RESEARCH ARTICLE

Bioactives of *Dendrophthoe trigona* (Wt. & Arn.) Danser for its Antioxidant and Antibacterial Activities

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Abstract

The natural phenolic compound 2,6-bis(1,1-dimethylethyl)-4-methylphenol, commonly known as butylated hydroxytoluene (BHT), and the fatty acid pentadecanoic acid was isolated from the methanol extract of *Dendrophthoe trigona* leaves, which parasitize *Ficus benghalensis*. The structures of these compounds were elucidated through nuclear magnetic resonance (NMR) and mass spectral (MS) analyses. This study assesses the total phenolic content, as well as the antioxidant and antibacterial properties of the isolated compounds. The total phenolic content for BHT and pentadecanoic acid was found to be 65.09 ± 0.24 and 52.47 ± 0.17 µg of gallic acid equivalents (GAE), respectively. In antioxidant assays, BHT exhibited greater activity than pentadecanoic acid, with both compounds demonstrating effectiveness comparable to standard ascorbic acid. Additionally, pentadecanoic acid showed superior antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* compared to BHT. These results indicate that *D. trigona* could serve as a natural source for the production of BHT and antimicrobial agents.

Keywords: *Dendrophthoe trigona*, Nuclear magnetic resonance, Butylated hydroxytoluene, antioxidant activity, antibacterial activity.

Highlights:

- *Dendrophthoe trigona*, a parasitic plant, was taken for the study of biological activities.
- Isolation of compounds was carried out.
- The isolated compounds were characterized by proton (1H) and Carbon (13C) NMR spectra.
- The antioxidant and antibacterial activities of isolated compounds were evaluated.

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Introduction

Plants have been utilized in traditional medicine for treating diseases, although their scientific validation is often lacking, which may lead to potential risks (Fabricant and Farnsworth, 2001; Alviano and Alviano, 2009). Approximately 80% of rural populations rely on medicinal plants for primary healthcare due to their biologically active compounds. In the USA, Europe, and other parts of the world, plants or their derivatives are widely used in the production of medicines (Sakarkar and Deshmukh, 2011). There are around 500,000 plant species on Earth, but only a small fraction has been examined for their therapeutic and pharmacological properties (Verpoote, 2000). Free radicals, such as hydroxyl radicals, superoxide, and nitric oxide, are implicated in the development of several diseases, including neurodegenerative diseases (Halliwell, 2006), anti-inflammatory conditions (Ferguson, 2010), cancer (Halliwell, 2012), atherosclerosis, arthritis, cataractogenesis, muscular dystrophy, diabetes, pulmonary dysfunction, and Alzheimer's disease (Di Matteo and Esposito, 2003). Antioxidants combat oxidative damage caused by these free radicals in the human body (Kaliora *et al.,* 2006). Many phenolic compounds, such as phenolic acids and flavonoids, exhibit antioxidant properties, including free radical scavenging, inhibition of lipid peroxidation, and metal chelation (Rice-Evans *et al.,* 1996). Consequently, researchers have screened various plants for their antioxidant activity (Katalinic *et al.,* 2006; Kavitha Chandran *et al.,* 2022).

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In the food industry, synthetic antioxidants like BHT, BHA, TBHQ (tertiary butylhydroquinone), and PG (propyl gallate) are frequently used to prevent the oxidation of lipids. Although these synthetic antioxidants help reduce the risk of various diseases, their toxicity can lead to carcinogenesis and liver damage. Consequently, the U.S. Food and Drug Administration (FDA) has restricted their use (Sasaki *et al.,* 2002), underscoring the need to identify natural antioxidants from plant sources.

Natural bioactive compounds play a crucial role in developing products with biological activities (Wu and Chappell, 2008). These compounds can be synthetically modified to enhance their therapeutic properties (Dias *et al.,* 2012). Over

the past 25 years, approximately 60-70% of drugs derived from natural bioactive compounds have been used to treat cancer and infectious diseases (Newman and Cragg, 2007; Dawurung *et al.,* 2021; Irinmwinuwa *et al.,* 2023). Many commercial drugs are derived from herbal medicines, such as digitoxin from *Digitalis purpurea*, ephedrine from *Ephedra sinica*, and reserpine from *Rauwolfia serpentina*. Various methods, including paper chromatography (PC), thin layer chromatography (TLC), gas-liquid chromatography (GLC), HPLC, and spectroscopic techniques (ultraviolet, infrared, NMR, and mass spectroscopy), are used to screen, isolate, and identify compounds from plants.

Plants exhibit different modes of adaptation, such as parasitic, epiphytic, mesophytic, and halophytic strategies. Among these, parasitic angiosperms are often considered unwanted plants because they depend on host plants for their needs and can damage economically important crops. Despite their destructive nature, there are reports of parasitic plants possessing various biological activities and being used in traditional medicine for their therapeutic properties (Osadebe *et al.,* 2004; Hasan *et al.,* 2006; Raut *et al.,* 2009; Zainuddin and Sulain, 2015; Elsyana *et al.,* 2016; Yee *et al.,* 2017; Maheshwari and Rothe, 2018).

Dendrophthoe species hold considerable medicinal value and are widely utilized in traditional medicine for treating a range of ailments. They are applied as remedies to enhance vitality, act as astringents, and provide narcotic and diuretic effects. These species are traditionally used to alleviate conditions such as asthma, menstrual irregularities, lung diseases like tuberculosis, ulcers, various skin conditions, impotence, paralysis, wounds, and even cancer. Additionally, they have been reported to possess antilithiatic, antihypertensive, antioxidant, antimicrobial, anticancer, antidiabetic, pain-relieving (antinociceptive), antiproliferative, and anthelmintic activities (Osadebe *et al.,* 2004; Hasan *et al.,* 2006; Raut *et al.,* 2009; Zainuddin and Sulain, 2015; Elsyana *et al.,* 2016; Yee *et al.,* 2017; Maheshwari and Rothe, 2018; Widowati *et al.,* 2011; Ain *et al.,* 2012; Pattanayak and Sunitha, 2008; Pattanayak *et al.,* 2012; Puneetha *et al.,* 2013).

In this study, we report the isolation and characterization of bioactive components from the methanol leaves extract of *D. trigona* and evaluate their antioxidant and antibacterial activities.

Materials and Methods

Plant Material Collection

The Western Ghats region of Karnataka served as the collection site for the *D. trigona* plant material and was subsequently transported to the laboratory, where it was thoroughly disinfected. An herbarium specimen was prepared, identified, and then deposited in the Herbarium of the Department of Studies in Botany at the University of Mysore, Manasagangotri, Mysore, under the voucher number UOMBOT23DT42.

Preparation Process for Extracts

The leaves of *D. trigona* were thoroughly rinsed with running tap water and then shade-dried at room temperature. Once dried, the leaves were ground into a fine powder using a blender and stored in an airtight container for subsequent use. A total of 40 g of dried leaf powder from each plant was successively extracted with 250 mL of various organic solvents in the following sequence: petroleum ether, chloroform, ethyl acetate, and methanol, using a soxhlet apparatus. After complete extraction, the respective solvent extracts were evaporated under reduced pressure, and the dried extracts were stored in airtight vials at 4°C for further studies (Jain and Sharma, 2009). The methanol extract was chosen for the isolation and characterization of compounds, as well as the evaluation of antioxidant and antibacterial activities.

Isolation and Characterization of Compounds from *D. trigona*

Purification and Isolation

The methanol extract was purified using silica gel column chromatography with chloroform and methanol as eluents. During the purification process, two fractions were collected at different polarities. The first fraction, eluted with 5% methanol in chloroform (MeOH: $CHCl_3$, 5:95), yielded compound 1 as a pale brown solid. The second fraction, eluted with 8% methanol in chloroform (MeOH: CHCl3, 8:92), yielded compound 2 as a brown solid. The isolated compounds were further characterized by NMR and mass spectrometry.

Nuclear Magnetic Resonance (NMR) Studies

The isolated compounds were characterized by proton (1H) and carbon (13C) NMR spectra using a Bruker Advance NMR Spectrophotometer operating at 400 and 100 MHz, respectively. The ^{1}H-NMR spectra were recorded using tetramethylsilane (TMS) as a reference and DMSO-d6 as a solvent at ambient temperature. Chemical shifts were given in parts per million (δ-scale), and coupling constants were given in Hertz.

Mass Spectra (MS) Studies

Mass spectrometry is an important tool for the identification and structural determination of compounds. The mass spectra of the compounds were recorded using ESI mass spectrometry.

Estimation of Total Phenolic Compounds

The total phenolic content in compounds of *D. trigona* was assessed using the method outlined by Singleton *et al*., (1999). A stock solution of the extract at 5 mg/mL concentration was prepared, from which 20 µL (equivalent to 1 mg/mL) was mixed with 0.75 mL of 20% sodium carbonate solution and 0.25 mL of Folin-Ciocalteu reagent. This reaction mixture was exposed to light for 3 minutes, followed by incubation in the dark for 2 hours. The resulting color change in the solution containing the extract was measured at 765 nm using a UV-visible spectrophotometer. A standard calibration curve was created by measuring the absorbance of known concentrations of gallic acid (0–100 µg/mL) to quantify the total phenolics. The results were expressed as micrograms of gallic acid equivalent (GAE) per 100 µg of plant extract. The experiment was conducted three times, with each trial containing three replicates.

Antioxidant Activity

2,2-Diphenyl-1-Picrylhydrazyl Scavenging Activity (DPPH)

The DPPH assay, as outlined by Sultanova *et al.,* (2001), was used to assess the free radical scavenging activity of *D. trigona*

compounds. A stock solution was prepared by dissolving 1 mg of each compound in 1-mL of the respective solvents to achieve a 1-mg/mL concentration. For the reaction mixture, 5 µL of the compound solution was added to 95 µL of DPPH solution (300 µM in methanol). Test samples were prepared at various concentrations (100–1000 µg/mL) while maintaining a constant DPPH concentration. The mixtures were incubated at 37°C for 30 minutes, and absorbance was measured at 517 nm. Methanol was used as the control, and ascorbic acid served as the positive control. Each experiment was conducted in triplicate, with three replicates per concentration. The percentage of DPPH radical scavenging activity (RSA) was then calculated using the specified formula:

$$\%DPPH\ Scavenging\ Activity = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

Nitric Oxide Radical Scavenging Assay

The NO radical scavenging activity of *D. trigona* compounds was evaluated following the method by Marcocci *et al.,* (1994), with minor modifications. In this assay, 1-mL of sodium nitroprusside solution (5 mM) in 0.5 M phosphate buffer was combined with 3 mL of each compound at varying concentrations (100–1000 µg/mL). The mixture was then incubated at 25°C for 150 minutes, after which absorbance was measured at 546 nm. Ascorbic acid served as the positive control. This experiment was conducted in triplicate, with three replicates for each concentration. The NO radical scavenging capacity was determined using the following formula:

$$\%\ NO\ Scavenging\ Activity = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of *D. trigona* compounds was assessed following the method by Czochra and Widensk (2002). In this assay, 0.6mL of hydrogen peroxide solution (40 mM) prepared in phosphate buffer (pH 7.4) was combined with 2.4 mL of each compound at different concentrations (100–1000 µg/mL). The mixtures were incubated for 10 minutes, and absorbance was then recorded at 230 nm. Ascorbic acid was used as a reference standard. Each test was conducted three times with three replicates per concentration. The hydrogen peroxide scavenging percentage was calculated using the following formula:

$$\%H_2O_2 Scavenging\ Activity = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

Total Antioxidant Capacity by Phosphomolybdenum Method

The phosphomolybdenum method, as outlined by Prieto *et al.,* (1999), was used to determine the total antioxidant capacity of *D. trigona* compounds. In this assay, 0.3 mL of each compound, at concentrations ranging from 100 to 1000 µg/mL, was mixed with 3 mL of a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixtures were then incubated at 95°C for 90 minutes, and the absorbance was measured at 695 nm. Ascorbic acid was used as the standard for comparison. Each test was conducted in triplicate, with three replicates for each concentration.

Reducing Power Assay

The reducing power of *D. trigona* compounds was assessed based on the method described by Nagulendran *et al.,* (2007), with slight modifications. A 0.75 mL aliquot from a 1-mg/mL stock solution of each compound was combined with 0.75 mL of 0.2 M phosphate buffer (pH 6.6) and 0.75 mL of 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes. After incubation, 0.75 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant (1.5 mL) was then mixed with 1.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ solution. Absorbance was measured at 700 nm using a UV-visible spectrophotometer, with phosphate buffer serving as the blank and butylated hydroxytoluene (BHT) as the reference standard. The experiment was performed in triplicate, with three replicates for each concentration.

Antibacterial Activity

The compounds of *D. trigona* were prepared in methanol at a concentration of 25 mg/mL for testing antibacterial activity, following the modified method of Singh *et al*., (2009). The antibacterial efficacy was assessed against both gram-positive bacteria (*Staphylococcus aureus* MTCC 7443 and *Bacillus subtilis* MTCC 121) and gram-negative bacteria (*Escherichia coli* MTCC 7410 and *Salmonella typhi* MTCC 733). Streptomycin (1-mg/mL) served as the reference antibacterial agent. The microbial cultures were adjusted to match the 0.5 McFarland standard, approximating a cell density of 1.5 x 10^8 cfu/mL. Nutrient agar plates (20 mL) were prepared and solidified under sterile conditions, followed by swabbing with 50 µL of each bacterial inoculum and a 15-minute incubation for adsorption. Sterile discs (6 mm diameter) were then infused with 0.5 and 0.75 mg of the compounds per disc and placed onto the agar surface. Plates were incubated at 37°C for 24 hours, and antibacterial activity was measured by observing the zone of inhibition around each disc. Methanol served as the negative control, while Streptomycin (10 µg/disc) was used as the positive control. The experiment was conducted three times, each with three replicates.

Statistical analysis

For statistical analysis, data obtained from three replicates of each experiment were processed. Analysis of variance (ANOVA) was conducted using SPSS software version 16.0 to evaluate the significance of treatment effects, with significance determined by F-values at a threshold of $p \leq 0.05$. Differences between treatment means were assessed using Tukey's Honestly Significant Difference (HSD) test.

Result

In this study, the methanolic extract of *D. trigona* leaves, which is rich in phytoconstituents, was used for the purification of bioactive compounds. The methanolic extract of *D. trigona* revealed the presence of two compounds *viz*., BHT and pentadecanoic acid, with different molecular weights, molecular formulas, and structures (Fig. 1). The ^{1}H NMR spectrum of BHT revealed the presence of aromatic protons, which was observed at 6.88 to 6.84 δ values, a singlet at chemical shift value of 6.5

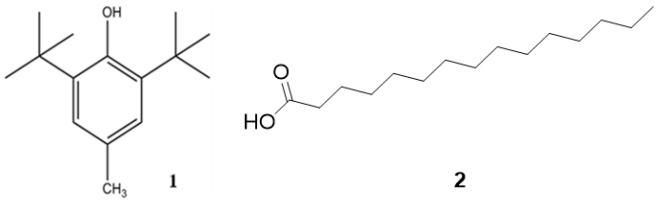

Fig. 1: Structure of isolated compounds [compound 1 (BHT) and compound 2 (Pentadecanoic acid)]

δ confirmed the presence of phenolic OH, a singlet at chemical shift value of 2.1 δ confirmed the presence of aromatic methyl (CH_3) protons and another singlet peak at 1.2 δ confirming the presence of *t*-butyl protons in the molecule. The mass spectrum complements the NMR spectra by giving an m/z value of 220 (Figs 1 and 2). The ^{1}H-NMR spectrum of pentadecanoic acid revealed the presence of an acid proton at 7.2 δ and peaks at aliphatic region 2.0 to 0.8 confirm the presence of alkyl protons. The mass spectrum showed a peak at m/z 242, which corresponds to the mass of pentadecanoic acid (Figs 3 and 4). From the analysis, it was revealed that molecular weight, molecular formula, and structure of the BHT and pentadecanoic acid are complementing with the structure of 2,6-Bis (1,1- dimethylethyl)-4-methylphenol (Butylated Hydroxytoluene- BHT), a phenolic compound and Pentadecanoic acid.

The two purified compounds of *D. trigona* were subjected to analysis of total phenolic content and antioxidant and antibacterial activity. The BHT and pentadecanoic acid were shown to contain 65.09 and 52.47 µg of GAE, respectively.

The DPPH, nitric oxide and hydrogen peroxide scavenging activity of BHT and pentadecanoic acid of *D. trigona* were carried out to determine the antioxidant activity. The DPPH activity of BHT and pentadecanoic acid showed an IC_{50} value of 21.26 µg/mL and 23.01 µg/mL, respectively, while standard ascorbic acid showed IC_{50} value of 40.06 µg/mL (Fig. 2A). The nitric oxide scavenging activity results indicated that BHT exhibited an IC_{50} value of 22.50 µg/mL, which was lower than that of pentadecanoic acid, with an IC_{50} value of 23.61 µg/mL. In comparison, the control, ascorbic acid, showed an IC_{50} value of 24.16 µg/mL (Fig. 2B). Similarly, the hydrogen peroxide activity of BHT exhibited a significant activity with an IC_{50} value of 23.05 µg/mL, while pentadecanoic acid showed an IC_{50} value of 25.45 µg/mL. The standard ascorbic acid showed IC_{50} value of 27.41 µg/mL concentration (Fig. 2C). The results indicated that the

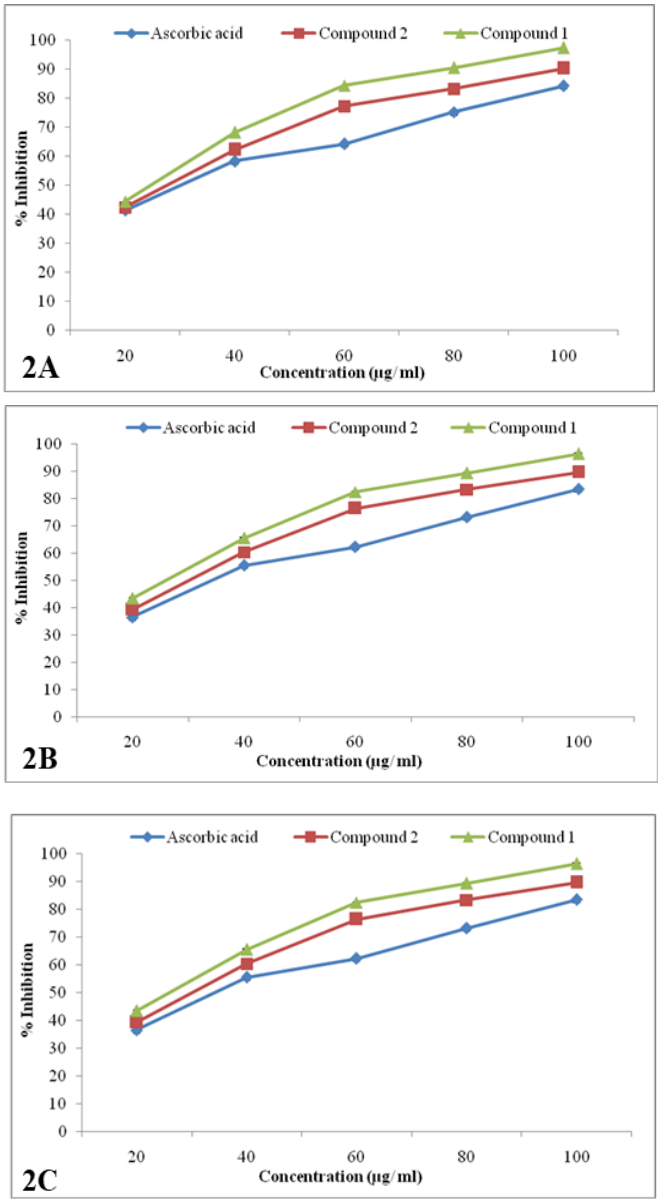

Fig. 2: Antioxidant activities of methanol extract of *D. trigona*. **2A:** DPPH scavenging activity; **2B:** Nitric oxide scavenging activity; **2C:** Hydrogen peroxide scavenging activity. The values represent the mean of three independent replicates, with error bars indicating the standard error of the mean.

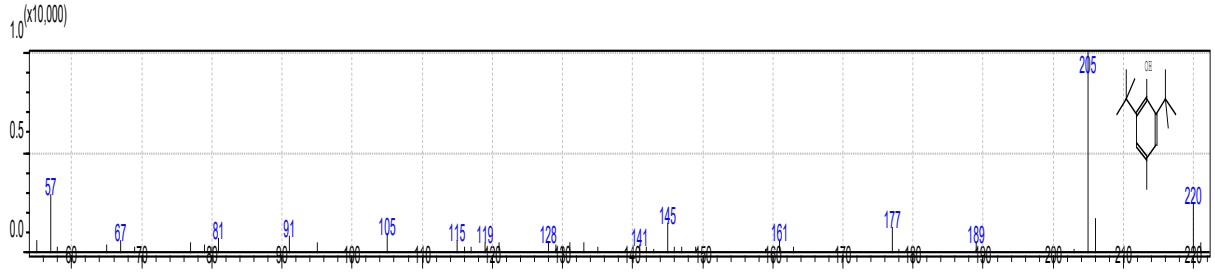

Fig. 3: ^{1}H-NMR Spectrum of compound 1, identified as butylated hydroxytoluene (BHT:2,6-Bis(1,1-dimethylethyl)-4-methylphenol).

antioxidant activity of BHT and pentadecanoic acid was almost similar to the standard ascorbic acid in nitric oxide and hydrogen peroxide activity. In the DPPH assay antioxidant activity of both compounds was almost half of the standard ascorbic acid. The antioxidant activity of BHT was higher than pentadecanoic acid in all three assays carried out, which corresponds to its total phenolic content.

The two purified compounds showed promising results in total antioxidant capacity by phosphomolybdenum method and reducing power assay. Ascorbic acid, used as a standard for total antioxidant capacity, showed an increase in absorbance from 0.201 at 20 µg/mL to 0.422 at 100 µg/mL and BHT was used as a standard for reducing power assay, which showed an increase in absorbance from 0.88 at 20 µg/mL to 1.452 at 100 µg/mL. The absorbance of BHT was higher than pentadecanoic acid at different concentrations tested. The absorbance of BHT increased from 0.205 at 20 to 0.491 at 100 µg/mL and 1.002 at 20 to 1.891 at 100 µg/mL concentration in total antioxidant capacity and reducing power assay, respectively. Similarly, the pentadecanoic acid also showed an increase in absorbance from 0.178 at 20 to 0.46 at 100 µg/mL and 0.864 at 20 to 1.67 at 100 µg/mL concentration in total antioxidant capacity and reducing power assay, respectively. The initial and final absorbance of BHT and pentadecanoic acid were on par with the ascorbic acid, and BHT used as standard in both assays (Figs 3 and 4).

The two purified compounds from the methanol extract of *D. trigona* showed good antibacterial activity. BHT and pentadecanoic acid were tested at 0.5 mg/disc and 0.75 mg/

Fig. 4: Mass spectrum of compound 1 butylated hydroxytoluene (BHT: 2,6-Bis (1,1- dimethylethyl)-4-methylphenol)

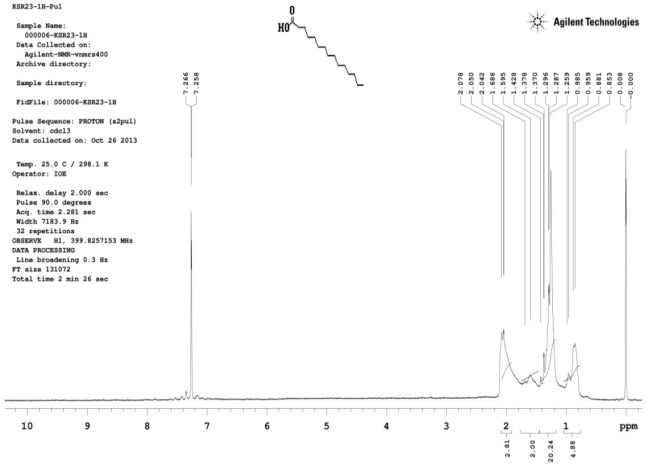

Fig. 5: ^{1}H-NMR spectrum of compound 2 pentadecanoic acid

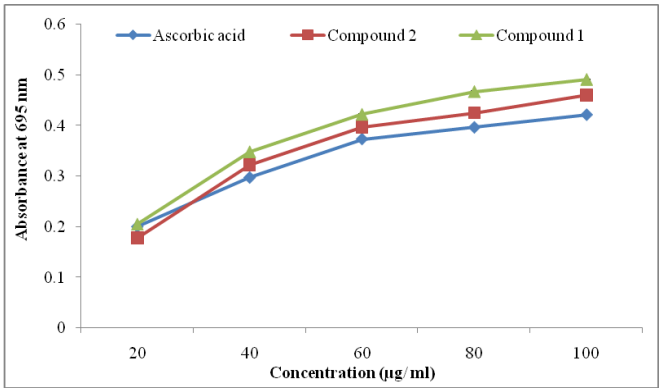

Fig. 6: Total antioxidant capacity of BHT and Pentadecanoic acid from methanol extract of *D. trigona*. The values represent the average of three independent replicates, with ± indicating the standard error.

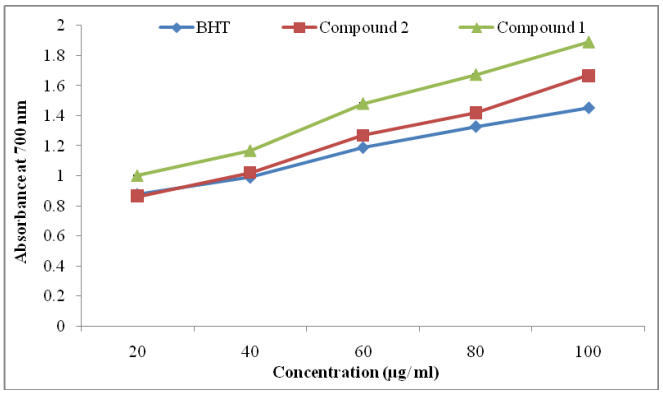

Fig. 7: Reducing power assay of BHT and Pentadecanoic acid from methanol extract of *D. trigona*. The values represent the average of three independent replicates, with ± indicating the standard error.

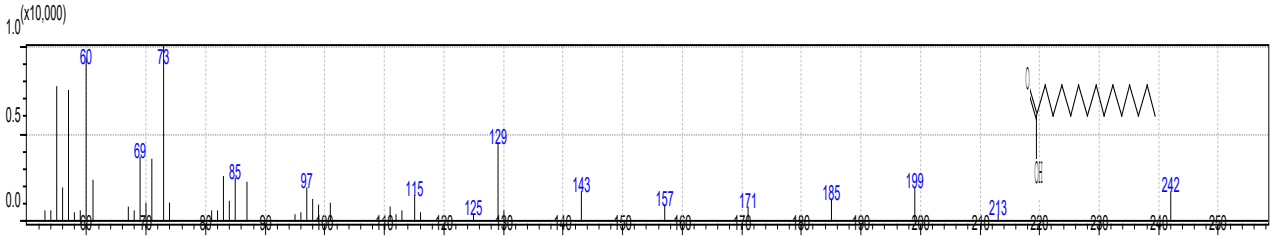

Fig. 8: Mass spectrum of compound 2 pentadecanoic acid

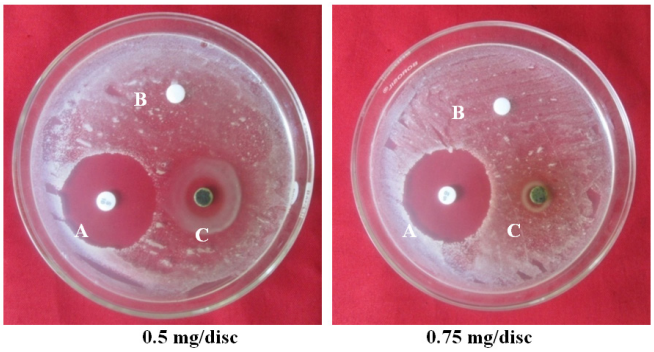

Fig. 9: Antibacterial activity of BHT from methanol extract of *D. trigona* against *S. aureus*. A. Streptomycin (10 µg/disc); B. Methanol control; C. BHT

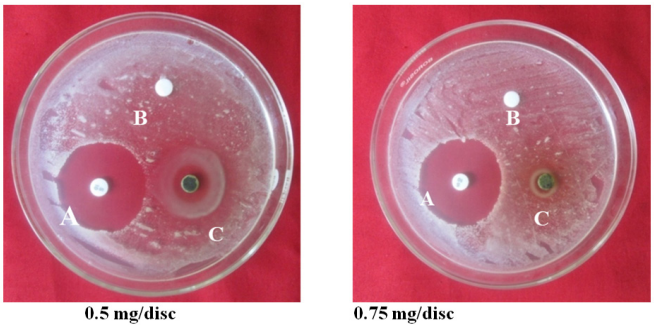

Fig. 10: Antibacterial activity Pentadecanoic acid from methanol extract of *D. trigona* against *S. aureus*. A. Streptomycin (10 µg/disc); B. Methanol control; C. Pentadecanoic acid

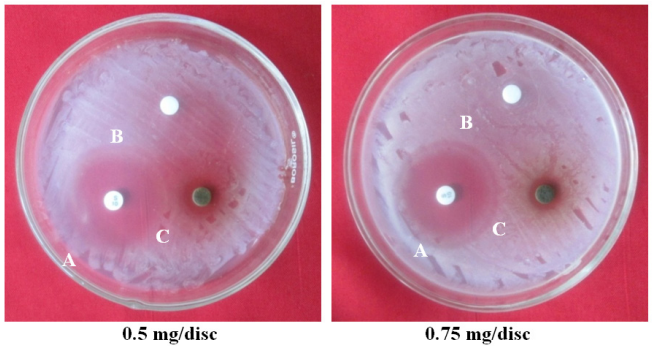

Fig. 11: Antibacterial activity of BHT acid from methanol extract of *D. trigona* against *B. subtilis***.** A. Streptomycin (10 µg/disc); B. Methanol control; C. BHT

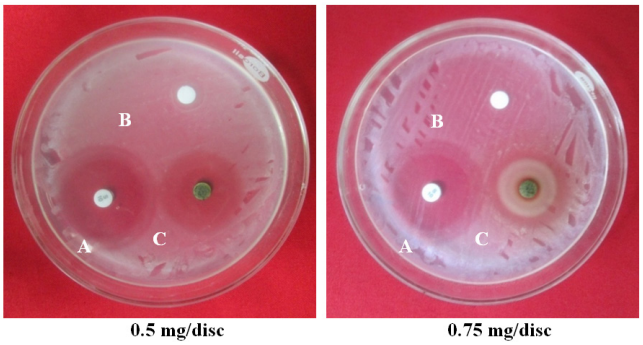

Fig. 12: Antibacterial activity of Pentadecanoic acid from methanol extract of *D. trigona* against *B. subtilis***.** A. Streptomycin (10 µg/disc); B. Methanol control; C. Pentadecanoic acid

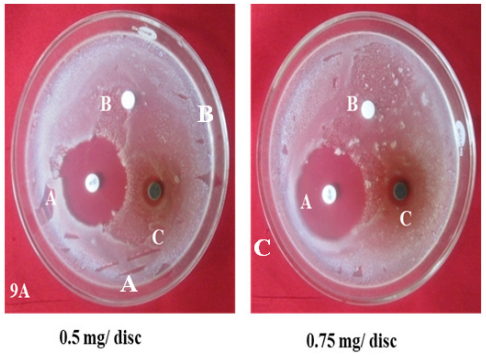

Fig. 13A: Antibacterial activity of BHT from methanol extract of *D. trigona* against *E. coli*. **A**: Streptomycin (10 µg/disc); **B**: Methanol control; **C**: BHT (0.5 mg and 0.75 mg/disc).

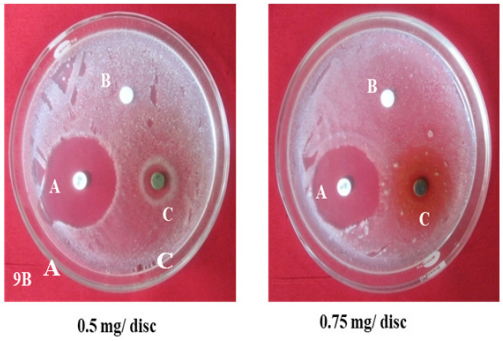

Fig. 13B: Antibacterial activity of pentadecanoic acid from methanol extract of *D. trigona* against *E. coli*. **A**: Streptomycin (10 µg/disc); **B**: Methanol control; **C**: Pentadecanoic acid (0.5 mg and 0.75 mg/disc).

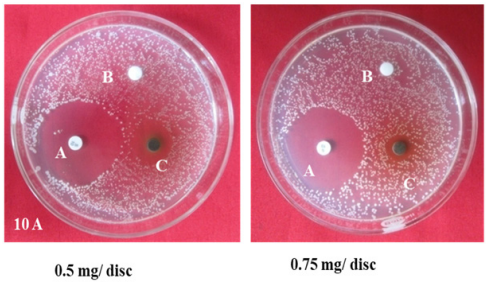

Fig. 14A: Antibacterial activity of BHT from methanol extract of *D. trigona* against *S. typhi*. A: Streptomycin (10 µg/disc); B: Methanol control; C: BHT (0.5 mg and 0.75 mg/disc).

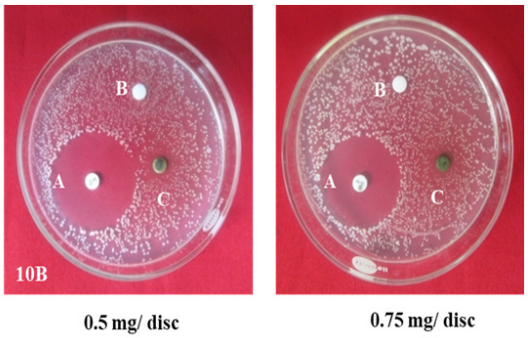

Fig. 14B: Antibacterial activity of pentadecanoic acid from methanol extract of *D. trigona* against *S. typhi*. A: Streptomycin (10 µg/disc); B: Methanol control; C: Pentadecanoic acid (0.5 mg and 0.75 mg/disc).

Table 1: Antibacterial activity of BHT and Pentadeconoic acid from methanol extract of *D. trigona* against test pathogens

Plants	Concentration (mg/disc)	Inhibition zone (mm)			
		S. aureus	*B. subtilis*	*E. coli*	*S. typhi*
BHT	0.5	15.36 ± 0.20	14.23 ± 0.20	11.10 ± 0.26	10.13 ± 0.15
	0.75	11.16 ± 0.20	12.26 ± 0.25	12.06 ± 0.20	-
Pentadeconoic acid	0.5	24.23 ± 0.15	29.4 ± 0.26	12.2 ± 0.3	-
	0.75	-	22.33 ± 0.15	12.26 ± 0.20	-
Solvent control		-	-	-	-
Streptomycin	10 µg/disc	29.56 ± 0.30	36.13 ± 0.20	27.46 ± 0.25	27.23 ± 0.15

The values represent the average of three independent replicates, with ± indicating the standard error. NA: Not applicable.

disc concentrations. Among the two concentrations tested 0.5 mg/disc concentration showed higher antibacterial activity than 0.75 mg/disc concentration in both the compounds, and comparatively, pentadecanoic acid showed higher antibacterial activity than BHT against *S. aureus*, *B. subtilis* and *E. coli*. *S. aureus* was susceptible to BHT at 0.5 mg/disc concentration, which was inhibited to a maximum extent (inhibition zone of 15.36 mm), followed by *B. subtilis* (inhibition zone of 14.23 mm), *E. coli* (inhibition zone of 11.1 mm) and S. typhi (inhibition zone of 10.13 mm), while at 0.75 mg/disc concentration, it was effective against *B. subtilis*, *E. coli* and *S. aureus* only with 12.26, 12.06 and 11.16 mm of inhibition zone, respectively. The pentadecanoic acid at 0.5 mg/disc concentration, *B. subtilis* showed the highest inhibition zone of 29.4 mm followed by *S. aureus* (inhibition zone of 24.23 mm) and *E. coli* (inhibition zone of 12.2 mm), while *S. typhi* remains unaffected. The pentadecanoic acid at 0.75 mg/disc concentration was efficient in inhibiting *B. subtilis* and *E. coli* with 22.33 and 12.26 mm of inhibition zone. The positive control streptomycin offered a maximum of 36.13 mm inhibition zone against *B. subtilis* and a minimum of 27.23 mm inhibition zone against *S. typhi* (Figs 5 to 14B and Table 1).

Discussion

Phenolic compounds possess redox characteristics that allow them to function as singlet oxygen quenchers, hydrogen donors, and reducing agents, which contribute to their antioxidant activity (Pietta, 2000). Additionally, they possess photosensitive reactions, antimicrobial properties, and protective effects against cancer and cardiovascular diseases, making them valuable in human diets and plant-based beverages (Nychas *et al.,* 2003). The synthetic phenolic antioxidant BTH, chemically known as 2,6-Bis(1,1-dimethylethyl)-4-methylphenolis known for its cancer-preventive properties (Slaga, 1995). This study confirms that *D. trigona* can naturally produce BHT. The research findings align with those of Bouftira *et al.,* (2007), who isolated BHT from the methanolic leaf extract of *Mesembryanthemum crystallinum*. BHT has also been identified in *Asclepias physocarpa* (Ma *et al*., 2006) and in hexane extracts of various algae, including *Botryococcus braunii*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, and *Oscillatoria* species (Babu and Wu, 2008) and diethyl ether-hexane extracts of leaf, stem, flower, and fruit of *Cytisus triflorus* (Aourahoun *et al.,* 2014).

Currently, available antibacterial agents are often limited due to their toxicity, reduced efficacy, and high cost for prolonged treatments. Hence, there is a need to develop new antibacterial agents that can meet current demands (Nair and Chanda, 2006).

Conclusion

The antibacterial activity of the purified compounds was comparable to that of standard antibiotics, suggesting that *D. trigona* leaves can yield highly potent antibacterial molecules in pure form. The compounds isolated from the methanol extract of *D. trigona* leaves have potential as therapeutic agents and can be used in pharmaceutical industries. It is concluded that the synergistic effect of both antioxidant and antibacterial activities enhances the medicinal value of *D. trigona*. Additionally, *D. trigona* can serve as a natural source of bioactive products, potentially leading to the development of new pharmaceuticals to address therapeutic needs.

Author's contributions

GKP carried out the sample collection, soxhlet extraction, antioxidant and antibacterial activities, and results in interpretation and write-up. KSS did NMR and MS analysis and interpretation. KNA guided me in analyzing the data. All authors read and approved the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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