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Original Article

PLANT-BASED SYNERGISTIC THERAPY OF *PTEROCARPUS SANTALINOIDES* AND *TETRAPLEURA TETRAPTERA* EXTRACTS AGAINST *STREPTOCOCCUS PNEUMONIAE*

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ABSTRACT

Objective: This study investigated the plant-based synergistic therapy of *Pterocarpus santalinoides* and *Tetrapleura tetraptera* against *Streptococcus pneumoniae*.

Methods: Qualitative phytochemical screening of bioactive extracts of both plants, and the sensitivity test against *Streptococcus pneumoniae* were conducted.

Results: Screening results revealed the presence of alkaloids, tannins, glycosides, saponins, flavonoids, and sterols in the extracts, with sterols absent in *T. tetraptera*. Sensitivity results showed that the minimum inhibitory concentrations (MICs) for *P. santalinoides*, *T. tetraptera*, and their combination were 25 mg/ml, 50 mg/ml, and 6.25 mg/ml, respectively.

Conclusion: The superior efficacy of the combined extract underscores the potential of phytochemical synergy in developing effective, low-cost therapeutic alternatives to synthetic antibiotics, particularly for use in rural settings with limited healthcare access. It has also highlighted the promise of such a combination as an efficacious phytomedicine to combat *S. pneumoniae* infections, by potentially reducing treatment time, increasing cure rates, and decreasing drug resistance.

Keywords: Antimicrobial synergy, Phytochemicals, Traditional medicine, Health risk

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INTRODUCTION

Plants have formed the basis of the traditional medicine system, which has been used for thousands of years. Conventional medicine refers to health practices, approaches, knowledge, and beliefs incorporating plant, animal, and mineral-based medicine, spiritual therapies, manual techniques, and exercises, applied singularly or combined to treat or diagnose and prevent illness or maintain well-being [1]. In developing countries where orthodox medicine is quite expensive, traditional medicine is widely practiced; thus, screening for antimicrobial active compounds from ethno-medical plants, is vital to ascertain genuine active plants and active compounds.

In Nigeria, *Pterocarpus santalinoides* is used as food or medicine. The tender leaves are used as a vegetable in soup preparation, while the stem bark is used in making pepper soup. An earlier screening activity by [2] reported the presence of alkaloids, tannins, steroids, flavonoids, terpenes, saponins, and glycosides, which are responsible for the wide therapeutic properties. The plants are used in treating rheumatism, diarrhea, dysentery, cough, asthma, diabetes, malaria, elephantiasis, cold, and other conditions [3]. There is a report on use of leaves in treating skin diseases such as eczema, candidiasis, and acne. The use of the concoction made from its root in treating asthmatic patients has also been reported. It is also used in treating diarrhea, which is a major cause of death, as it has a proven anti-enteropathogenic activity in traditional medicine [4]. The antimalarial activity has been reported in treating infertility in females [5]. The stem bark extract is also used in the treatment of cough and diabetes. The leaves are used in veterinary medicine to reduce abdominal pains in goat and also menses in females [4].

Tetrapleura tetraptera is one such medicinal plant commonly found in Nigeria, which possesses antibacterial properties [6]. This plant owes its therapeutic properties to the presence of bioactive compounds such as saponins, tannins, flavonoids, cyanogenic glycosides, alkaloids, and steroids [7]. Research indicates that *Tetrapleura tetraptera* has been used in the treatment of various conditions, including cardiovascular diseases, neuromuscular disorders, convulsions, ulcers, hypoglycemia, inflammation, rheumatoid pain, and microbial infections [6, 8, 9].

Streptococcuspneumoniae is a g-positive coccus, α-hemolytic, catalase-negative, and optochin-sensitive bacterium, surrounded by a polysaccharide capsule that allows this microorganism to avoid phagocytosis, representing a major virulence factor. The ability to adhere to mucosal linings is another important virulence factor. Pneumonia causes life-threatening diseases, a major causative agent of human diseases, which include pneumonia, meningitis, septicemia, meningitis, chronic otitis media, and sinusitis in pediatric and elderly populations, and is one of the major contributors to mortality and morbidity with a significant impact on health and the economy of the world. Pneumococcal diseases kill about 1.6 million people in developing countries each year, of whom one million are children younger than 5 years [10].

The increasing resistance, and associated health risks of synthetic antibiotics have become a major challenge in modern medicine, pushing researchers toward plant-based alternatives. Hence, this work is aimed at evaluating the synergistic activity of bioactive of *Pterocarpus santalinoides* and *Tetrapleura tetraptera* extracts against *Streptococcus pneumoniae*.

MATERIALS AND METHODS

Plant material preparation

The stem bark and leaves of *Pterocarpussantalinoides* were collected at Umulolo, while pods of *Tetrapleura tetraptera* were obtained from commercial sources (Eke Okigwe) in Okigwe Local Government Area of Imo State, Southern part of Nigeria. Identification was done at the National Horticulture Research Institute in Imo State (NIHORT) by a botanist. The stem bark, leaves (*Pterocarpussantalinoide*), and pods (*Tetrapleura tetraptera*) were washed with sterile water, cut into smaller pieces, further dried, and were grinded to a fine powder using a high-power electric grinder.

Bioactive extraction

Fifty 50 g of milled powders were soaked in 250 ml of 70 % v/v ethanol for 72 h at room temperature to obtain the ethanolic extract. After soaking, the extract was filtered using a muslin cloth, followed by Whatman No.1 filter paper. The solvent was evaporated in an evaporator to obtain dry extracts. The dried extract was resuspended in dimethyl sulfoxide (DMSO) to obtain various concentrations of the ethanolic extract, which was used in the experiment.

Qualitative phytochemical screening of *Pterocarpus santalinoides* and *Tetrapleura tetraptera* extracts

Extract components were subjected to standard phytochemical screening using standard methods as described by [11]. The presence of the following constituents: alkaloids, tannins, saponins, glycosides, flavonoids, and sterols were determined.

Preparation of test microorganism and sensitivity test

The overnight culture of *Streptococcus pneumoniae* was sub-cultured into fresh broth and incubated for three (3) h and standardized to a 0.5 McFarland solution.

The disc diffusion method, as described by [12, 13], was employed. A 16-18 h culture was diluted with sterile physiological saline solution (0.85 % (w/v) sodium chloride) with reference to McFarland standard. A 0.5 McFarland standard is equivalent to a bacterial suspension that represents 1.5×10^8, containing between 1×10^8 and 2×10^8 CFU/ml of *E. coli*. The standard was prepared by adding an aliquot of 0.5 ml of $BaCl_2$ (1.175% $^w/_v$ $BaCl_2.2H_20$) to 99.5 ml of H_2SO_4 (1%$^v/_v$) with constant stirring to achieve an inoculum of approximately 1×10^8CFU/ml.

Using spread plate technique, prepared media were poured to an approximate depth of 4 mm (approximately 25 ml of liquid agar for 100-mm plates), after solidifying, a sterile swab was dipped into the inoculum tube, rotated at the side of the tube to remove excess fluid, and then inoculated by streaking the swab three times over the entire agar surface of a pre-dried Muller Hinton agar plates. The plates were rotated at approximately 60 degrees each time to ensure even distribution of the inoculum. The inoculated plates were rimmed with the swab to pick up any excess liquid, and the swab was discarded into an appropriate container. The plates had the lid slightly ajar and were allowed to stand at room temperature for at least 3 to 5 min for the surface of the agar plate to dry.

The extracts of *P. santalinoides* and *T. tetraptera*, were weighed separately in one part, and weighed in equal portions and mixed together in another part, dissolved in DMSO to make distinct concentrations of 3.13, 6.25, 12.5, 25, 50, 100, 150, 200 in g/ml according to National Committee for Clinical Laboratory Standards (NCCLS) recommendation before testing.

Sterile commercial filter paper discs of 6-8 mm were used to absorb the extract samples, re-suspended in DMSO. The appropriate antimicrobial-impregnated disks were placed on the surface of the agar, using forceps to dispense each antimicrobial disk one at a time. After allowing for an hour contact time at room temperature for the samples to diffuse across the surface, the plates were incubated at 37 °C for 24 h. The zone of inhibition was measured in millimetres with a meter rule or Vernier calliper.

Statistical analysis

All data obtained in this study were analyzed using SPSS version 21 (Statistical Package for the Social Sciences).

RESULTS

The qualitative screening of *P. santalinoide, T. tetraptera*, and composite (P and T) is shown in table 1. The phytochemical screening of the trio revealed the presence of bioactives such as alkaloids, tannins, glycosides, saponins, and flavonoids in all extracts, with sterols only present in *P. santalinoide* and in composites of P and T.

These extracts were tested against *S. pneumoniae,* and the activities of the extracts were reported in table 2. The extracts showed some biological activity towards the test organism, both at single and combined effects. The combined effect showed more potency, stronger synergy, and susceptibility of the test organism to the extract. The concentration of the extracts ranged from 3.13 to 200 mg/ml. The minimum inhibitory concentration (MIC) for both single and combined effects against *S. pneumonia* is as shown: *P. santalinoides* (25 mg/ml), *T. tetraptera* (50 mg/ml), and composite "P and T"(6.25 mg/ml). All data obtained were statistically analyzed and compared for efficient reporting.

Table 1: ***Phytochemical screening results***

Test compounds	*P. santalinoide*	*T. tetraptera*	Composite P andT
Alkaloids	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Flavonoid	+ve	+ve	+ve
Sterols	+ve	-ve	+ve

Legend:+ve = present and –ve = absent, P= *P. santalinoide,* T= *T. tetraptera*

Table 2: Minimum inhibitory concentration of single and combined effects of the extract compound

Test organism	Concentrations (mg/ml)								
	3.13	6.25	12.5	25	50	100	150	200	MIC
	Pterocarpus santalinoides								
S. pneumoniae	+	+	+	-	-	-	-	-	25
Tetrapleura tetraptera									
S. pneumoniae	+	+	+	+	-	-	-	-	50
Composite (*Pterocarpus santalinoide+Tetrapleura tetraptera*)									
S. pneumoniae	+	-	-	-	-	-	-	-	6.25

Key:-means no growth, +means growth

DISCUSSION

The phytochemicals detected in the extracts were alkaloids, tannins, glycosides, saponins, flavonoids, and sterols, and they possess medicinal properties. These bioactives were extracted using ethanol, which has been considered a better extracting solution than other classes of alcohols [14]. The biological activities of these plant extracts were attributed to the occurrence of these natural compounds [15]. Alkaloids are an assembly of naturally occurring chemical compound composites that typically comprise basic nitrogen atoms with pharmacological properties such as hypotensive, anticonvulsant, anti-protozoal, antimicrobial, and antimalarial activities [16]. Tannins are important natural bioactive compounds found in different forms. They are reported to possess antitumor, antiviral, and antibacterial activities [17]. Saponins possess antioxidant, anticancer, anti-inflammatory, hyperglycemic, and antifungal properties [18]. Flavonoids are natural substances with variable polyphenolic structure and have been reported to possess antiallergic, antioxidant, and anticancer properties [19]. These secondary metabolites will confer on these extracts some biological activity. Glycosides are the plant's secondary metabolites that exhibit diverse medicinal properties due to their chemical structure and source, which include anti-inflammatory, antioxidant, antidiabetic, anti-thrombotic, antibacterial, antifungal, antiviral, anticancer, laxative, and cardiovascular effects [17]. Sterols are a type of lipid with potential medicinal properties, particularly plant-derived sterols known as phytosterols. They are known for their cholesterol-lowering, anti-inflammatory, antioxidant, and anticancer properties [20].

The concentration of the extracts was varied from 3.13 mg/ml to 200 mg/ml. The MIC, which is the lowest concentration of a chemical that prevents the visible growth of a bacterium, was measured. The MIC varies for each extract against the test microorganism, with the combined (P and T) compound showing the best sensitivity effect of the test organism against the extract. The lower the MIC, the more potent and effective, is an extract compound.

It was observed that the activity of the extracts increased with increasing concentration, which was consistent with expectations. The antimicrobial activity of the most effective antibacterial agents increases linearly with concentration. This implied that the higher the concentration of the extract, the greater the zone of inhibition. The bioactivities of the extracts were compared individually in single and combined effects. The synergistic effect of *P. santalinoide* and *T. tetraptera* was positive and effective, as was observed in the minimum inhibitory concentration that was able to inhibit the growth of *S. pneumoniae*, the causative agent of pneumonia. The MIC of the combined effect was very low compared to the single standards. This indicated that the effectiveness of the combined extracts was synergistic and effective, with very low MIC compared to the single standards. This corroborates the statement on the features of drugs/chemotherapeutic agents that they are biologically active at low concentrations.

P. santalinoide and *T. tetraptera* have demonstrated significant antimicrobial properties, inhibiting the growth of *S. pneumoniae* as identified in this study. Given these findings, and the associated risk resulting from consuming synthetic drugs, there is need fora shift to natural products from plant sources in the treatment of pneumonia in most rural communities where access to standard health care is not affordable. Similar reports on the single potency of *P. santalinoide,* or *T. tetraptera*, on selected bacteria isolates were reported by [21, 14, 8, 6, 2].

CONCLUSION

Medicinal plants are plants whose different parts, such as fruit, leaves, stem, bark, flower, root, and juice, have therapeutic purposes or precursor substances for the synthesis of useful drugs. Aside from the associated risk resulting from consuming synthetic drugs, natural products of plant origin are safer and more efficacious in the treatment of diseases, hence the shift to phyto-medicine. The potential of *P. santalinoides and T. tetraptera* extracts has revealed their inhibitory activity against *S. pneumoniae, which w*as a result of the presence of phytochemicals. The antimicrobial activity of individual plants is low in activity against *S. pneumoniae*, and when combined, it revealed higher antimicrobial activity. The combinations of these plants act complementary and synergistic in the antimicrobial activity. The synergy and indifference extract activity of these plants could presenta better advantage in drug development. The combination of *P. santalinoide and T. tetraptera* could be a potent ingredient for a phyto-medicinethat is more efficacious, with shortened treatment time, increased cure rate, and decreased drug resistance to *S pneumoniae*. This approach would support better health with reduced dependence on costly healthcare services.

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AUTHORS CONTRIBUTIONS

All the authors contributed meaningfully

CONFLICT OF INTERESTS

There is no conflict of interest

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