

Antioxidant and Antimicrobial Activities of β -Caryophyllene dominated Leaf Essential oil of *Trichilia monadelpha* (Thonn.) JJ De Wilde

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Abstract

Trichilia monadelpha (Thonn) JJ De Wilde (Meliaceae) is an essential plant of diverse medicinal uses in folk medicine. It is deployed in the treatment of ulcers/epidermal infections, epilepsy, gonorrhoea and inflammatory disorders such as arthritis etc. This study investigated the leaf essential oil constituents of *T. monadelpha* along with the corresponding antioxidant and antimicrobial activities. The essential oil of fresh leaves of *T. monadelpha* was isolated by hydrodistillation using all glass Clevenger distillation apparatus while the identification and characterization was done by Gas Chromatography-Mass Spectrometry (GC-MS). Antioxidant activity was carried out by scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) at 517nm in a UV-Visible spectrophotometric assay with Ascorbic acid, Butylated hydroxyanisole (BHA) and α -Tocopherol as reference standards. Antimicrobial assay was examined by Agar diffusion method. A total of fifteen compounds were identified in the essential oil with β -Caryophyllene (35.41%) as the major constituent. The oil demonstrated moderate inhibition against seven of the ten microorganisms used for the assay, namely: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Rhizopus stolonifer*. The antioxidant activity of the essential oil (IC₅₀ of 3.72 mg/ml) though lower than that of ascorbic acid (IC₅₀ of 1.47 mg/ml) and BHA (IC₅₀ of 1.88 mg/ml) was however better than that of α -Tocopherol (IC₅₀ of 4.83 mg/ml). The results obtained corroborated the uses of *Trichilia monadelpha* in folk medicine.

Keywords: Gas Chromatography-Mass Spectrometry, Essential oil, β -Caryophyllene, Antioxidant and Antimicrobial Activities.

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1. Introduction

Plants have been the major source in the treatment of various diseases, especially in developing countries (Benoit-Vical, 2006). Some of these plant extracts and phytochemicals are known to have antimicrobial properties and are of great significance in therapeutic treatments. A number of studies have been conducted in different countries to demonstrate such efficacy (Senatore et al, 2007; Langseth et al, 1996). Medicinal plant constituents have a long history of use in old and modern medicines. They are the source of important medications such as vincristine, codeine, quinine, morphine, digoxin, atropine and others, some of which are still currently in use (Raskin et al, 2002).

Essential oils from aromatic plants and spices have a long history of use as natural microbial agents in folk medicine. They also have wide applications in food, cosmetics and pharmaceutical industries as these oils are known to possess antimicrobial, antiviral and antioxidant properties. Essential oils are employed as antioxidants due to their small molecular size and their ability to easily penetrate the skin tissue. They are lipid soluble and are capable of penetrating the membranes easily even in conditions where oxygen deficiency leads to hardening of membranes. Studies reveal that essential oils serve as powerful antioxidants that produce adverse environment for damaging free radicals, thus preventing mutations and oxidants in cells. They therefore function as scavengers for free radicals. Essential oils are extracted from different plant parts: fruits, leaves, flowers, barks, roots and seeds with each part possessing unique characteristics (Bako et al, 2005; Bray, 1999; Potterat, 1997).

Oxidative damage in human cells or tissues is mainly due to the uncontrolled production of oxygen from free radicals. These are harmful substances produced in the body during the normal metabolic processes. Bacterial, fungal and viral infections have also been reported to produce reactive oxygen species. Environmental chemicals

such as pesticides and other chemicals like alcohol, cigarette smoking, burning of organic matter, UV radiations and automobile pollution also contribute to free radical generation (Sato et al, 1995). Medicinal plants with antibacterial and antioxidant potentials are thus used as alternative therapy for bacterial infections and many kind of degenerative diseases in folk medicine (Cai, 1996).

However, the need for new antioxidant and antimicrobial agents has increased remarkably over the years due to multiple drug resistance and fewer side effects of drugs from natural sources as compared to synthetic drugs. *T. monadelpha* (Thonn) JJ De Wilde (Meliaceae) is a medium-sized tree that grows 12–20 m high, distributed in deciduous and semi-deciduous secondary forests, often in wet places in West Africa. *T. monadelpha* has diverse application in folklore in the treatment of ailments namely: Yaws, Ulcers, abortion, arthritis, cough, gonorrhoea, syphilis, chancres and syphilitic sores, wounds/cuts/epidermal infections, rickets, sterility, amenorrhoea, stomach pain, gastrointestinal pain and disorders, dysentery and fever (Burkill, 1997; Abbiw, 1990).

Earlier preliminary phytochemical screening of different stem bark extracts of *T. monadelpha* revealed the presence of Alkaloids, saponins, flavonoids, tannins, glycosides, terpenoids and sterols. Some of the investigated pharmacological activities of *T. monadelpha* include: antioxidant, wound healing, analgesic, anti-inflammatory, and anti-anaphylactic properties (Ben et al., 2013; Woode et al., 2012 & 2013; Tuwari et al., 2011; Kokate, 2005), analgesic (Dos Santos et al., 2010), antidepressant (KwamiEdem et al., 2018), antimicrobial and antioxidant (Quartey, 2014). In a continuing search for more potent natural products with fewer side effects (Odeja et al., 2020; Oloyede et al., 2020; Onocha et al., 2016), we present the result of the investigation of the chemical constituent, antioxidant and antimicrobial activities of the leaf essential oil of *T. monadelpha* which is being reported for the first time.

2. Materials and methods

2.1 Plant material

Fresh leaves of *T. monadelpha* were collected from botanical garden of University of Ibadan, Ibadan. Specimens were identified and authenticated at Forest Research Institute of Nigeria (FRIN), Oyo State, Nigeria where a voucher specimen with herbarium number FHI 112655 was deposited in the FRIN herbarium. The leaves were chopped and hydro-distilled immediately.

2.2 Chemicals and reagents

Hexane, methanol, butylated hydroxyanisole (BHA) and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), ascorbic acid and α -tocopherol were obtained from Sigma Chemical Co. (Germany).

2.3 Equipment and Apparatus

UV-Visible Spectrophotometer (Uicol 200 and Perkin Elmer lambda 25 models), GC-Mass spectrophotometer (Agilent Technologies), Hydro distiller-all glass Clavenger apparatus.

2.4 Isolation of essential oil

The oil was obtained by hydro-distillation on all glass Clavenger distillation apparatus for 4hrs in accordance with the British pharmacopoeia specifications (1980). The essential oil (TML) was collected and stored at 4°C prior to analysis.

2.5 Gas Chromatography-Mass Spectrometry

GC-MS analyses of the essential oil was analyzed on an Agilent Technologies 7890A GC system coupled to a 5975c VLMSD mass spectrometer with an injector 7683b series device. An Agilent (9091)-413:325°C HP-5 column (30 m x 320 μ m x 0.25 μ m) was used with helium as carrier gas at flow rate of 3.3245 mL min⁻¹. The GC oven temperature was initially programmed at 50°C (hold for 1 min) and finally at 300°C (hold for 5mins) at a rate of 80°C min⁻¹ while the final temperature was 37.25°C. The column heater was set at 250°C and was a split less mode while the pressure was 10.153 psi with an average velocity of 66.45 cm sec⁻¹ and a hold-up time of 0.75245 min was recorded. Mass spectrometry was run in the Electron Impact mode (EI) at 70 eV. The percentage compositions were obtained from electronic integration measurements using Flame Ionization Detector (FID) set at 250°C. The peak numbers and relative percentages of the characterized components are given in Table 1.

2.6 Antioxidant Activity

A 0.5 ml of the radical source of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) solution in methanol was prepared and 3 ml of this solution was mixed with 1 ml of the oil sample in methanol (Oloyede et al., 2011; Onocha et al., 2011). The decrease in absorption (A) at 517 nm of DPPH was measured after 10 mins of incubation. The actual decrease in absorption was measured against that of control and the percentage inhibition (% I) was also calculated. The same experiment was carried out on ascorbic acid, α -Tocopherol and butylated hydroxyanisole (BHA) which are known antioxidants. All test and analysis were run in triplicates and the result obtained were averaged. The activities were determined as a function of their % inhibition which was calculated using the formula:

$$\% I = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.7 Antimicrobial Activity

All microorganisms were clinical isolates obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. The leaf essential oil of *T. monadelpha* was tested against 10 strains of microorganisms consisting of 7 bacteria: 5 Gram negative (*Escherichia coli* and *Klebsiella pneumonia*, *Salmonella typhi*, *Klebsiellae pneumonia*, *Pseudomonas aeruginosa*), 2 Gram-positive: (*Staphylococcus aureus* and *Bacillus subtilis*) and 3 fungi (*Candida albicans*, *Penicillium notatum* and *Aspergillus niger*). Agar diffusion method (Pour broth dilutions and surface plate for antibacterial and antifungal activities, respectively) was employed (Kalemba & Kunicka, 2003; Okpala et al., 2019). Oil sample was prepared such that 1 ml of the oil was regarded as 100 % concentration. 0.5 ml into 0.5 ml of Hexane gave 50 % concentration and 25 % concentration was obtained using serial dilution. Hexane was used as a negative control, Gentamicin (10 $\mu\text{g}/\text{mL}$) as a positive control for bacteria and Tioconazole (0.7 mg/mL) as positive control for Fungi. All tests were carried out in triplicates. Observed zones of inhibition of growth were measured and recorded in millimetre (mm).

3. Results and Discussion

3.1 Identification of components

The individual constituents of the oil were identified on the basis of their retention indices, determined with reference to a homologous series of n-alkanes and by comparison of their mass spectral fragmentation patterns (NIST 08.L database/chemstation data system) with data previously reported in literature by Adams (2001), Joulain & Konig, (1998) and McLafferty & Stauffer (1998). The hydrodistillation of the leaves of *T. monadelpha* gave a high level of sesquiterpenes hydrocarbon in the essential oil. Fifteen compounds were identified in the essential oil representing a total percentage composition of 89.44% with β -Caryophyllene (35.41%) as the major compound. Other constituents identified include: 1-Aminomethyl cyclododecanol (15.14%), Cyclohexadecane (8.22%), α -Cubebene (6.56%), 1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene (3.04), Isopropyl ester (3.11%), Cycloeicosane (2.75%), (Z,Z)-2,6-Farnesol (2.61%), 1-(1,5-Dimethylhexyl)-4-(4-methylpentyl)cyclohexane (2.21%), Squalene (2.06%) etc. The volatile metabolites were predominantly sesquiterpenes hydrocarbon in nature (45.01 %) and contained 2.62 % sesquiterpenoids, while the non-terpene derivatives comprises of 37.79% as shown in Table 1. β -Caryophyllene is a bicyclic sesquiterpene reported to be one of the major active components of essential oils derived from a large number of spice and food plants such as: basil (*Ocimum* spp.), cinnamon (*Cinnamomum* spp.), black pepper (*Piper nigrum* L.), cloves (*Syzygium aromaticum*), cannabis (*Cannabis sativa* L.), lavender (*Lavandula angustifolia*), oregano (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis*) (Fidy et al., 2016). Its medicinal implication has also been reported. These include: anti-inflammatory (Medeiros et al., 2007), anticarcinogenic (Langhasova et al., 2014), antimicrobial (Sabulal et al., 2006), antioxidative (Singh et al., 2006) and analgesic activities (Klauke et al., 2013).

Table 1: Chemical composition of the leaf essential oils of *Trichilia monadelpha*

S/N	Retention Index	Compounds	% Composition
1	-	9-(1-Methylethylidene)-bicyclo[6.1.0]nonane	2.07
2	-	1-(1,5-Dimethylhexyl)-4-(4-methylpentyl)cyclohexane	2.21
3	-	Bromoacetic acid	1.6
4	-	Cycloeicosane	2.75
5	-	1-Aminomethyl cyclododecanol	15.14
6	1476	β-Caryophyllene	35.41
7	1519	α -Cubebene	6.56
8	1519	1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene	3.04
9	1695	(Z,Z)-2,6-Farnesol	2.61
10	1883	Cyclohexadecane	8.22
11	1921	6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one	1.09
12	2023.5	Isopropyl ester	3.11
13	2828	Squalene	2.06
14	2835.8	Spinacene	1.97
15	3745.68	Octadecyl ester	1.6
Total			89.44

3.2 Antioxidant Activity

Antioxidant activity (scavenging assay) of essential oil of *T. monadelpha* leaves was determined with DPPH method. The scavenging assay was carried out to determine the ability of the essential oil in inhibiting free radicals. The leaf essential oil of *T. monadelpha* displayed a moderate free radical scavenging activity at all the concentrations varying from 60.5 % to 53.6% as shown in Table 2. The antioxidant activity of the essential oil though lower than that of ascorbic acid and BHA was however better than that of α -Tocopherol. Furthermore, the scavenging activity effectiveness was measured through IC₅₀ (the concentration of the samples required to scavenge 50 % of the hydroxyl radicals. The lower the IC₅₀, the greater the overall effectiveness of the suspected antioxidant potency. From the results obtained, the antioxidant effectiveness is as follows: ascorbic acid (1.47 mg/ml) > butylated hydroxyanisole (1.88 mg/ml) > TML (3.72 mg/ml) > α -Tocopherol (4.83 mg/ml). The percentage inhibition is presented in Table 2 and Fig. 1.

Table 2: DPPH radical scavenging activity of leaf essential oil (TML) and standards

Concentration (mg/ml)	Absorbance values from scavenging effects on DPPH at 517nm			
	TML	BHA	Ascorbic Acid	α -Tocopherol
1.0	60.5	84.7	92.1	53.7
0.5	59.7	82.8	92.0	53.6
0.25	58.3	75.2	91.2	52.2
0.125	58.2	81.2	91.1	48.2
0.0625	53.6	75.2	91.1	45.0
IC ₅₀	3.72	1.88	1.47	4.83

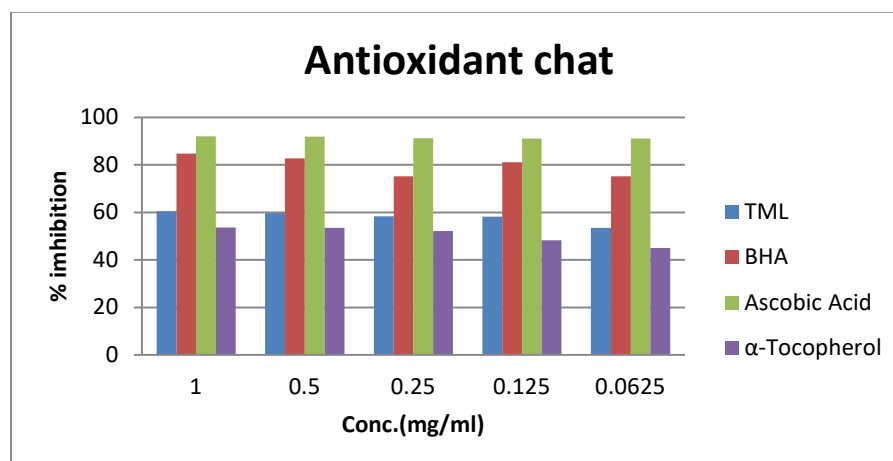


Fig 1: % Inhibition of leaf essential oil (TML) and standards

3.3 Antimicrobial Activity

The antimicrobial activity of the essential oil was determined against ten microorganisms. *In vitro* antimicrobial activity of the leaf essential oil of *T. monadelphpha* resulted in a range of growth inhibition patterns against the studied pathogenic microorganism. The antimicrobial assay showed that at high concentration of 200 µg/mL, 100 µg/mL and 50 µg/mL the essential oil was moderately active against *Staphylococcus aureus*, *Esherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Rhizopus stolonifer*. At 200 µg/mL, the range was 20 mm to 16 mm, at 100 µg/mL, the range was 18 mm to 14 mm while 50 µg/mL, the range was 14 mm to 10 mm zone of inhibition. However, the extract showed more activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, *Aspergillus niger*. At lower concentration of 25 µg/mL, the zone of inhibition was between 12 mm to 10 mm for *Staphylococcus aureus*, *Esherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*. At 12.5 µg/mL, the extract showed activity only against *Pseudomonas aeruginosa*, the zone of inhibition was 10 mm. At 6.25 µg/mL the extract showed no inhibition against all the tested micro-organisms. The diameter of zone of inhibition is presented in Table 3.

Table 3: Antimicrobial analysis of the leaf essential oils of *Trichilia monadelphpha* (TML)

Concentration(µg/mL)	Diameter of zone of inhibition of microorganisms (mm)									
	S.A	E.C	B.S	P.A	S.T	KP	C.A	A.N	RS	PN
200	16	16	18	20	-	-	18	18	16	-
100	14	14	14	18	-	-	14	14	14	-
50	12	12	12	14	-	-	12	12	10	-
25	10	10	10	12	-	-	10	10	-	-
12.5	-	-	-	10	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	40	38	40	38	40	38	28	28	28	26

SA: *Staphylococcus aureus*, EC: *Eshericha coli*, B.S: *Bacillus subtilis*, PA: *Pseudomonas aeruginosa*, ST: *Salmonella typhi*, KP: *Klebsiellae Pneumonia*, CA: *Candida albicans*, AN: *Aspergillus niger*, PN: *Penicillium notatum*, RS: *Rhizopus stolonifer*, -ve: Negative control (Hexane), +ve: Positive control; Gentamicin 10 µg/mL (bacterial) and Tioconazole 0.7 mg/mL (fungi).

4. Conclusion

Fifteen compounds were identified in the essential oil from the leaves of *T. monadelphpha* wherein β-Caryophyllene was the most abundant compound. The predominance of the bioactive β-Caryophyllene which is known for its

diverse pharmacological properties: antioxidant, wound healing, analgesic, anti-inflammatory, anti-anaphylactic, antidepressant and antimicrobial, in the leaf essential oil could be responsible for the diverse medicinal application of the plant in folk medicine. The results obtained justifies the uses of *Trichilia monadelpha* in folk medicine.

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Conflict of interest

The authors declare that there is no conflict of interest.

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