

Chemical composition and antimicrobial activity of *Eleusine indica* leaf essential oil

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Abstract

Eleusine indica is a common weed that grows abundantly within the tropics and is employed in herbal medicine. The present work is aimed at determination of the chemical composition and antimicrobial activity of its leaf essential oil. The constituents of the essential oil were separated by gas chromatography and identified by mass spectrometry. Antimicrobial susceptibility test was done by agar disc diffusion method while the minimum inhibitory concentrations were carried out using broth dilution method. Twenty seven organic compounds were identified for the first time in *Eleusine indica* leaf essential oil. The oil exhibited antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas areuginosa*, *Candida albican* and *Penicillium glabrum*. Twenty seven components have been identified for the first time in *Eleusine indica* leaf essential oil. The oil will therefore serve as an alternative source of new antimicrobial agents.

Keywords: *Eleusine indica*, essential oil, antimicrobial activity, chemical composition

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1. Introduction

Eleusine indica is an annual weed and medicinal plant found all over the tropics and subtropical regions. It serves as an antidiarrheal [1], diuretic and anthelmintic agent [2,3] and it is used in management of fertility [4]. It has also been reported to have anti-plasmodial and anti-diabetic activity as well as anti-oxidant and anti-inflammatory activity [5]. It has been showed that it has *in vivo* anti-hypertensive activity [6]. It in addition has diuretic and urolithiatic activity [6].

Aqueous and methanol extracts of *Eleusine indica* have been reported to have anthelmintic activity against *Strongyloides stercoralis* and antimicrobial activity against both gram positive and gram negative pathogenic bacteria [7,8]. To the best of our knowledge there is no previous report on the essential oil of this plant. The present work is therefore aimed at the determination of the chemical composition and antimicrobial activity of *Eleusine indica* leaf essential oil.

2. Materials and Method

Fresh leaves of *Eleusine indica* were plucked within the visibility of the Department of Chemistry, University of Calabar. The plant was authenticated by staff of the Herbarium unit, Botany Department, University of Calabar.

The leaves were rinsed with distilled water and crushed, while still fresh, it was immediately steam-distilled for about 2h and the distillate collected in a separatory funnel. The lower aqueous layer was run off while the upper layer was collected as the essential oil.

2.1. GC-MS Analysis

The organic compounds, contained in the essential oil, were separated by gas chromatography while the individual constituents were identified by mass spectrometric analysis [9]. The compound identification was done by comparison of the obtained mass spectra with those of standard mass spectra of organic compounds from National Institute of Standard and Technology [9].

2.2. Antimicrobial Susceptibility Test

Agar disc diffusion method was used for antimicrobial susceptibility test against *Staphylococcus aureus*, *Candida albicans*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Penicillium glabrum*. These microbes are all clinical isolates obtained from the Pathology Department, University of Calabar Teaching Hospital (UCTH). The microbes were maintained by the method from the National Committee for Clinical Laboratory Standard [10]. The extract was diluted with distilled water to give 0.6, 1.2, 2.4, 4.8 and 9.6 mg cm⁻³ solutions of the *E. indica* essential oil. Mueller Hinton agar was used for both bacterial and fungi tests. Sterilized whatman filter paper discs were separately soaked in the different solutions of different concentrations of the extract. These were placed in different agar plates which contained the different test organisms. They were incubated for 24h at 37°C. At the end of the incubation period the zone of inhibition was measured for the different plates.

2.3. MIC, MCB and MFC Determination

The determination of minimum inhibitory, minimum bactericidal and minimum fungicidal concentrations (MIC/MBC/MFC) was carried out for the extract using chloramphenicol and nystatin as standards for bacteria and fungi respectively. 0.6, 1.2, 2.4, 4.8, 9.6 and 19.2 mgcm⁻³ solutions of the *E. indica* essential oil were placed in different test tubes. 1cm³ of water was added to each of the test tubes. 4cm³ of peptone water (Mueller

Hinton broth) was added followed by addition of 24h broth culture of the microorganisms. The test tubes were all sealed with sterile cork and incubated for 24h for bacteria and 48h for fungi. There after the test tubes were observed for clearance and turbidity. The first test tube with high degree of clearance was taken as the minimum inhibitory concentration (MIC) while the one preceding it is the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) for bacteria and fungi respectively [10]. The entire process was repeated using chloramphenicol and nystatin as control for bacteria and fungi respectively.

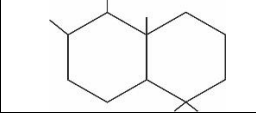

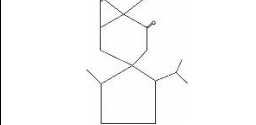


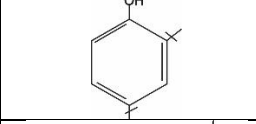




3. Results and Discussions

3.1. Results Gas Chromatography

Table 1 shows that *Eleusine indica* leaf essential oil contains twenty seven organic compounds. Eleven of these are hydrocarbons while thirteen others are oxygenated.

Table 2 shows that the inhibition of the growth of the *P. vulgaris* and the *Penicillium glabrum* occurs below 9.6 and 4.8mgcm⁻³ respectively. The inhibition of growth of the test microbes is concentration dependent. Fig. 3 shows the minimum inhibitory concentration, minimum bactericidal and minimum fungicidal concentrations of the essential oil on the selected test organisms. Fig. 4 shows the MIC, MBC and MFC of the control against these organisms.

Table 1: Gas chromatography-mass spectroscopy analysis of *Eleusine indica* leaf essential oil

S/N	Compound Name	Retention Time (Minutes)	Molecular Formula	Relative Molecular Mass	Percentage Composition	Chemical Structure
1	Decahydro-4,4,8,9,10-pentamethylnaphthalene	23.696	C ₁₅ H ₂₈	208	0.281	
2	2,4,6-trimethyldodecane	24.317	C ₁₅ H ₃₂	212	0.648	
3	Spiro[4,5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl	26.642	C ₁₅ H ₂₄ O ₂	236	0.366	
4	2,6,10-trimethylpentadecane	36.783	C ₁₇ H ₃₆	240	1.244	
5	Nonadecane	36.834	C ₁₉ H ₄₀	363	4.264	
6	2,4-bis(1,1)dimethylphenol	28.666	C ₁₄ H ₂₂ O	206	0.400	
7	Tert-hexadecanethiol	337.922	C ₁₆ H ₃₄ S	258	7.670	
8	2-methylhexadecan-1-ol	30.805	C ₁₇ H ₃₆ O	256	2.18	
9	Hexadecane	31.025	C ₁₆ H ₃₄	226	2.056	
10	2,6,10-trimethyltetradecane	26.987	C ₁₇ H ₃₆	240	10.507	

11	Nonadecane	38.001	C ₁₉ H ₄₀	268	5.240	
12	2,6,10,14-tetramethylpentadecane	33.924	C ₁₉ H ₄₀	268	0.702	
13	2-methylhexan-1-ol	35.314	C ₁₇ H ₃₆ O	256	6.613	
14	Cis-13-eicosenic	36.484	C ₂₀ H ₃₈ O	310	3.709	
15	z-(hexadec-8-enyloxy) ethanol	37.506	C ₂₀ H ₄₀ O ₂	312	0.336	
16	2,4,7,14-tetramethyl-3-vinyl-tricyclo[5,4,3(9,8)]tetradecan-6-ol	37.631	C ₂₀ H ₃₄ O	290	0.336	
17	7-methyl-8Z-tetradecen-1-ol acetate	38.480	C ₁₇ H ₃₂ O ₂	268	0.566	
18	2-bromooctadecanal	38.582	C ₁₈ H ₃₅ Br	347	0.864	
19	Docosanol	38.556	C ₂₂ H ₄₂ O	326	4.820nm	
20	2-(octadecyloxy) ethanol	39.556	C ₂₀ H ₄₂ O ₂	314	1.487	
21	1,2,4-trioxdane-2-octanoic acid 5-octyl-methyl ester	39.729	C ₁₉ H ₃₆ O ₅	344	0.759	
22	Pentatriacotene	40.148	C ₃₅ H ₇₀	490	12.165	
23	Heptacosane	41.103	C ₂₇ H ₅₆	380	16.501	
24	Pirotxin	38.401	C ₃₀ H ₃₄ O ₅	602	1.344	
25	Cyclopropane carboxylic acid	38.276	C ₄ H ₆ O ₂	86.09	1.271	
26	3,8,8-trimethoxy-3-piperidyl-2,2-binaphthalene-1,1,4,4-tetrone	42.015	C ₂₈ H ₂₅ NO ₇	487	1.162	
27	Octadecane,3-ethyl-5-(2-ethylbutyl)	42.329	C ₂₆ H ₅₄	366	0.638	

Table 2: Antimicrobial susceptibility of the test microbes to *Eleusine indica* leaf essential oil

Test microbes	Zone of inhibition (mm)				
	1.2mgcm ⁻³	2.4mgcm ⁻³	4.8mgcm ⁻³	9.6mgcm ⁻³	19.2mgcm ⁻³
-					
<i>E. coli</i>	4.80	7.10	9.00	10.40	12.03
<i>P. vulgaris</i>	-	-	-	8.00	10.00
<i>S. aureus</i>	10.05	12.00	14.07	16.00	16.82
<i>P. aeruginosa</i>	6.0	8.0	10.00	12.00	13.25
<i>C. albican</i>	8.00	9.00	12.00	16.00	17.20
<i>Penicillium glabrum</i>	-	-	8.0	10.10	10.30

Table 3: MIC and MBC/MFC of *Eleusine indica* leaf essential oil

Test organisms	MIC	MBC	MFC
<i>E. coli</i>	4.8mgcm ⁻³	9.6mgcm ⁻³	-
<i>P. vulgaris</i>	9.6mgcm ⁻³	19.2mgcm ⁻³	-
<i>S. aureus</i>	1.2mgcm ⁻³	2.4mgcm ⁻³	-
<i>P. aeruginosa</i>	2.4mgcm ⁻³	4.8mgcm ⁻³	-
<i>C. albican</i>	2.4mgcm ⁻³	-	4.8mgcm ⁻³
<i>Penicillium glabrum</i>	4.8mgcm ⁻³	-	9.6mgcm ⁻³

Table 4: MIC and MBC/MFC of chloramphenicol and nystatin control

Test organisms	MIC	MBC	MFC
<i>E. coli</i>	2.4mgcm ⁻³ *	4.8mgcm ⁻³	-
<i>P. vulgaris</i>	9.6mgcm ⁻³ *	19.2mgcm ⁻³	-
<i>P. aeruginosa</i>	2.4mgcm ⁻³ *	4.8mgcm ⁻³	-
<i>S. aureus</i>	1.2mgcm ⁻³ *	2.4mgcm ⁻³	-
<i>C. albican</i>	1.2mgcm ⁻³ **	-	2.4mgcm ⁻³
<i>Penicillium glabrum</i>	2.4mgcm ⁻³ **	-	4.8mgcm ⁻³

*with chloramphenicol and **with nystatin

Discussions

Eleusine indica leaf essential oil contains twenty seven organic compounds which are being reported for the first time in the essential oil. These compounds include pirotoxin (1.344%), 3,6,10-trimethylpentadecane (1.244%), nordecane (4.264%), tert-hexadecanethoil (9.670%), 2-methylhexa decan-1-ol (2.18%), nonadecane (5.24%), 2,6,10,14-tetramethylpentadecane (10.507%), heptacosane (10.50%) and 3,8,8-trimethoxy-3-piperedy-2,2-binaphthalene-1,1,4,4-tetrone (1.662%).

Some of these compounds have important industrial and medicinal applications. For example 3,8,8-trimethoxy-3-piperidy-2,2-bynaphalene-1,1,4,4-tetaone is an anti-sickling agent of *Aloe vera* [11]. Pirotoxin is used to treat erosive esophagitic, and slower acidity of the stomach [12]. Spiro [4, 5] decan-7-one and 1,3-dimethyl-8,9-epoxy-4-isopropyl are known anti-inflammatory agents [13] etc. It is these chemical constituents that are responsible for its biological activities. Although one is tempted to attribute these activities to the major constituents, it is a known fact that such biological activities are modulated by minor constituents through several antagonistic, synergistic and additive effects [14].

Table 2 shows that the essential oil is active against both bacteria and fungi. However *Proteus vulgaris* and *Penicillium glabrum* did not show much sensitivity to the essential oil. Table 3 shows that the minimum inhibitory concentration (MIC) of 1.24mgcm⁻³ was observed for *E. coli* while that of 6.96mgcm⁻³ was observed for *P. vulgaris*. These are not far from the values obtained for the reference drug chloramphenicol for bacteria and nystatin for fungi. The *E. indica* leaf essential oil will serve as a suitable substitute to the conventional drugs employed in orthodox medicine. Use of herbs in control of infectious diseases is becoming increasing popular and it has the added advantage of being readily available, environmentally friendly and cheap [15].

Conclusion

Eleusine indica leaf essential oil contains twenty seven compounds which are being identified for the first

time in *Eleusine indica* leaf essential oil. The essential oil has both antibacterial and antifungal activities which are attributed to its chemical constituents.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. E. Lans (2007). Comparison of plants used for skin and stomach problems in Trinidad and Tobago. *Ethanobiol. Ethnomed.* 3(3): 1-12.
2. M. Gunjar, L. Karna, K. Daylan and P. Sasigaren (2012). A review of search of phytomedicine used by traditional people in Malaysia. *International Jour. Phytotherapy Res.* 2(3): 26-41.
3. C.A. Gruyal, R.D. Roasona and N.D. Palmec (2014). Ethnomedical plants used by residents of Nothern surigeo del sur Philippines. *Natural Product Chemistry and Res.* 2(4): 1-5.
4. J.S. Ashidi, F.A. Olaosho and E. Ayodele (2013). Ethnobotanical survey of plants in the management of fertility and preliminary phytochemical survey of *Albelmuchus esclents*. *Journal of Pharmacognosy and Phytotherapy.* 9(5): 164-169.
5. M.I.G. Valentino, R. Milton, R. Dulay and J.M.E. Lindin (2018). Phytochemicals antioxidant potential and total phenolic of *Eleusine indica*. *International Jour. Bio. Res.* 13(5):317-321.
6. A.V. Desai, V.M. Patil, S.I. Patil and K.A. Kningralkar (2017). Phytochemical investigation of *Eleusine indica* for *in vivo* anti-hypertensive activity. *International Jour. Innovative Sci. Res. Tech.* 2(6): 405-409.
7. F.N.I. Morah and M.E. Otuk (2015). Antimicrobial and anthelmintic activity of *Eleusine indica*. *Acta Scientiae et Intellectus.* 1(4): 28-32.
8. E.O. Ettobong and A.I. Bassey (2017). *In vivo* antibacterial evaluation of whole plant extract of *Eleusine indica*. *Jour. Med. Plant Studies.* 5(4): 99-102.

9. F.N.I. Morah, A.E. Ogar, E.I. Eyong and H.A. Nathaniel (2019). Chemical composition, anthelmintic, insecticidal and antibacterial activities of *Veronia colorata* essential oil. International Jour. Herb. Med. 74: 45-50.
10. NCCS, National Committee for Clinical Standards (1997). Methods for dilution antimicrobial susceptibility for bacteria that grow aerobically. 4th ed. NCCS approved standards. NCCS Document M7-A4.
11. A.E. Ejele and N.J. Aneke (2011). Anti-sickling potential of *Aloe vera* extract III: Gas chromatography/mass spectrometry characterization of neutral metabolite. International Jour. Trop. Agric. And Food Systems. 5(11): 31-36.
12. M.G. Papich (2002). Saunders handbook of veterinary drugs. 4th edn. Elsevier uk. 933pp.
13. H. Hussein, V.M. Hadi and I.A. Hameed (2016). A study of chemical composition of *Foeniculum vulgai* using fourier transformer IR spectrophotometer and GC-MS. International Jour. Phamacognosy and Phytotherapy. 8(3): 60-69.
14. FNI. Morah, EP. Emehige, MM. Mowang (2017). Chemical composition and antimicrobial activity of *Nauclea latifolia* leaf essential oil. International Jour. of Chemical and Biochemical Science. 11: 44-50.
15. F.N.I. Morah, A.P. Ekanem and E.N. Michael (2016). Ichthyotoxic effect of *Phyllantus niruri* leaf. Acta Scientiae et Intellectus. 2(2): 39-41.