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Chemical constituents, antioxidant and antimicrobial activities of essential oil from stem of *Napoleonaea vogeli*

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ABSTRACT

Background: Medicinal plant is the foundation of traditional medicine practice. *Napoleonaea vogelii* is used in traditional medicine practice for the treatment of pain and inflammatory disorders. *Napoleonaea vogelii* is rarely used and little is known about its pharmacological foundation in traditional medicine practice. The aim of this study is to determine the chemical constituents of the essential oil from the stem of *Napoleonaea vogelii* to justify its use in traditional medicine practice.

Methods: *Stem of Napoleonaea vogelii* collected from Southwestern, Nigeria was subjected to soxhlet extraction method, for the extraction of its essential oil. The Essential oil extracted was analyzed using gas chromatography mass spectrometry technique and investigated for antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The essential oil was also investigated for antimicrobial activities using different clinical isolates of bacterial strains viz., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium* and with fungi strain *Aspergillus niger,* and *Penicillium sp.*

Results: Stigmasterol (7.68%), 9,12- octadecanoic acid (7.63%), tetradecanoic acid (5.62%) were the major constituents while phytol (0.62%), squalene (0.74%), eicosane (0.14%) were found to be the minor constituents of the essential oil. The antioxidant activities of the essential oil revealed that at 100 µg/ml, the % radical scavenging activity at 79.36 % is significant and comparable to the % radical scavenging activity of the standard ascorbic acid. At 25 to 50 µg/ml, the % radical scavenging activity was also comparable to the ascorbic acid values. The IC_{50} value was also significant and at a moderate value of 35.56 µg/ml. At concentrations of 50 to100 mg/ ml, the essential oil was active against all bacteria and fungi except *Aspergillus Niger*.

Conclusion: This study revealed that *Napoleonaea vogelii* is a good source of bioactive compounds with antimicrobial and antioxidant activities and justifies the use of the plant in the treatment of ailments in traditional medicine practice.

Keywords: Antioxidant, antimicrobial, *Napoleonaea vogelii,* essential oil

1. INTRODUCTION

A medicinal plant is any plant which one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for chemo-pharmaceutical semi synthesis [1]. Before the introduction of orthodox medicine, humans rely on healing properties of medicinal plants. Medicinal plants remain the foundation of traditional medicinal practices.About 3. 3 billion people in the less developed countries make use of medicinal plants to treat aulmemt amd diseases. [2]. Medicinal plants is an important resource drug discovery with 80 % of all synthetic drugs , derived from them. [3]. Essential oil is a complex mixture of volatile constituents biosynthesized by living organism [4]. Essential oils consist of organic volatile compounds. They are found partly in vapour state due to their vapour pressure which is sufficiently high at atmospheric pressure and room temperature. They are soluble in alcohol, ether but insoluble in water. They have a characteristic odour and are responsible for different scents that plants emit [5]. *Napoleonaea vogelii* belongs to the family lecythindaceae. It is a tropical evergreen shrub; that grow up to 15 m high with fibrous and alternate leaves (7.5 to 15cm long and 3.25 to 7.5 cm wide). Its fruits are reddish orange, edible [6]. It is used in traditional medicine practice for the treatment of pain and inflammatory

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disorders [7]. There is no literature study on the chemical constituents of the essential oil from the stem of *Napoleonaea vogelii* in Nigeria. Hence the aim of this study is to determine the chemical constituents of the essential oil from the stem of *Napoleonaea vogelii* and to determine the antioxidant, antimicrobial activities of the essentialoil.

2. MATERIALS AND METHODS

2.1 Materials

Diphenyl-1,1-picrylhydrazine (DPPH), methanol, ascorbic acid, Dimethyl sulfoxide, pefloxacin, ciprofloxacin, chloramphenicol, Petri plates, agar solution, Ultraviolet spectrometer, Gas chromatography mass spectrometer.

2.2. Methods

Napoleonaea vogelii plant was collected at Ikire, Osun State in Southwestern, Nigeria with (7023'12.49" N Lat, 4013'6.75"E long). The plant was identified and authenticated at Forestry Research Institute of Nigeria with voucher specimen No FHI 113753 deposited. The stem was then air dried and pulverized. Air dried pulverized stem was then subjected to soxhlet extraction procedure, for essential oil extraction. The stem (100 g) was air dried and pulverized. Extraction of essential oil from the stem of *Napoleonaea vogelii* was carried out using the method described by Association of official Analytical chemists [8]. The essential oil obtained was preserved on a glass tube at 4 0C.

2.2.1. Gas Chromatography Mass Spectrometry Analysis [9]

The Gas chromatography mass spectrometry analysis was carried out on an Agilent 7820A gas chromatography coupled to 5975C inert mass spectrometer with electron impact source. HP-5 capillary column coated with 5% Phenyl methylsiloxane (30 m length x 0.32 mm diameter x 0.25 µm film thickness) was used as the stationary phase. Helium was used as the carrier gas, at constant flow of 1.4871 ml/min at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. 1ml of the sample was injected in splitless mode at an injection temperature of 300^0C. Purge flow to spilt vent was 15 ml/min at 0.75min with a total flow of 16.654 ml/min gas saver mode was switched off. Oven was initially programmed at 40^0C for (1min), then ramped at12^0C/min to 300^0C (10min). Run time was 32.667min with a 5min solvent delay. The mass spectrometer was operated in electron impact- ionization mode at 70 eV with an ion source temperature of 230^0C, quadrupoles temperature of 150^0C and transfer line temperature of 280^0C. Ion acquisition was via scan mode. (Scanning from m/z 45 to 550 amu at 2.0 s/scan rate). The identification of the oil constituents was based on a comparison of their retention time and retention indices, with those of literature by matching their mass spectra fragmentation patterns with corresponding data (Wiley 275 L. library) and other published mass spectra.

2.2.2. Antioxidant Tests [10]

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Capacity of the essential oil fraction

The effect of the essential oil extract on DPPH radical was estimated. 0.1 mM solution of DPPH in methanol was prepared and 1.0mL of this solution was mixed with 1.0 mL of extract in methanol containing different concentrations of extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature or 30 min. The absorbance of the mixture wasmeasured spectrophotometrically at 517nm. Ascorbic acid was used as standard.
The percent DPPH scavenging effect was calculated using the following equationDPPH

$$\text{Scavenging effect (\%)} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard sample or extract. The IC_{50} value represented the concentration of the compounds thatcaused 50% inhibition of DPPH radical formation.

2.2.3. Antimicrobial tests [11]

The antimicrobial activities of varied concentrations of the essential oil from the stem of *Napoleonaea vogelii* were carried out using standard bacteria *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and* with fungi strain*Aspergillus niger*, and *Penicillium sp* were obtained from the Department of Microbiology, University of Lagos, Akoka, Nigeria. Agar well diffusion technique was used as described [11].. The innocula were prepared from the bacteria and yeast cultures of and were maintained in glycerol-peptone water at 4 0c as a pure culture and were sub cultured into sterile peptones waterin a Mccartney

bottles. One hundred microliter (100ml) of innocula (test organisms) was introduced into the sterile agar plates and were spread evenly with sterile swab stick and 8mm cork-borer was used to bore holes. The essential oil was diluted with Dimethyl sulfoxide (DMSO) to achieve different concentrations. One hundred micro litre of the variousconcentrations of the sample were introduced into the bored holes in duplicate plates. The innoculated plates were allowed to stand for 30 mins for proper diffusion of the sample into the medium. These inoculated plates were incubated at 37^0C for 24 hours and examined for zones of inhibition. Zone of inhibition was measured in millimeter with a ruler at 90^0 perpendicular to each other and the mean of the readings were then calculated. Pefloxacin and ciprofloxacin was as positive control while Dimethoxy served as negative control.
Negative control was prepared using the respective solvent. Chloramphenicol was used as a positive control for bacteria, ketoconazole as a positive control for fungi and DMSO was also used as a negative control. They were incubated for 24 hours. Observations were made to check for organism growth.

Minimum Inhibitory Concentration: Petri dish plates were prepared with 20 mL of sterile Muller Hinton Agar (MDA) for bacteria and 20 mL of Potato Dextrose Agar (PDA) for fungi. The 24- hour prepared test cultures of inoculums were swabbed on top of the solidified media andallowed to dry for 10 min. The tests were conducted with a cork borer of 8.0mm. Solutions of plant extracts aseptically were filled into the hole of agar well at the different dilutions of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml concentrations previously prepared were introduced into Mueller Hinton agar (MHA) plates and Potato Dextrose Agar already swabbed with the different selected organisms in duplicates aseptically. All appropriate controls- Chloramphenicol, ketoconazole, and DMSO were also inoculated on Mueller Hinton agar in duplicates. The plates were placed on the surface bench and left for 30 minutes at room temperature for compound diffusion. The plates were then incubated for 24 hrs at 37 °C for bacteria and 4 days at 28 °C for fungi, respectively. The zone of inhibition was recorded by measuring the clear zone of growth inhibition on the MHA and PDA surface around the holes in millimeters.

Minimum Bactericidal Concentration: Pour plate techniques were used by inoculating 0.1ml of plant extracts at the different dilutions of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml concentrations previously prepared into sterile Petri dishes. Freshly prepared Mueller Hinton Agar (MHA) and Potato Dextrose Agar were added into the inoculum in the plates aseptically. The plates were incubated for 24 hrs at 37 °C for bacteria and 4 days at 28 °C for fungi, respectively. The growth rate was recorded aseptically.

2.3. Statistical analysis

All data were expressed as mean of three replicates. Significant levels were tested at $P< 0.05$.

3. RESULTS

Table 1: Chemical constituents from the essential oil from stem of *Napoleonaea vogelii*

Constituents	%Composition	Molecular formular
Phytol	0.62	$C_{20}H_{40}O_2$
Squalene	0.74	$C_{30}H_{50}$
Hexadecanoic acid	3.98	$C_{16}H_{32}O_2$
Tetradecanoic acid	5.62	$C_{14}H_{28}O_2$
Docosene	5.62	$C_{22}H_{44}$
Eicosene	0.14	$C_{20}H_{42}$
Ergosterol	1.06	$C_{28}H_{44}O$
Lanosterol	0.07	$C_{30}H_{50}O$
Stigmasterol	7.68	$C_{29}H_{48}O$
Tocopherol	2.4	$C_{29}H_{50}O_2$
Octadecanoic acid	0.91	$C_{18}H_{36}$
Docosanoic acid	0.27	$C_{22}H_{44}O_2$
Methyl sterate	0.29	$C_{19}H_{38}O_2$
9,12, octadecaclicnoic acid ethyl ester	34.59%	$C_{20}H_{36}O_2$

Stigmastan-7-one	0.18	$C_{29}H_{50}O_2$
Linolelaidic acid	7.07	$C_{18}H_{32}O_2$
Hexadecanoic acid methyl ester	0.81	$C_{17}H_{34}O_2$
Pentadecanoic acid	0.47	$C_{15}H_{30}O_2$
Dodecanoic acid	4.53	$C_{12}H_{24}O_2$
Dodecanoic methyl ester	0.39	$C_{13}H_{26}O_2$
Methyl tetradecanoate	0.47	$C_{15}H_{30}O_2$
9,12-octadecadienoic acid	7.3%	$C_{18}H_{32}O_2$
Ergost-5-22-dien-3-ol	0.55	$C_{28}H_{46}O_2$

Table 2: Antioxidant activities of essential oil from stem of Napoleonaea vogeli*i*

Concentrations µg/ml	Sample	Ascorbic acid
	% radical scavenging activities	
100	79.36	83.94
75	69.85	75.44
50	52.60	62.58
25	47.17	36.54

IC_{50}= 35.56µg/ml

Table 3: Antimicrobial activities of essential oil from stem of *Napoleonaea vogelii*

Microorganisms	Concentrations (mg/ml) 100	50	25	12.5	DMSO	CHL	KET	MIC	MBC
	Zone of inhibition of Pathogens in mm								
E.coli	12.0	7.0	0.0	0.0	0.0	10	-	100	100
S. aureus	10.0	6.0	3.0	0.0	0.0	12.0	0.0	100	100
P. aeruginosa	14.0	10.0	0.0	0.0	0.0	0.0	0.0	60.0	100
S. typhimurium99s	5.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. niger	0.0	0.0	0.0	0.0	0.0	0.0	18.0	0.0	0.0
Penicillium sp	60.0	25.0	0.0	0.0	0.0	0.0	0.0	50.0	100

NOTE: CHL-Chloramphenicol, KET- ketoconazole, 0 – No zone of inhibition, MIC- minimum inhibitory concentration, minimum bactericidal concentration, Diameter of Zone of inhibition - 8mm

4. DISCUSSION

Fatty acids 9, 12,- octadecadienoic acid (7.3%), tetradecanoic acid (5.62%), a sterol stigmasterol (7.68%), docosene (5.60%), linoleladic acid (7.07%) were the predominant constituents in the essential oil from the stem of *Napoleonaea vogelii*. A Diterpene alcohol Phytol (0.62%), a triterpenoid squalene (0.74%), an alkane eicosane (0.14%), a fatty acid octadecanoic acid (0.91%) were the minor constituents in this essential oil. From previous studies, hexadecanoic acid (5.39%), methyl-hexadecanoate (3.75%), limonene (3.48%) and geranial (39.51%) were identified and reported in the essential oil fraction from another specie in the family of lecythindaceae, which is *Naipoleona imperials [*9]. Hexadecanoic acid is a common constituent with the essential oil from *Napoleonaea vogelii* and *Napoleonaea Imperials*. The total constituents of the essential oil from *Napoleonaea vogelii* are fatty acid 30.44%, fatty acid ester 36.26%, sterol 11.94%, diterpene alcohol 0.62%, triterpenoid 0.74 % and alkane 7.13 %. In this study, the chemical composition of the essential oil from stem of *Napoleonaea vogelii* in Nigeria is reported. The antioxidant activities of the essential oil revealed at 100 µg/ml, the % radical scavenging activity at 79.36% is significant and comparable to the standard ascorbic acid. At 50 to 25 µg/ml, the % radical scavenging activity was also comparable to the ascorbic acid values. The IC_{50} value was also significant and at a moderate value of 35.56 µg/ml. It was reported that hexadecanoic acids possesses antioxidant, pesticide and nematicidal activity [12], Also phytol and octadecanoic acid were reported to possess anti-inflammatory activities. [13]. Tocopherol is also a known antioxidant [14]. The antioxidant activities of the essential oil maybe due to the presence of its constituents (tocopherol , hexadecanoic acids) that possess

antioxidant activities. The essential oil was investigated for antimicrobial activities using different clinical isolates of bacterial strains viz., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimuriumand* with fungi strain *Aspergillus niger*, and *Penicillium sp*. At concentrations of 100 to 50 mg/ ml, the essential oil was moderately active against all bacteria and fungi except Aspergillusniger. At concentration of 25 mg/ ml, it was moderately active against *S. aureus* only but not active against the other microorganisms used.

5. CONCLUSION

The chemical composition of the essential oil from stem of *Napoleonaea vogelii* in Nigeria is reported in this study. This study revealed that *Napoleonaea vogelii* is a good source of bioactive compounds with antimicrobial and antioxidant activities

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Conflict of Interest

No potential conflict of interest was reported by the authors

Contributions of the Authors

Conceptualization, Odusina B.O; Methodology, Odusina B.O, Ajimosun I.E, Kunade R.A, Analysis, Odusina B.O;Writing original draft, Odusina B.O; Resources Odusina B.O, Ajimosun I.E, Kunade R.A; Writing review and editing, Odusina B.O; Supervision, Odusina B.O; Funding acquisition. Odusina B.O, Ajimosun I.E, Kunade R.A.

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