

Antimicrobial and Antioxidant Activities of Sesquiterpenes-Rich Essential Oil of *Trichilia monadelpha* (Thonn) J.J. De Wilde of Root Bark

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Abstract

Trichilia monadelpha (Thonn) J.J. De Wilde (Meliaceae) is used in traditional medicine for the treatment of ulcers, cough and inflammatory disorders such as arthritis. In this present study, the essential oil of *T. monadelpha* root bark was obtained by hydrodistillation using an all-glass Clevenger apparatus. The identification and characterisation were done using Gas Chromatography-Mass Spectrometry. We also, aimed to evaluate the antimicrobial activity of the essential oil against ten micro-organisms using the Agar diffusion method and the free radical scavenging capacity was determined using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) methods. Twenty (20) different compounds made up the hydrodistilled essential oil, which made up 99.0% of the entire oil content. Sesquiterpene compounds made up about 73.3% of the essential oil from the root bark of *T. monadelpha*, which was described as sesquiterpenes-rich. Sesquiterpenes' most abundant constituents include (*E*)-Longipinene (18%), 10s,11s-Himachala-3-(12),4-diene (15.26%), Aromadendrene (11.12%), Alloaromadendrene oxide-(1) (8.82%), and β -Caryophyllene (5.92%). The essential oil inhibited growth against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Klebsiella Pneumonia* and *Salmonella typhi* at concentrations of 200 mg/mL to 50 mg/mL with an inhibitory zone of 20 – 10 mm while the antioxidant analysis of the essential oil revealed low scavenging activity which reveals that the synthetic antioxidants were more effective with an Ascorbic acid; IC₅₀ value of 1.47 mgmL⁻¹, Butyl hydroxyanisole; 1.88 mgmL⁻¹, α -Tocopherol; 4.83 mgmL⁻¹ followed by the essential oil with an IC₅₀ value of 9.95 mgmL⁻¹. *T. monadelpha* root bark essential oil contains notable chemical compounds that are responsible for its antioxidant and antimicrobial activities.

Keywords: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH); Antimicrobial; Essential oil; Sesquiterpenes; *Trichilia monadelpha*.

Abbreviations: DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; BHA: Butyl hydroxyanisole; RI: Retention Index; FRIN: Forest Research Institute of Nigeria; TMRO: *T. monadelpha* essential Root Bark Essential Oil

INTRODUCTION

Almost every culture in the world has evolved an indigenous system of traditional healing (Abbiw 1990). Plants are an important source of drugs, especially in traditional medicines (Akihisa *et al.* 2001). Atropine from *Atropa belladana*; morphine and codeine from *Papaver somniferum*; quinine and quinidine from *Cinchona spp* and digoxin from *Digitalis spp* are examples of drugs with plant sources. Approximately 60% of all the anti-infectious and anti-tumor drugs already on the market or under clinical trials are estimated to be of natural origin (Bako *et al.* 2005).

The role of free radicals in many disease conditions has been established. Various biochemical reactions in the human body generate reactive oxygen species and

these are capable of damaging important bio-molecules leading to diseased conditions if they are not effectively scavenged by cellular constituents (Halliwell and Gutteridge 1990; Okpala *et al.* 2019). These radicals include superoxide anions, hydroxyl, nitric oxide and hydrogen peroxide radicals (Halliwell and Gutteridge 1992; Raghuvver and Tandon 2009). Antioxidants of natural and synthetic origin prevent free radical damage by their protective role such as reacting with them, chelating catalytic metals and acting as oxygen scavengers. Ingestion of several synthetic antioxidants such as Butylhydroxyl toluene (BHT) and Butylated hydroxyanisole (BHA) has been reported as toxic to man (Hosseinimehr *et al.* 2007). The need for the discovery of

more natural antioxidants is therefore of uttermost importance Labo *et al.* 2010).

Trichilia monadelphica (Thonn) JJ De Wilde has a wide application in traditional medicine for the following conditions namely: yaws, ulcers, abortion, arthritis; cough, gonorrhoea, syphilis, chancres and syphilitic sores, wounds/cuts/epidermal infections, rickets, sterility, amenorrhoea, stomach pain, gastrointestinal pain, disorders, dysentery and fever (Abbiw 1990; Msbana *et al.* 2000; Lemmens 2008).

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

T. monadelphica was found to exhibit antioxidant, wound healing, anti-inflammatory, and anti-anaphylactic properties (Tiwari *et al.* 2011; Woode *et al.* 2012; Ben *et al.* 2013, Kokate 2014), analgesic (Dos Santos *et al.* 2010), antimicrobial and antioxidant (Quarthey 2014). In this research article, we report the antimicrobial, antioxidant and cytotoxicity activities of *T. monadelphica* root bark essential oil along with its chemical composition.

MATERIALS AND METHODS

Plant Material

The fresh root bark of *T. monadelphica* was collected from the botanical garden of the University of Ibadan, Ibadan. Specimens were identified and authenticated at the Forest Research Institute of Nigeria (FRIN), Oyo State, Nigeria where a voucher specimen with herbarium number FHI 112655 was deposited in the FRIN herbarium.

Reagents

Hexane, Methanol, Hydrogen peroxide, Butylated Hydroxyanisole (BHA), Ascorbic acid, α -tocopherol, DMSO and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), were obtained from Sigma Chemical Co. (Germany).

Major equipment used

UV-visible spectrophotometer (Unicol 200 and Perkin Elmer lambda 25 models), GC-Mass spectrophotometer (Agilent Technologies) and Hydro distillation-Clevenger apparatus.

Preparation and Isolation of essential oil from the plant material

The fresh root bark of *T. monadelphica* was chopped into pieces using a knife and the essential oil was immediately collected from the fresh plant material by hydro-distillation on a Clevenger-type apparatus for 4 hrs in accordance with the British pharmacopoeia specifications (1980). The essential oil was collected and stored at 4 °C before analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil was analysed by GC-MS using Agilent 7890A Gas Chromatograph coupled with MS Agilent Technologies 5975 series MSD. The capillary column type was an HP-5 MS with a column length of 30 m, an internal diameter of 0.32 mm and a film thickness of 0.25 μ m. The carrier gas was helium at a constant flow rate of 1.4123 mL/min, an average velocity of 43.311 cm/sec and the pressure was put at 1.5 psi. The temperature of the column was initially set at 80°C for 2 min and then increased to 240°C at the rate of 10°C/min for 30 min. The volume of each sample injected was 3 μ L. Identification of the constituents of the oils was carried out by comparing the mass spectra data of the compounds with a pre-installed NIST MS search 2.0 data bank and with the data previously reported in the literature (Adam 2001), Linear retention index experimentally determined using homologous series of C₆-C₃₀ alkanes.

Antimicrobial Screening of the essential oil of *T. monadelphica* root bark

The root bark essential oil of *T. monadelphica* were tested against 10 strains of micro-organisms consisting of 6 bacteria; 4 Gram-negative (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*), 2 Gram-positive: (*Staphylococcus aureus* and *Bacillus subtilis*) and 4 fungi (*Candida albicans*, *Penicillium notatum*, *Rhizopus* spp. and *Aspergillus niger*). The Agar diffusion method was employed (Kalemba and Kunicka, 2003; Okpala *et al.* 2019). The oil sample was prepared such that 1 mL of the oil was regarded as 100% concentration. 0.5 mL into 0.5 mL of *n*-Hexane gave 50% concentration and 25% concentration was obtained using serial dilution. Hexane was used as a negative control, Gentamicin (10 μ g/mL) as a positive control for bacteria and Tioconazole (0.7 mg/mL) as a positive control for Fungi. All tests were carried out in triplicates. Observed zones of inhibition of growth were measured and recorded in millimeter (mm).

Scavenging of DPPH on the essential oils

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 hydroxyl anisole (BHA) which are known antioxidants. All tests and analyses were run in triplicates and the result obtained was averaged. The activities were determined as a function of their % inhibition which was also calculated using the formula:

$$\% \text{ Scavenging (DPPH)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

RESULT AND DISCUSSION

Chemical Constituents of root bark of *T. monadelpha* Essential Oil

A total of 20 compounds comprising 99% of the essential oil was identified based on their retention time determined regarding 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 the literature (Joulain and Konig 1998; Adam 2001).

The hydro-distillation of the root bark of *T. monadelpha* gave a high level of sesquiterpenes hydrocarbon in the essential oil with (E)-Longipinene (18 %) as the major compound; Other constituents identified include 10s,11s-Himachala-3-(12),4-diene (15.26 %), Aromadendrene (11.12 %), Alloaromadendrene oxide-

(1) (8.82 %), [E]-4-Hexadecen-6-yne (6.55 %), Caryophyllene (5.91), (+)- α -Himachalene (5.47), (-)-Eremophyllene (4.83 %), α -Cyperene (4.39) and p-Cymen-7-ol (4.04). The essential oils were predominantly sesquiterpenes hydrocarbon in nature (73.3 %), Oxygenated Sesquiterpenes (10.23 %), Non-terpenes derivative (10.1 %), Oxygenated Monoterpenes (4.04 %) and 1.33 % Apocarotenes as shown in Table 1.

The most abundant constituent in the root oil (E)-Longipinene is known to be an anti-cancer agent, insect repellent and cytotoxic (Javaprakasha *et al.* 2002; Liao *et al.*, 2013). The compound 10s,11s-Himachala-3-(12),4-diene is reported to exhibit insecticidal activities against ticks and mosquitoes (Tawatsin *et al.* 2006), while Alloaromadendrene oxide-1 possesses a wide range of pharmacological activities such as antioxidant, anti-inflammatory, anti-tumor, anti-proliferative and anti-depressant activity (Liao *et al.* 2013). The pharmacological activities of the chemical composition of the essential oil justify the ethnomedicinal usage of the root of *T. monadelpha*.

Also, the essential oil of the root of *T. monadelpha* is characterised as sesquiterpenes-rich (Table 2), having comprised of about 73.3% sesquiterpenes compounds. Some of the most dominant constituent of sesquiterpenes include (E)-Longipinene (18%), 10s,11s-Himachala-3-(12),4-diene (15.26%), Aromadendrene (11.12%), Alloaromadendrene oxide-(1) (8.82%) and β -Caryophyllene (5.92%). Phytochemical analyses of numerous plants' essential oils has been reported to be dominated by sesquiterpenes; *T. polium*, *T. flavum*, *T. montanum* and *T. chamaedrys* were characterised by a high percentage of the sesquiterpene hydrocarbons such as β -caryophyllene and germacrene D (Nada *et al.* 2011). The essential oils from the investigated Croatian Teucrium species exhibited antiphytoviral activity (Nada *et al.* 2011).

Table 1. Chemical composition of the essential oils from the root of *Trichilia monadelpha* (TMRO).

S/N	RI	Compound	% Composition
1	-	1-(1-methylene-2-propenyl)-Cyclopentanol	2.01
2	-	1,2-Cyclohexanedicarboxylic acid, di(2-chlorophenyl) ester	0.54
3	1028	p-Cymen-7-ol	4.04
4	1330	(E)-Longipinene	18
5	1398	α -Cyperene	4.39
6	1399	10s,11s-Himachala-3-(12),4-diene	15.26
7	1418	Ylangene	3.31
8	1428	(+)- α -Himachalene	5.47
9	1445	γ -Elemene	0.85
10	1459	Aromadendrene	11.12
11	1475	(-)- α -Helmiscapene	1.21
12	1476	β -Caryophyllene	5.91
13	1486	(-)-Eremophyllene	4.83
14	1510	Germacrene A	2.33
15	1519	α -Cubebene	0.8
16	1519	1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene	1.77
17	1534	γ -Muurolene	0.38
18	1613	(-)-Caryophyllene oxide	1.41
19	1702	Alloaromadendrene oxide-(1)	8.82
20	1737	[E]-4-Hexadecen-6-yne	6.55
TOTAL			99%

RI: Retention Index.

Table 2. Classes of Compounds identified from the *Trichilia monadelpha* root bark.

Classes of Compounds identified	% Composition of the Identified Compounds
Oxygenated Monoterpenes	4.04
Sesquiterpenes Hydrocarbon	73.3
Oxygenated Sesquiterpenes	10.23
Non-terpenes derivative	10.1
Apocarotenes	2.33

The antimicrobial activity of *T. monadelpha* essential Root Bark Essential Oil (TMRO)

The root essential oil inhibited growth against *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, *C. albicans*, *A. niger*, and *K. Pneumonia*, *S.typhi* at concentrations of 200 mg/mL to 50 mg/mL with an inhibitory zone of 20 – 10 mm (Table 3). At a lower concentration of 25 mg/mL, the zone of inhibition was between 12 - 10 mm for *S. aureus*, *B.subtilis*, *E. coli*, *P. aeruginosa*, *A. niger* and *C. albicans*. The oil extract showed inhibition against *P. aeruginosa* with a zone of inhibition of 10 mm at 12.5

mg/mL, while no inhibition was observed at 0.0625 mg/mL against all the tested micro-organisms.

Essential oil has bioactive constituents that are crucial in pharmacological and therapeutic use such as 10s,11s-Himachala-3-(12),4-diene was one of the major constituent identified in the essential oil of *Erythrina caffra*, which inhibited antibactericidal activities (Wintola *et al.* 2021). However, the good antibacterial activity of the *T. monadelpha* root bark essential oil observed may be associated with the presence of Aromadendrene, (E)-Longipinene, Aromadendrene oxide, (+)- α -Himachalene and β -caryophyllene, which were reported to have antibacterial effects against many bacterial strains (Filipowicz *et al.* 2013; Bonikowski *et al.* 2015; Dahham *et al.* 2015; Gordien *et al.* 2019). However, synergistic effect between constituents should not be neglected, since it may cause a much more noticeable effect than single component (Oukerrou *et al.* 2017; Moa *et al.* 2019).

Table 3. Antimicrobial analysis of the root bark essential oils of *T. monadelpha* (TMRO).

Conc.(mg/ml)	SA	EC	BA	PA	ST	KP	CA	AN	PN	RS
200	20	18	20	18	18	16	14	14	-	-
100	18	14	18	14	14	14	12	12	-	-
50	14	12	14	12	12	12	10	10	-	-
25	12	10	10	10	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*, BS: *Bacillus subtilis*, PA: *Pseudomonas aeruginosa*, ST: *Salmonella typhi*, KP: *Klebsiellae Pneumonia*, CA: *Candida albicans*, AN: *Aspergillus niger*, PN: *Penicillium notatum*, RS: *Rhizopus* spp., -ve: Negative control (*n*-Hexane), +ve: Positive control; Gentamicin 10 μ g/mL (bacterial) and Tiocosnazole 70 % (fungi).

DPPH Free Radical Scavenging Activity of *T. monadelpha* root bark Essential oil

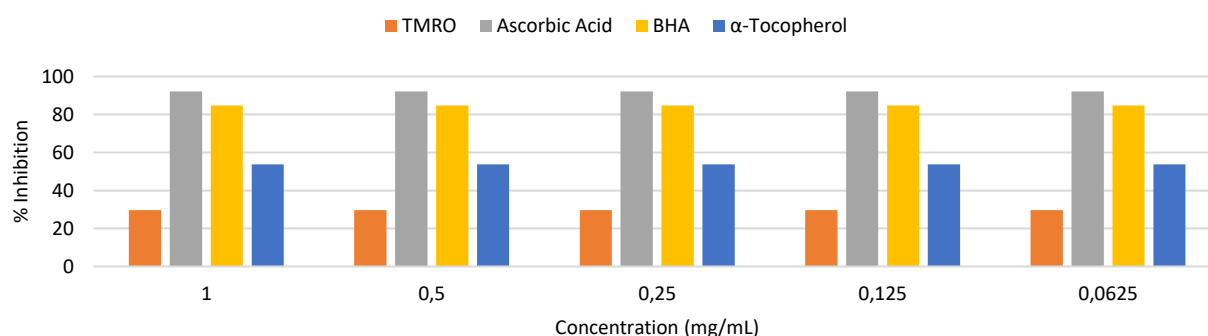
DPPH is a stable free radical compound widely employed to study the free radical scavenging ability of various samples (Benvenuti *et al.*, 2004). Figure 1 and Table 4 show the scavenging ability of *T. monadelpha* root bark essential oil, ascorbic acid, butylated hydroxyanisole (BHA) and α -tocopherol on DPPH radicals at various concentrations. The essential oil showed lower percentage inhibition at various concentrations (29.6% - 28.50%) while ascorbic acid, BHA and α -tocopherol, a concentration at 1.0 mgmL⁻¹ was required to achieve the inhibition of DPPH radicals of 92.1%, 84.70% and 53.7%, respectively. The result obtained revealed that the synthetic antioxidants were

more effective with an IC₅₀ value of 1.47 mgmL⁻¹, 1.88 mgmL⁻¹, 4.83 mgmL⁻¹ followed by the essential oil with an IC₅₀ value of 9.95 mgmL⁻¹. The low inhibition of DPPH free radical scavenger might be due to the low concentration of polar or phenolic moiety among the chemical components present in the oil.

The low antioxidant activities observed in the *T. monadelpha* root bark essential oil can be attributed to the absence of phenolic compounds or hydroxyl group's compounds. However, the presence of Aromadendrene, (E)-Longipinene, Aromadendrene oxide, 10s,11s-Himachala-3-(12),4-diene, (+)- α -Himachalene and β -caryophyllene, also in synergy of other component identified in this study contributed to the free radical scavenging activity.

Table 4. The Absorbance Reading at 517 nm of the Essential oil and standards at different concentrations.

CONC. (mg/ml)	TMRO	Ascorbic Acid	BHA	α -tocopherol
1.0	0.983±0.006	0.110±0.002	0.214±0.006	0.646±0.004
0.5	0.988±0.003	0.113±0.005	0.240±0.003	0.648±0.002
0.25	0.992±0.007	0.123±0.003	0.261±0.000	0.668±0.002
0.125	0.993±0.003	0.124±0.004	0.263±0.005	0.722±0.005
0.0625	0.998±0.001	0.214±0.002	0.346±0.002	0.768±0.001

**Figure 1.** DPPH radical scavenging activity of root bark essential oil (TMRO) and standards.

CONCLUSION

The *T. monadelpha* root bark essential oil contains notable chemical compounds that are responsible for its antioxidant and antimicrobial activities. However, the results obtained from this study suggest *T. monadelpha* root bark is a potential source of natural antioxidants and antimicrobials. This result also supports the ethnomedicinal application of the plant.

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