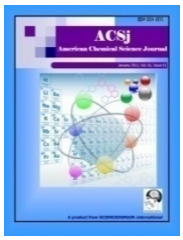

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Assaying Antioxidant and Antimicrobial Activities of 1-phenyl-3-naphthoic acid Derivatives

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors designed the study. Author RPM wrote the first draft of the manuscript and authors RPM and NSK managed the analysis of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Laboratory synthesized 1-phenyl naphthoic acid systems (substituted naphthalenes or lignans) were evaluated for their antioxidant and antimicrobial potential. All the phenyl naphthoic acids showed significant to moderate antioxidant activity in DPPH (2, 2-diphenyl-1-picrylhydrazyl) and $FeCl_3$ (ferric chloride) assay.
All the lignan compounds exhibited significant to moderate antimicrobial and antifungal activity except one of the compounds which showed no toxicity to the fungal species.

Keywords: 1-phenylnaphthoic acids; antioxidant potential; antimicrobial potential.

1. INTRODUCTION

An antioxidant or a free radical scavenger is defined as a substance which at low concentrations prevents or delays the oxidation of an oxidizable substrate like proteins, carbohydrates, lipids, DNA and other cell constituents [1]. Antioxidants help prevent

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oxidation, the common pathway for cancer, aging, and a variety of diseases and may help increase immune function, and possibly decrease risk of infection and cancer [2].

The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins.

The antioxidant activity (chain breaking) of natural antioxidants can be expressed in terms of radical scavenging capacity, in which the antioxidant reacts with a specific radical under controlled conditions. One of the well-established methods for determining the antioxidant activity is the spectrophotometric monitoring of the concentration of the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH·) [3,4]. In recent years, an increase in the number of studies of the antioxidant activity of lignans or lignan-rich extracts has been reported [5–8]. Using more than one assay for measuring antioxidants in foods has been recommended by some researchers [9,10].

With the indiscriminate use of drugs and antibiotics, microorganisms are attaining resistance to commonly used antibiotics, which leads to downfall of effectiveness of conventional medicines and therefore, search for new antimicrobial agents has become necessary [11-13]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Such an alarming situation has made its way to the discovery of new effective antimicrobial substances for the treatment of infectious diseases with good therapeutic values. Despite the existence of potent antibiotics and antibacterial agents, resistant or multi-resistant strains are continuously appearing. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered eligible for developing new antimicrobial drugs, may it be a newly synthesized compound or medicinal plant. There is a constant need of new and effective therapeutic agents with no harm to the host [14].

Many lignans and neolignans have been used for the development of new drugs. Lignans have been used for medicinal purposes dating back to many thousand years. Lignans have been of great importance with regard to their synthesis & studies since recent years due to their medically important biological activities like antioxidant and antimicrobial property [5–8] to name a few. So the present work was designed to evaluate the antioxidant and antimicrobial potentials of the lignan compounds [Fig. 1] namely, 1-phenyl-6,7-methylenedioxynaphthalene-3-carboxylic acid (1a), 1-phenyl-6,7,8-trimethoxynaphthalene-3-carboxylic acid (1b), 1-phenyl-6,7-dimethoxynaphthalene-3-carboxylic acid (1c) and 1-phenyl-6-methoxy-7-hydroxynaphthalene-3-carboxylic acid (1d)that had been synthesized [15] earlier in our laboratory and have shown significant anti-inflammatory activity [16].

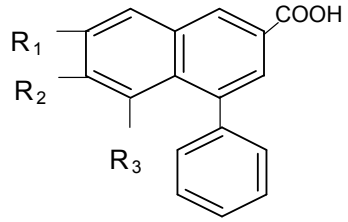


Fig. 1. Derivatives of 1 - phenyl naphthalene -3- carboxylic acids

a) $R_1 = R_2 = O – CH_2 – O$, $R_3 = H$,
b) $R_1 = R_2 = R_3 = OCH_3$,
c) $R_1 = R_2 = OCH_3$, $R_3 = H$,
d) $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$,

2. MATERIALS AND METHODS

All the chemicals and reagents used were of analytical or reagent grade and were not additionally purified.

The monitoring of antioxidant potentials of the test compounds (1 a-d) was carried out by DPPH· and $FeCl_3$ assay. The DPPH free radical scavenging of the test compounds was determined by the method described by Ganesan et al. [17]. And the reducing power of the test compounds was determined according to the method of Oyaizu [18].

2.1 Experimental Section

2.1.1 DPPH free radical scavenging activity

DPPH· radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1mM solution of DPPH· in methanol was prepared and 3ml of this solution was added to the test tube containing 1ml of sample solutions in methanol or methanol : water (1:1) at different concentrations (5, 10, 20μg/ml). Thirty minutes later after incubation at room temperature, the absorbance was measured at 517nm. The absorbance of similar reaction mixtures of methanol or methanol: water (1:1) (without test compounds) with DPPH· served as control.

The capability of the extracts to scavenge the DPPH radical was calculated using the following equation.

DPPH· Scavenged (%) = A (cont) - A (test) x 100/A (cont)
A (cont) – Absorbance of control
A (test) – Absorbance of the test extracts

2.1.2 $FeCl_3$ assay

Briefly, 1.0ml of methanol or methanol: water (1:1) containing different concentrations of test compounds (5, 10, 20μg/ml) were mixed with 2.5ml of phosphate buffer (0.2M, pH6.6) and 2.5ml potassium ferricyanide (1%). Reaction mixture was incubated at 50ºC for 20min. After incubation, 2.5ml of trichloroacetic acid (10%) was added and centrifuged for 10min. From the upper layer, 2.5ml solution was mixed with 2.5ml distilled water and 0.5ml $FeCl_3$ (0.1%). Absorbance of all the sample solutions was measured at 700nm. The absorbance of the similar reaction mixtures without test compounds served as control. Increased absorbance indicated increased reducing power.

2.1.3 Antimicrobial assay [19]

Test organisms taken for study were *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Candida albicans.*

2.1.3.1 Determination of zone of inhibition

Freshly prepared suspensions in sterile water (Optical Density: 0.6) of pure isolated cultures of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Candida albicans* were

mixed with the sterilized nutrient agar and Sabouraud dextrose agar maintained at 42.0±2.0ºC and poured in petri dish (6inch) and allowed to solidify. Five wells of 6mm diameter were bored in the medium with the help of sterile cork-borer having 6mm diameter and were labelled properly. 50, 100, and 200µg/ml of the working solution /vehicle and same volume of extraction solvent for control, as well as 25µg/ml of the standard (Bacitracin) was filled in these wells with the help of micropipette. Similar sets were made for other test compounds. Petri dishes containing nutrient agar for microbial and sabouraud dextrose agar for fungal growth were incubated at 37±2.0ºC for 2 days and 24±2.0ºC for 5 days respectively. Plates were observed for zone of inhibition.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Assay

The *In vitro* tests carried out with the test compounds (lignans) (1a-d) showed significant antioxidant activity as shown in graphs below (Graphs 1 and 2). From the percentage DPPH· free radical scavenging values (Graph 1), it was found that the compound 1d was the most potent scavenger followed by compound 1b, 1c and 1a at 20µg/ml concentration.

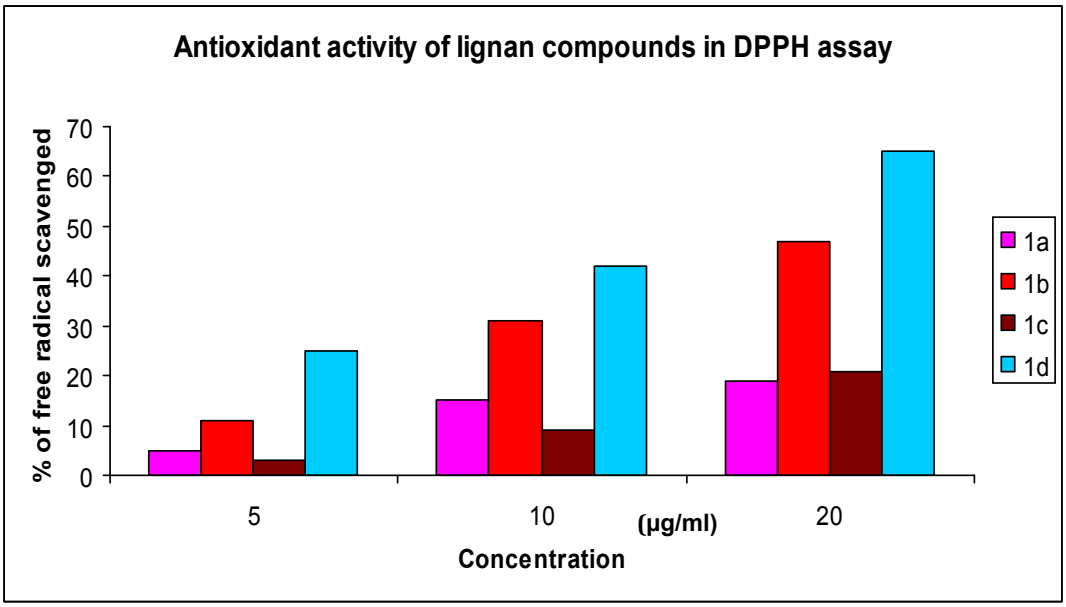
However at 5µg/ml concentration the compound 1a was a good scavenger than compound 1c, but at 20µg/ml compound 1c was more effective than compound 1a.

From the percentage scavenging values in $FeCl_3$ assay (Graph 2), it was found that the compound 1b was the most effective at reducing the iron (III), followed by compounds 1a, 1c and 1d at 20µg/ml concentration.

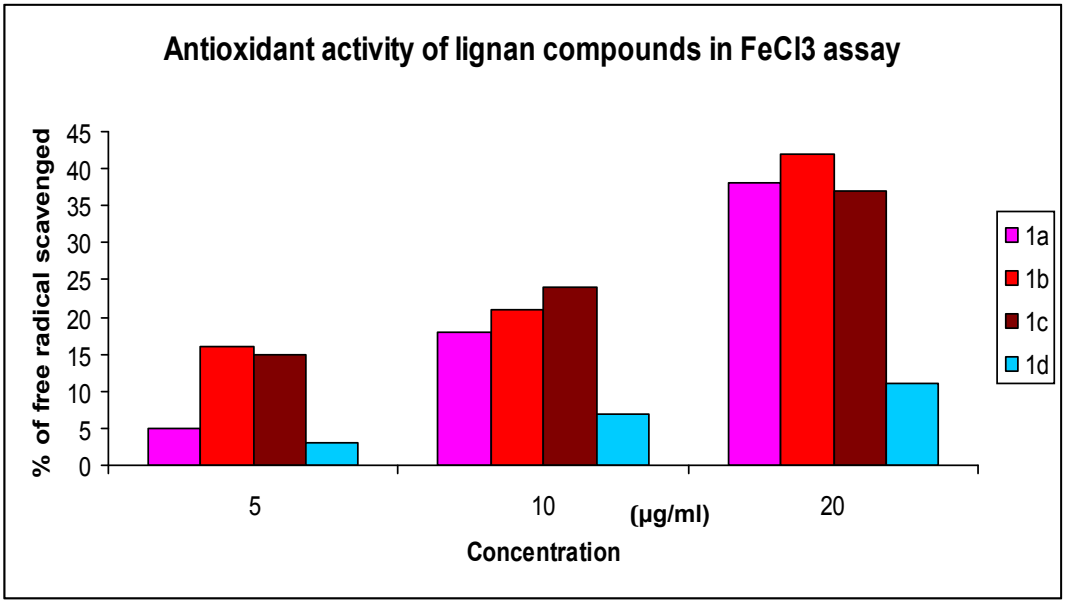
3.2 Antimicrobial Assay

Among the gram-positive bacteria taken for study, the growth of *Staphylococcus aureus and Bacillus subtilis was* inhibited by all the test compounds. Among gram-negative bacteria taken for assay, growth of *Escherichia coli* was also inhibited by all the test compounds.

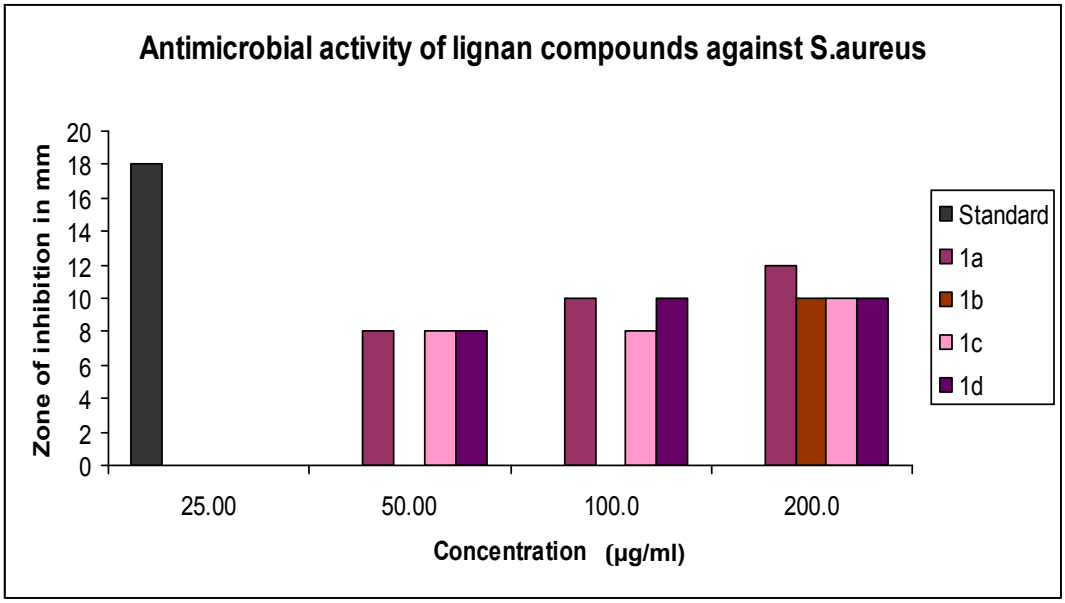
The zone of inhibition was shown by only three lignan compounds (1a, 1c and 1d) against the only fungal studied *Candida albicans*. Bacitracin was taken as standard. From (Table 1) and (Graphs 3, 4, 5, 6) it can be seen that the compound 1a showed strong antimicrobial activity against *S. aureus* than 1b, 1c and 1d which were equally toxic to *S. aureus*. Compounds 1a and 1c showed strong antimicrobial activity against *B. subtilis* than 1b and 1d. Compounds 1a, 1b and 1d showed strong antimicrobial activity against *E. coli* than the compound 1c. Compound 1c showed strong antifungal activity against *C. albicans* than 1a and 1d which were equally toxic to *C. albicans*. The increase in concentration of these compounds increased their potency. Compound 1b didn't show antifungal activity against *C. albicans* at any concentration. All the values were compared with the standard used.



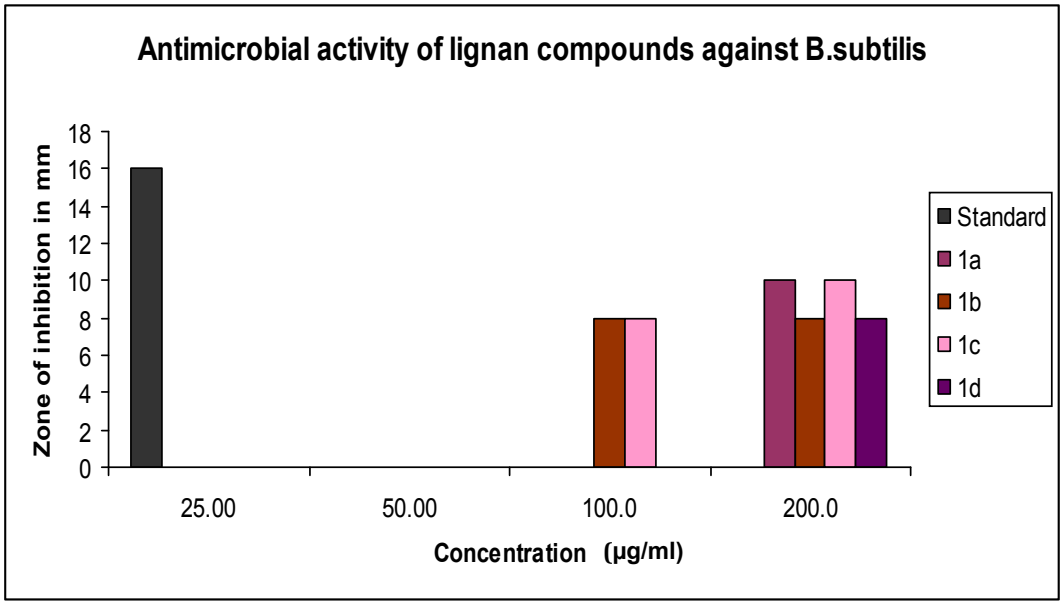
Graph 1. Antioxidant activity of lignan compounds in DPPH assay



Graph 2. Antioxidant activity of lignan compounds in $FeCl_3$ assay



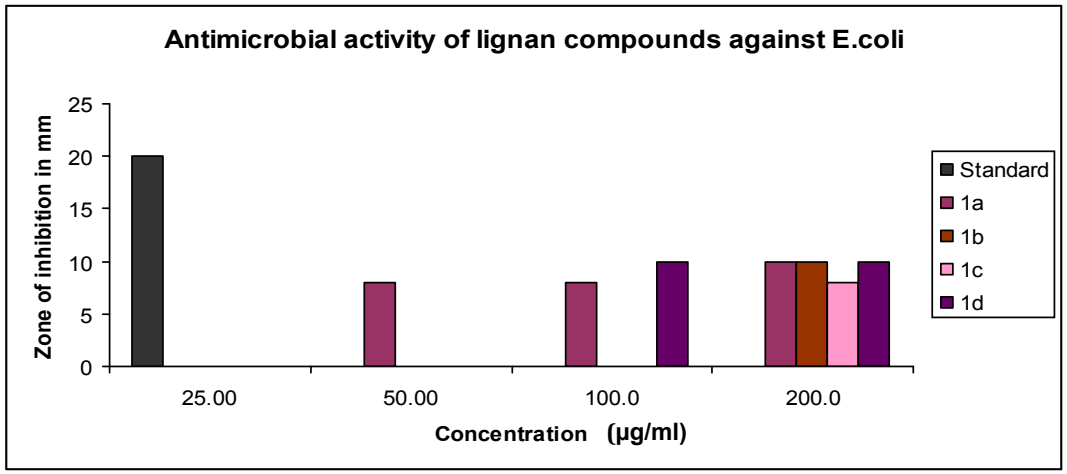
Graph 3. Antimicrobial activity of lignan compounds against *S. aureus*



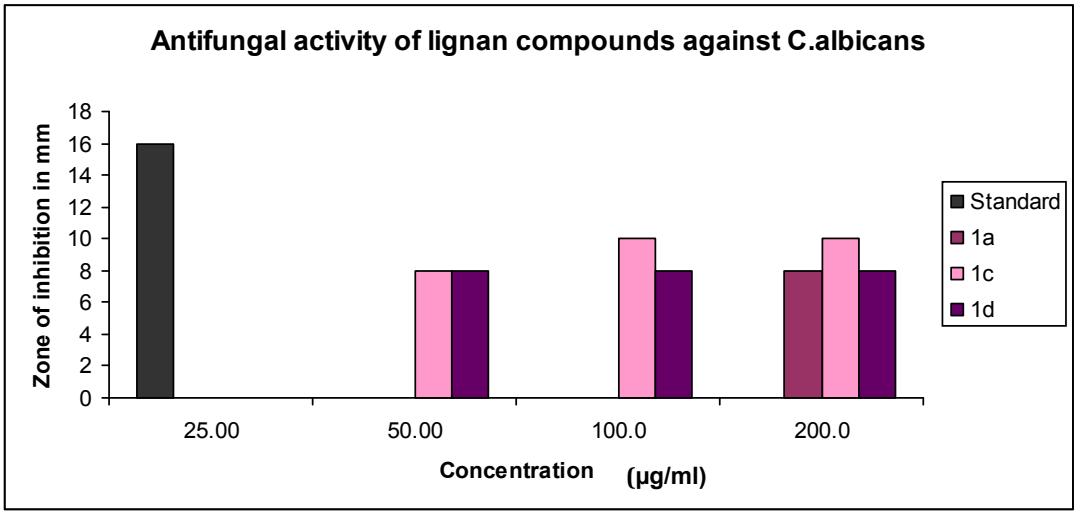
Graph 4. Antimicrobial activity of lignan compounds against *B. subtilis*

Table 1. Zone of inhibition

Sl. No	Compounds	Concentration	Solvent	Zone of Inhibition (mm)			
				S. aureus	*B. subtilis*	*E. coli*	*C. albicans*
1.	Bacitracin (Standard)	25μg/ml		18	16	20	16
2.	1a	50 μg/ml	Methanol	8	0	8	0
		100 μg/ml	Methanol	10	0	8	0
		200 μg/ml	Methanol	12	10	10	8
3.	1b	50 μg/ml	Methanol: Water (1:1)	0	0	0	0
		100 μg/ml	Methanol: Water (1:1)	0	8	0	0
		200 μg/ml	Methanol: Water (1:1)	10	8	10	0
4.	1c	50 μg/ml	Methanol: Water (1:1)	8	0	0	8
		100 μg/ml	Methanol: Water (1:1)	8	8	0	10
		200 μg/ml	Methanol: Water (1:1)	10	10	8	10
5.	1d	50 μg/ml	Methanol	8	0	0	8
		100 μg/ml	Methanol	10	0	10	8
		200 μg/ml	Methanol	10	8	10	8



Graph 5. Antimicrobial activity of lignan compounds against *E. coli*



Graph 6. Antifungal activity of lignan compounds against *C. albicans*

4. CONCLUSION

The antioxidant and antimicrobial activity for all the compounds was evaluated. All the phenylnaphthoic acids (1a-d) have shown 18% to 65% scavenging in DPPH assay and 10% to 42% reduction of iron (III) in $FeCl_3$ assay. The compound 1d was found to be the most active antioxidizing agent. All the phenylnaphthoic acids (1a-d) have shown considerable toxicity in high concentrations towards the tested the gm+ ve and gm– ve bacteria. All the phenylnaphthoic acids have shown considerable toxicity to the fungal species tested. Unexpectedly the compound 1b tested showed no toxicity to the fungal species. The compound 1a was found to be the most active antibacterial agent and compound 1c was found to be the most active antifungal agent.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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