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Phytochemical screening of *Leucaena leucocephala* leaf essential oil and its antibacterial potentials

Abstract

Objective: The study aimed to identify the phytochemical composition of Leucaena leococephala leaf and evaluate the antimicrobial potential of the essential oil from the leaf. Material and Methods: The sample was subjected to Clevenger apparatus to extract the oil. The essential oil was characterized by chromatography method (GC-MS). The GC-MS was performed on Perkin Elmer gas chromatography model Clarus 680 equipped with HP-5 fused capillary column (5%) phenylmethyl polysiloxane stationary phase with 30m length, 0.25µm of film thickness and 0.25oc and 280oc respectively. Antibacterial activity using Agar Disc Method. Result: The result obtained from the GC-MS presented thirty phytochemicals of which Neophytadiene (9.48%), Octadecane (3.15%), 1-Octadecyne (3.85%), Phytol (52.51%) and Hexacosane (7.26%) are major. The antibacterial potential activities were observed in various ways with zone of inhibition diameters ranging from 0.70±0.00mm to1.27±0.06mm for staphylococcus aureus and Klebsielia pneumonia respectively among the six-concentration selected (25, 50, 100, 250, 500, and 1000ppm). Conclusion: It is investigated in this present studies that Leucaena leococephala essential oil can be utilized against the management of antibacterial diseases particularly Klebsielia pneumonia and Staphylococcus aureusas well as used in the Pharmaceutical and Cosmetics industry.

Keywords: phytochemical, *Leucaena leucocephala*, essential oil, antibacterial

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Isaac John Umaru,[1],[2] Benedict Samling,[1] Hauwa A Umaru[3]

[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Malaysia
[2]Department of Biochemistry, Federal University Wukari, Nigeria
[3]Department of Biochemistry, Moddibo Adama University Yola Adamawa State, Nigeria

Correspondence: Isaac John Umaru, Faculty of Resource Science and Technology, University Malaysia, Malaysia, Email umaroisaac@gmail.com

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Introduction

Essential oils were rated as one of the most important perfume component in the industry for cosmetics and scent preparation because of their floral scent and sweet pleasant odour.[1–3] It was observed from history that extraction of organic products from plants parts has been acknowledged by local people right from time immemorial for their scent, ointment and for food.

The analysis of essential oil has become emanate to increase the discovery of phytochemical component for industrial demands. Qualitative and quantitative analysis give a concise results and steps for understanding the phytochemicals in the essential oil.[4,5]

The plant *Leucaena leucocephala* (*Fabaceae*) was reported as a nitrogen-fixing plant, it belongs to the family Leguminosae.[6,7] the farmers cultivate the plant for animal feeds as well as vegetable for man. It was reported to have vitamin K as well as used for green manure.

Among the traditional usage of the plant *Leucaena leucocephala*, is its use as wind break, as well as reforestation. The plant was reported to be in used in as food in Northern American,[8,9] *Leucaena leucocephala* (*Fabaceae*) is endowed with rich component as an agent for diseases and ailments.[10]

The plant was reported to grow wild beyond control in tropical and sub-tropical region, it was observed by many researcher as one of the worst Allien species which when established it becomes difficult to quartile the plant, it tends to dominate the environ making the place inaccessible though it was said to have a prolonged period of germination because of the hard seed.[11]

Leucaena leucocephala was found to be different from other species by its intermediate leaflets, large cluster pod, smaller thornless tree as well as the presence of creamy white flower. Medically the plant has been used for its antimicrobial, anthelmintic, antibacterial, anti-proliferative and antidiabetic, anticancer, cancer preventive, diuretic, anti-inflammatory, antioxidant; antitumor, antihistaminic, nematicide, pesticide, anti-androgenic, hypocholesterolemic, and hepatoprotective properties.[12]

It was reported by Mohammed et al.[13] that the plant consists of phytochemical compound like sterols, terpenes flavonoids and coumarins. Its potential as an antioxidant gave the plant the activity to inhibit the propagation of free radicals' reaction.[13]

The purpose of this research work was undertaken phytochemical Screening of *Leucaena leucocephala* Leaf Essential Oil and its microbial activity of its natural raw material.

Material and Methods

Plant Material

The plant parts used for this studies (Leaves) was obtained from the uncultivated land of the University Malaysia Sarawak Kota-Samarahan. The plant materials were taxonomically identified and confirmed by Benedict samling and were deposited in the department of Botany Universiti Malaysia Sarawak.

Sample extraction

Approximately 100g of the sample of *Leucaena leucocephala* of the leaves were hydro distilled using Clevenger apparatus to extract the essential oils. After 8 hours of distillation, a pale yellowish essential oil was obtained from the leaves. The essential oil where stored in the refrigerator prior to use.

GCMS Reports

The gas chromatography report was performed by using a non-polar DB-5 cross-linked column with an initial temperature of 50oc stable for two minutes and then increased to 300°c at a rate of 6.5°c per minutes as well as the final temperature at 10min was stable. The temperature of the injector and detector were set at 280°c and 300°c respectively, 1µl of the fractions was diluted in 100µl hexane was introduced into the gas chromatography. The gas used as the carrier was Helium. Interpretation of mass-spectrum was conducted using the database of National Institute Standard and Technology (NIST). The spectrum used to identify the compound was used by internal library standard of the gas chromatography.

Bacteria

The bacterial used for this extract are Isolates of *staphylococcus aureus* and *Klebsielia pneumonia*.

Preparation of test samples

Essential oil from *Leucaena leucocephala* was used as an agent for bacterial assay and was tested for its potency using disc diffusion method on an agar medium.[14] Five (5mg) of the essential oil was dissolved in 5mL of methanol to give a final concentration of 1000ppm. Subsequent concentrations are 50ppm, 100ppm, 200ppm, 300ppm, 400ppm and 500ppm, i.e. five different volumes from the stock solution were taken for the studies.

Preparation of agar plates

The agar plates were prepared as reported by Isaac et al.[14] the powdered agar about 14g was mixed into 500mL distilled water. The agar solution was heated until boiling followed by sterilization in autoclave at 121°C. The mixture was poured into a sterile petri plate and allowed to cool down forming a gel. The plate was divided into eight sections for 50ppm, 100ppm, 200ppm, 300ppm 400ppm and 500ppm samples, tetracycline 30µg which was purchased from Oxoid Company Ltd was used as the positive control and methanol was used as (negative control) respectively.

Broth preparation

The pathogens used for this test were obtained from virology Lab Faculty of Resource Science Universiti Malaysia. 2.6g of the broth powder was used to prepare the broth dissolved in 200mL of distilled water and then sterilized at 121°c in an autoclave for 15min, it was allowed to cool and bacterial was introduced in the universal vail and put on a shaker for 16hrs in an incubation at 37°c for 16hrs after which the optical density was measure using spectrophotometer.[14] The measurement of the optical density was performed at wavelength 575nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6to0.9. Nutrient broth was used to adjust the turbidity.

Plate inoculation

The plate inoculation was done using the bacterial broth, the broth was streaked on the agar plate in all four directions to obtain uniformity of the bacteria on the agar. It was allowed to acclimatised for 10min before applying the extract concentration as well as the control agents. Positive control was the tetracycline while the negative control was the methanol. The prepared plate was put in the incubator and left after 24hrs the result of the growth of inhibition measured (mm) each crude extract was tested in triplicated.

Statistical analysis

The result in triplicate was analysed of its Mean standard deviation for each experiment using one-way ANOVA.

Result and discussion

Results

Table 1: presented the phytochemical composition obtained from essential oil of *Leucaena leucocephala* when subjected to GC-MS, where the active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented. The chromatogram from the result showed the most abundant compound found while some are in limited amount among of this phytochemicals are; 9.48% Neophytadiene, 52.51% Phytol, 7.26% Hexacosane, 7.08% Hexacosane, 4.61% Hexacosane, 3.85% 1-Octadecyne, 3.15% Octadecane, 1.47% 1-Deconol, 2-hexyl 1.14% Hexadecanal, 1.05% Z, Z, Z-4,6,7-Nonadecatriene, and others are less than 1%.

Table 2: presented the growth inhibition rate of the essential oil of *Leucaena leucocephala* within the range of 0.33±0.12mm at 50ppm to 1.27±0.06mm at 400ppm

Discussion

The phytochemical profiling in plant species (*Leucaena leucocephala*) leaf essential oil as reported by GCMS screening identified many bioactive compounds with biological properties as shown in Figure 1. The screening of the essential oil from *Leucaena leucocephala* leaf gives an inside of the most composition of this oil as an antibacterial (Table 1) as well as an ingredient for pharmaceutical and cosmetic industries.

The presence of phytol identified as one of the major compound of the leaf extract with about 57% indicated the potency of this plant extract to have biological activities. The compound was reported to have a potential for bacterial and as a precursor for vitamins E and K1. Its antibacterial activity against *Staphylococcus aureus*there by causing damage to cell membranes of the specified bacteria.[15] This research work agrees with this reported because of the significant effect of the extract on *staphylococcus aureus*and *Kliebselia pneumonia* as shown in Table 2.

The compounds identified using GCMS screening analysis was observed to have much medicinal potential which agrees with the report of grace et al.[16] who confirmed the effect of this oil on the selected bacterial with a significant growth inhibition.

The study of this essential oil on pathogen showed that the levels growth inhibition ranged from 50ppm to 500ppm. Maximum Inhibition Concentration was observed with concentration 500ppm essential oil dilution, while the Minimum Inhibition Concentration was observed with concentration 50ppm with *Klebsielia pneumonia* having the lowest inhibition (0.33±0.12mm) when compared to *staphylococcus aureus*as well as the control with 1.79±0.00mm (Table 2). The leaf oil is a potentially useful source of antimicrobial compounds. In the study maximum inhibition was observed with *Staphylococcus aureus*with 1.27±0.06mm, followed by 1.13±0.21mm for *Klebsielia pneumonia* at 500ppm when compared with the control. The least of the activity of this oil was observed with *Klebsielia pneumonia* at 50ppm (0.33±0.12mm). However, the rates of inhibition increase with increase in concentration.

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Table 1 Peak Report TIC

Peak#	R Time	Area	Area %	Height	Height %	Name
1	17.709	454811	0.61	281198	1.14	Hexadecanal
2	18.901	3292358	4.41	2344526	9.48	Neophytadiene
3	19.015	340965	0.46	229325	0.93	2-undecanone,6,10-dimethyl-
4	19.152	70832	0.09	45576	0.18	1-Tetradecyne
5	19.371	1974739	2.65	951885	3.85	1-Octadecyne
6	19.561	1123560	1.51	778093	3.15	Octadecane
7	19.608	180997	0.24	112901	0.46	9,17-Octadecadienal(Z)-8
8	19.678	531103	0.71	268918	1.05	Z,z,z-4,6,7-Nonadecatriene
9	9.875	175427	0.24	112901	0.48	Hexadecanal
10	20.065	253131	0.34	158813	0.64	Isophytol
11	20.368	154140	0.21	66173	0.27	n-Hexadecanoic acid
12	21.147	83784	0.11	46106	0.19	Kaur-15-ene,(5.alpha,9-alpha.10beta
13	21.499	158315	0.21	97494	0.39	Octadecane, 2-methyl-
14	21.59	141993	0.19	43061	0.17	3-Pentadecanone
15	21.753	55955081	75.02	12986435	52.51	Phytol
16	22.019	202273	0.27	126985	0.51	2-Piperdinone, N-[4-brom0-n-butyl]-
17	22.223	165943	0.22	98482	0.4	9,12,15-Octadedecatrienoic acid,(ZZZ)
18	23.283	360732	0.48	244678	0.99	Heneicosane
19	24.113	198985	0.27	75650	0.31	Eicosane
20	24.477	67892	0.09	39802	0.16	Hexadecanal
21	24.935	2456499	3.29	1794710	7.26	Hexacosane
22	25.69	432741	0.58	215480	0.87	Tetratetracontane
23	26.45	2449218	3.28	1750824	7.08	Hexacosane
24	27.154	634965	0.85	362724	1.47	1-Deconol, 2-hexyl
25	27.315	130615	0.18	77016	0.31	Supraene
26	27.939	1852668	2.48	1140943	4.61	Hexacosane
27	28.783	68874	0.09	33469	0.14	1-Decanol, 2=hexyl
28	29.368	193955	0.26	84071	0.34	Octadecanal
29	29.794	78750	0.11	34903	0,14	Hexatriacontane
30	31.851	400385	0.54	130963	0.53	Octacosanal
		74585731	100	24730956	100	

Table 2 Effect of Leucaena leucocephala essential oil on Bacteria

Concentration (ppm)						
Organism	**Control**	**50**	**100pm**	**200pm**	**300ppm**	**400ppm**	**500ppm**
Staphylococcus aureus	1.83±0.11	0.42a±0.00	0.43±0.12	0.70±0.40	0.86±0.05[a]	1.06±0.02[a]	1.27±0.06[a]
Klebsielia Pneumonia	1.79±0.00	0.33±0.12	0.37±0.31	0.50±0.13	0.67±0.13	1.04±0.00	1.13±0.21

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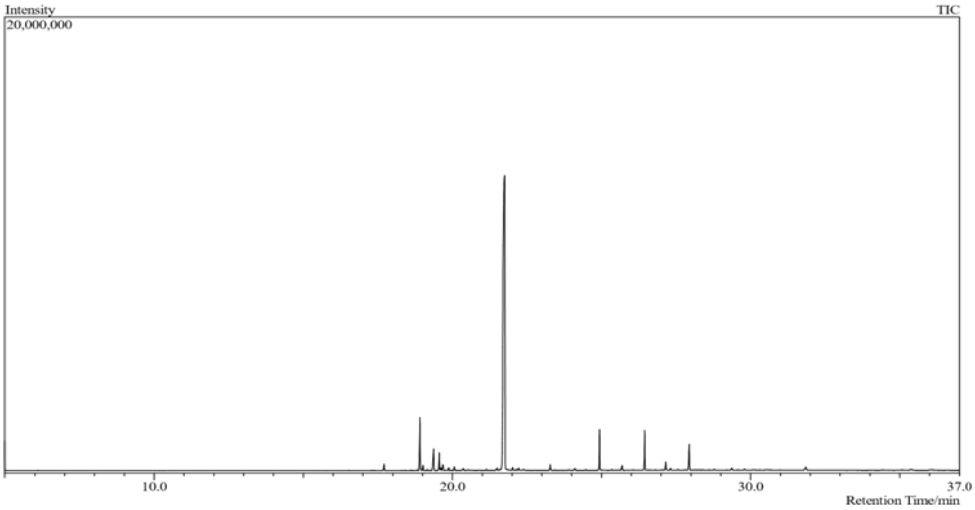

Figure 1 GC-MS Chromatogram.

However, the combination between essential oil and conventional antimicrobial drugs has been referred to as a strategy to bring about maximum of therapeutic efficacy by additive or synergistic effect.[17] It will be a good idea if this plant endowed with a lot of compound which have antibacterial activity could have applied to address the resistant bacteria against the modern medicine.

Thus this essential oil could be used as an antibacterial agent in the pharmaceutical industries as well as in cosmetics company.

Conclusion

The GCMS analysis of the essential oil of *Leucaena leucocephala* leaf has unveil the medicinal potential and the availability of this bioactive compound which include saponins, tannins, alkaloids, terpenoid, steroids and flavonoids and the determination of diameters of growth inhibition zones on the selected bacteria as well as the maximum inhibitory concentrations. indicates that this essential oil obtained from the leaves of *Leucaena leucocephala* unveiled its antibacterial properties and could be used in the development of novel antibacterial agents.

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Conflict of interest

The authors declare no conflict of interest.

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