus* L.	*Aspleniaceae*	EC, PA, SA, SP	[14]
5	*Capsicum chinense* Jacq.	*Solanaceae*	EC, KP, SA, SP, ST, BC, CA, AF	[15]
6	*Citrus sinensis* L. *Citrus aurantium* L.	*Rutaceae* *Rutaceae*	EC, SA, MRSA	[1]
7	*Cyclea peltata* Lam.	*Menispermaceae*	EC, SA, BC, KP, PA, ST, SH, PV, SM, PR, VC	[16]
8	*Garcinia lancifolia* Roxb.	*Clusiaceae*	BS, SA, KP, EC	[17]
9	*Lavandula angustifolia* Mill.	*Lamiaceae*	SA, PA, CA	[18]
10	*Melia azedarach* L.	*Meliaceae*	BC, SA, EC, PA, AG, AF, FO, RS	[19]
11	*Nyctanthes arbortristis* L.	*Oleaceae*	SF, SB, SEn, SP, PA, CF, MM, PV	[20]
12	*Ocimum basilicum* L., *Cymbopogon citratus* L., *Morinda citrifolia* L., *Triticum aestivum* L.	*Lamiaceae* *Poaceae* *Rubiaceae* *Poaceae*	PA, SA, BC, ST, EC, KP, CA, SP	[21]
13	*Parthenium hysterophorus* L.	*Asteraceae*	EC, PA, SC, CA, BS, SA	[22]
14	*Ricinus communis* L., *Pterocarpus santalinus* L., *Terminalia bellirica* (Gaertn.) Roxb.	*Euphorbiaceae* *Fabaceae* *Combretaceae*	BS, SA, EC, PA, CA, AN, SAb, AS, LA	[23]
15	*Svensonia hyderobadensis* Walp.	*Verbenaceae*	EC, PA, BS, KP, PV, FO, CL, RA, AF	[24]
16	*Swertia chirayita* L. *Dendrobium amoenum* L.	*Gentianaceae* *Orchidaceae*	SA, EC, KP, SP, ST, PA	[25]
17	*Tinospora cordifolia* willd *Phyllanthus niruri* L. *Abrus precatorius* L.	*Menispermaceae* *Phyllanthaceae* *Papilionaceae*	EC, AN, EF	[26]
18	*Terminalia catappa.* L., *Colocasia esculenta* L.	*Combretaceae* *Araceae*	SA, BS, BC, SAb, SE, PA, ST, EC, BM, CR, LM, MF, PM, PV, PMi, EA, KP, CF, KA, CA, CN, CG, CE, PB, TB	[27]
19	*Toddalia asiatica* L.	*Rutaceae*	BS, SA, KP, PA, ST, SE, SP, SF, ML, EA Clinical isolates EC, KP, CA	[28]
20	*Zingiber officinale* Rosc., *Thymus vulgaris* L., *Cinnamomum zeylanicum* Breyn. Syn.	*Zingiberaceae* *Lamiaceae* *Lauraceae*	SA	[4]
21	*Marselia minuta* L.	*Marsileaceae*	EC, PA, SA, SPn	[29]
22	*Anogeissus latifolia* Roxb. *Glycyrrhiza glabra* Linn.	*Combretaceae* *Leguminosae*	SA, EFe, EC, KP, AN, CA	[30]
23	*Alstonia scholaris* L.	*Apocynaceae*	BS, SP, EC, KP, PA, PM	[31]
24	*Centratherum punctatum* Cass.	*Asteraceae*	PA, AB, SA, EC, BS, PR	[32]

AB: *Acinetobacter baumannii*, AF: *Aspergillus flavus*, AN: *Aspergillus niger,* AS: *Alternaria solani*, BC: *Bacillus cereus*, BM: *Bacillus megaterium*, BS: *Bacillus subtilis*, CA: *Candida albicans,* CE: *Candida epicola,* CF: *Citrobacter freundii*, CG: *Candida glabrata*, CL: *Curvularia lunata*, CN: *Cryptococcus neoformans,* CR: *Corallium rubrum*, EA: *Enterobacter aerogenes*, EC: *Escherichia coli*, EF: *Epidermophyton floccosum*, EFe: *Enterococcus faecalis,* FO: *Fusarium oxysporum*, KA: *Klebsiella aerogenes,* KP: *Klebsiella pneumoniae*, LA: *Lasiodiplodia abnormis,* LM: *Listeria monocytogenes*, MF: *Myotis flavus*, MM: *Morganella morganii*, ML: Micrococcus luteus, MRSA: Methicillin-resistant *Staphylococcus aureus*, PA: *Pseudomonas aeruginosa*, PM: *Proteus morganii,* PMi: *Proteus mirabilis,* PR: *Providencia rettgiri*, PV: *Proteus vulgaris*, RA: *Rhizopus arrhizus*, RS: *Rhizopus stolonifer*, SA: *Staphylococcus aureus,* SAb: *Salmonella abony,* SB: *Shigella boydii,* SC: *Saccharomyces cerevisiae*, SE: *Staphylococcus epidermidis*, SEn: *Salmonella enteritidis*, SF: *Shigella flexneri,* SH: *Staphylococcus haemolyticus*, SM: *Serratia marcescens*, SP: *Streptococcus pyogenes*, SPa: *Salmonella paratyphi*, SPn: *Streptococcus pneumonia*, ST: Salmonella typhimurium, TB: *Trichosporon beigelii*, VC: *Vibrio cholerae*

when young, reaching 5 m (16 ft) tall in 3 years, even on degraded soils. It is not frost tolerant, being killed by temperatures of −1°C. The leaves are alternate, 3-9 cm long, trifoliate with three leaflets. The flowers are produced in short racemes. The fruit is a pod 6-9 cm long containing one or two seeds.

- Vernacular name: Rakta Chandan
- Part used: Leaves, stem, and bark
- Uses: It is an astringent and a cooling agent and is used in several skincare preparations. It is used in the treatment of pimples, acne, and wrinkles. It is also used internally in chronic bronchitis,

gonorrhea and gleet, and chronic cystitis with benzoic and boric acids. Much used as a perfume for different purposes. The wood is used for making fancy articles and is much carved. It has been used in Ayurvedic medicine as an antiseptic, wound-healing agent and in anti-acne treatment

- Commercial utility: Red sandalwood with wavy grain margins sells at higher prices than the standard wood.

METHODS

Plant collection

The leaf, stem, and bark of *P. santalinus* Linn. f. were collected in August 2015 from Surat, Gujarat, India. They were thoroughly washed with tap water and dried under shade. The dried plant parts were homogenized to a fine powder and stored in airtight bottles and later used for extraction.

Extraction: Decoction method

About 5 g of dried powder of leaf, stem, and bark was extracted with 100 ml of deionized water at 100°C for 30 minutes in a water bath [33]. It was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm in centrifuge (Remi centrifuge, India) for 10 minutes. The supernatant was collected, and the solvent was evaporated to dryness. The residue was weighed to obtain extractive yield, and it was stored in an airtight bottle at 4°C.

Determination of total phenol content

The amount of total phenol content was determined by Folin-Ciocalteu's reagent method [34]. The extract 0.5 ml and 0.l ml of Folin-Ciocalteu's reagent (0.5 N) was mixed, and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml of sodium carbonate (2 M) solution was added and further incubated for 30 min at room temperature, and the absorbance was measured at 760 nm (Systronics, India), against a blank sample. Total phenol content is expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compound). The assay was carried out in triplicate, and the mean values with ± standard error of mean are presented.

Antimicrobial activity

Antimicrobial activity was measured by agar well diffusion method against Gram-positive bacteria, Gram-negative bacteria, and fungal strains.

Microorganisms tested

The microorganisms were obtained from the National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4°C. The bacteria and fungi were maintained on nutrient agar and MGYP medium (Hi Media, India), respectively. The Gram-positive bacteria studied were *Bacillus cereus* (BC) ATCC11778, *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* (SA) ATCC29737, and *Corynebacterium rubrum* ATCC14898. The Gram-negative bacteria were *Escherichia coli* (EC) NCIM2931, *Pseudomonas aeruginosa* (PA) ATCC27S53, *Salmonella typhimurium* ATCC23564, and *Klebsiella pneumoniae* (KP) NCIM2719. Yeasts were *Candida albicans* (CA) ATCC209l, *Cryptococcus neoformans* (CN) NCIM3542, *Candida glabrata* (CG) NCIM3448, and *Candida epicola* (CN) NCIMB367 and four clinical fungal isolates (C1, C2, C3, and C4). The microorganisms used in the study are clinically important pathogens which are causing several infections and food-borne diseases.

Agar well diffusion assay

In vitro antimicrobial activity of leaf, stem, and bark of *P. santalinus* was determined by agar well diffusion assay [35,36]. Mueller-Hinton agar (MHA) and sabouraud dextrose agar (40-42°C) were seeded with 200 µl of inoculums (1×10^8 cfu/ml) and poured into Petri dishes. The media were allowed to solidify, and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). The plant parts extracts were dissolved in 100% dimethyl sulfoxide (DMSO) at a concentration of 20 mg/ml, and from this, 100 µl of extract was added into the well. The plates were incubated at 37°C and 28°C for 24 and 48 hrs for bacteria and fungi, respectively. DMSO was used as a negative control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimeters. The experiment was done in triplicate and the mean values are presented for antibacterial activity.

Antibiotics used

The antibiotics ampicillin (AMP), polymyxin-B (PB^{100}), clotrimazole (CC^{10}), and fluconazole (FLC^{10}) were purchased from Hi-Media Laboratory Pvt. Ltd., Mumbai, India.

Agar disc diffusion assay

Synergistic antimicrobial activity of *P. santalinus* with antibiotics (AMP, PB, CC, and FLC) was assessed against two Gram-positive bacteria, two Gram-negative bacteria, and two fungi using disc diffusion method [37]. The Petri plates were prepared by pouring 20 ml of sterilized molten MHA for bacteria and 20 ml Sabouraud dextrose agar for fungi, seeded with 200 µl test culture containing 1×10^8 cfu/ml as McFarland 0.5 turbidity standard. Plates were allowed to solidify. Sterile filter paper discs (6 mm) were impregnated with 20 µl of each plant extract separately. The antibiotic disc was impregnated with 20 µl of plant extract and allowed to saturate for 30 minutes and were placed on the surface of the agar plates which had previously been inoculated with test microorganisms. All the plates were incubated for 24 hrs at 37°C for bacteria and for 48 hrs at 30°C for fungi. Results were recorded by measuring the zone of inhibition appearing around the discs. All the tests were performed in triplicate, and the mean values are presented. DMSO was used as negative control.

RESULTS AND DISCUSSION

The extractive yield by decoction extraction method of different parts of *P. santalinus* is given in Fig. 1a and total phenol content in Fig. 1b. Maximum extractive yield was in stem, followed by leaf; bark had very less extractive yield (Fig. 1a). The extractive yield is different in different parts of the same plants, and it is also affected by the solvents and methods used for extraction [38]. All the three parts had almost same total phenol content (Fig. 1b). The extractive yield of bark was very much less than stem, but total phenol content was almost same like that of stem. Therefore, it can be stated that higher yield does not imply a higher level of activity and vice versa.

Antimicrobial activity

Antimicrobial activity of leaf, stem, and bark decoction extracts of *P. santalinus* against four Gram-positive bacteria, four Gram-negative bacteria, four fungi, and four clinical isolates is given in Fig. 2. The antibacterial activity against Gram-positive bacteria is given in Fig. 2a. The stem and bark extracts of *P. santalinus* inhibited all the four Gram-positive bacteria. The leaf extract did not inhibit any bacteria. All the four bacteria were resistant to leaf extract. The stem extract showed slightly higher zone of inhibition as compared to the bark extract (Fig. 2a). The antibacterial activity against Gram-negative bacteria is given in Fig. 2b.

The leaf, stem, and bark extracts of *P. santalinus* inhibited PA while EC and KP were inhibited by leaf and bark extracts. *S. typhimurium* was inhibited by stem and bark extracts (Fig. 2b). Among the three parts, bark extract showed best antibacterial activity, while leaf extract showed minimum activity. Among8 Gram-positive and Gram-negative organisms, PA was the most susceptible organism.

The antifungal activity of the three parts is given in Fig. 2c. The leaf, stem, and bark extracts of *P. santalinus* inhibited CA while CG was inhibited by leaf and stem extracts. CN and CE were inhibited by only stem extract (Fig. 2c). CA was the most susceptible fungal strain. The antifungal activity of the three parts against four clinical isolates is given in Fig. 2d. All the three extracts of *P. santalinus* inhibited the clinical isolate C1 while clinical isolates C2, C3, and C4 were inhibited by stem and bark extracts. They were resistant to leaf extract. Stem and bark extracts showed good antifungal activity (Fig. 2d).

Different parts of *P. santalinus* showed inhibition more against bacteria than fungi. The plant extracts showed slightly more antibacterial activity toward Gram-positive bacteria than Gram-negative bacteria. The bark extract showed best antibacterial activity followed by stem extract. There are two reasons for the differential activity of different parts of the same plant. The phytochemical compounds present in them may be different and also in different amounts. The inhibition level is different because the Gram-positive bacteria, Gram-negative bacteria, and fungi differ in their cell wall structure. The Gram-negative bacteria possess a tougher cell wall than Gram-positive bacteria and the fungi possess the toughest cell wall. The plant extracts generally inhibit Gram-positive bacteria as reported by many researchers [31,39-41].

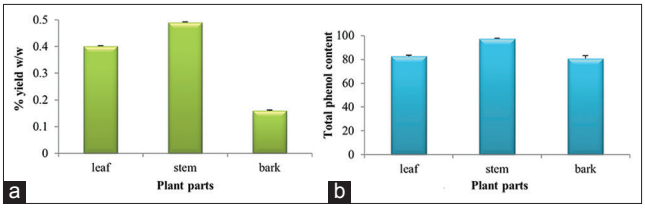

Fig. 1: (a) The extractive yield of different parts of *Pterocarpus santalinus*, (b) total phenol content of different parts of *P. santalinus*

Synergistic antimicrobial activity

Synergistic antimicrobial activity of different parts (leaf, stem, and bark) of *P. santalinus* is given in Figs. 3 and 4. *P. santalinus* leaf, stem, and bark extracts did not show inhibitory activity against all the four bacteria. AMP antibiotic showed inhibitory activity against SA and PA. PB antibiotic showed inhibitory activity against BC and PA. AMP antibiotic plus all the three parts extracts did not show inhibitory activity against all the four bacteria. PB antibiotic plus all the three parts extracts showed good inhibitory activity against all the four bacteria (Fig. 3). It appears that the plant extracts contain some phytocompounds that enhance the antibacterial efficacy of PB antibiotic, i.e. they work synergistically while they decrease the efficacy of AMP antibiotic.

Leaf, stem, and bark extracts of *P. santalinus* did not show antifungal activity against both the fungi. CC antibiotic alone showed inhibitory activity against both the fungi though the activity was more against CA. Unlike CC antibiotic, FLC antibiotic alone showed inhibition only against CN. The synergistic activity of stem and bark extract was slightly more than that of leaf extract. CC antibiotic plus leaf, stem, and bark extracts showed antagonistic activity against CA. FLC antibiotic plus all the extracts showed good synergetic activity against both the fungi. The stem and bark extracts showed better synergistic activity against both fungi than leaf extract (Fig. 4). In combination studies, both the antibiotics showed different activity. CC antibiotic with plant extracts showed antagonistic activity while FLC antibiotic with the same plant extracts showed synergistic activity against CA. Similar results, i.e., synergistic antimicrobial activity of plants extracts and antibiotics are also reported by others in literature [42-45]. The combination of plant extract with antibiotics can result in antagonistic or synergistic effect depending on the part of the plant used and the antibiotic used. The same plant extract may enhance the activity of some antibiotic while it may decrease the activity of another antibiotic. Similar results are reported in *P. cincinnata* [46]. It can also be stated that synergistic or combination therapy is a better option to tackle the problem of ever increasing drug resistance in bacteria and fungi [43,47].

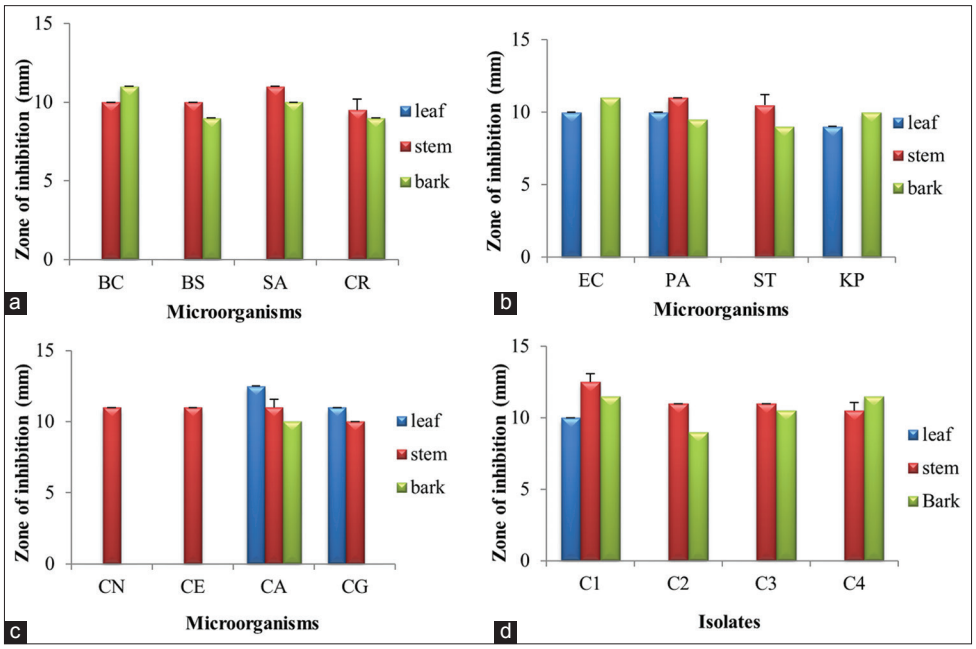

Fig. 2: Antimicrobial activity of different parts of *Pterocarpus santalinus*; (a) Gram-positive bacteria; (b) Gram-negative bacteria; (c) fungi; (d) clinical isolates

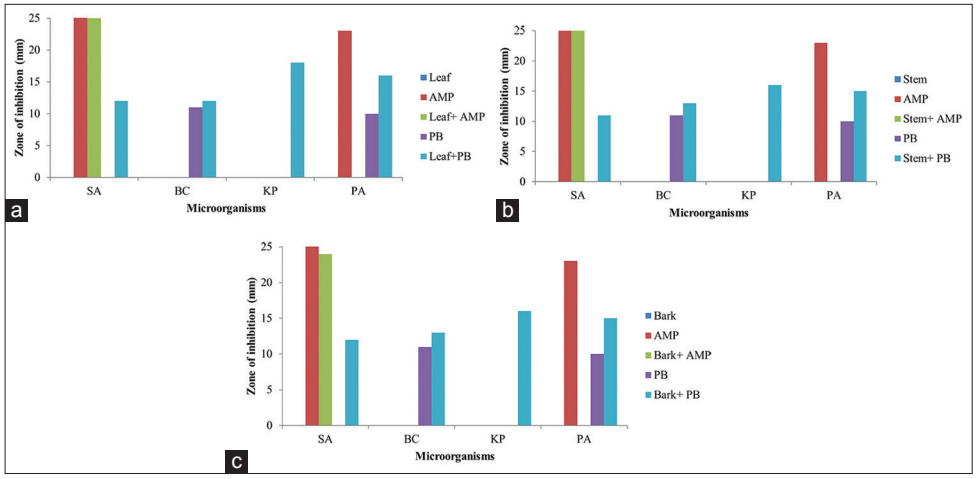

Fig. 3: Synergistic antimicrobial activity of *Pterocarpus santalinus,* (a) leaf, (b) stem, (c) bark against Gram-positive and Gram-negative bacteria

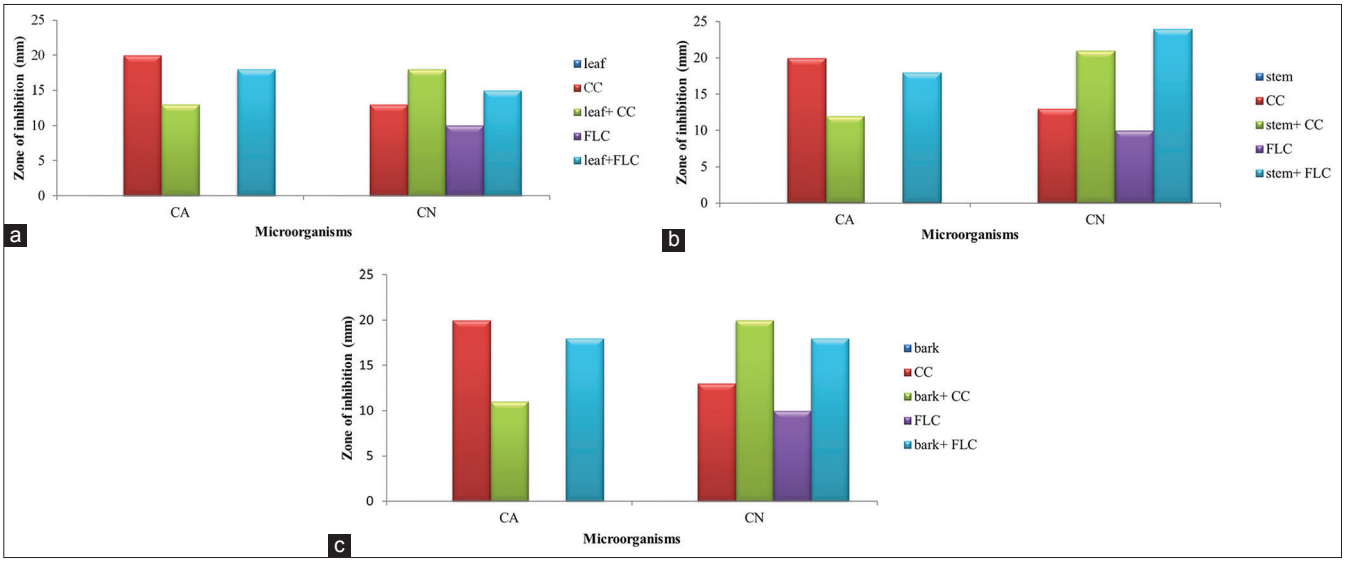

Fig. 4: Synergistic antimicrobial activity of *Pterocarpus santalinus,* (a) leaf, (b) stem, (c) bark against fungi

CONCLUSION

Plant extracts alone did not show any antimicrobial activity, but when combined with antibiotics, they showed synergistic antimicrobial activity. It can be emphatically stated that the three plant extracts possess some phytocompounds that enhance the antimicrobial efficacy of the antibiotics against some microorganisms. Hence, these plant extracts can be developed as a new therapeutic weapon against infectious diseases.

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