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Phytochemical screening, antioxidant, antifungal potentials of *Acacia auriculiformis* floral Scent composition

Abstract

Objectives: *Acacia auriculiformis* is a valuable and evergreen tree of family Mimosaceae. The plant is used as a folk medicine to treat aches, sore eyes, inflammation, malaria, skin. The aim of this study was to identify the flora scent composition using GCMS as well as understanding the antioxidant and antifungal potential of the flora scent from *Acacia auriculiformis* Flower.

Materials and methods: The floral scent of the plant was collected using headspace samples for thermodesorption and flowers were enclosed (Trapped) in polyester oven bags for a minimum of 10 min and up to 120 min, depending on the intensity of scent from the life plant. The concentrated floral volatiles were trapped by pulling air from the bag through small adsorbent tubes for 2 min and up to 30 min using a membrane pump and stored in a sample vail and kept in the refrigerator until use. GC-MS (Shimadzu QP2010 Plus) was performed by using non-polar DBX-5 cross-linked column was used to analysis the sample, antioxidant test using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and for antifungal agar disc method using some selected pathogens.

Results: The GC-MS screening suggest high composition of phytochemicals like carbohydrates, phenols, flavonoids, saponins and steroids. ……. which happen to have higher potential of biological activities. Conclusion: Extensive literature survey on this Floral-scent revealed that, no information about the phytochemical, antioxidant and fungal potentials was available. Therefore, the findings of this study will give an inside of the potential of this Floral scent as an agent of pathogen in the pharmaceutical and cosmetics industry.

Keywords: phytochemical, acacia auriculiformis, flora, antioxidant, antifungal

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Benedict Samling,[1] Isaac John Umaru[1,2]
[1]Faculty of Resource Science and Technology, University of Malaysia, Malaysia
[2]Department of Biochemistry, Federal University Wukari, Nigeria

Correspondence: Benedict Samling, Faculty of Resource Science and Technology, University of Malaysia, 94300 Kota Samarahan, Sarawak, Malaysia, Tel 168395004, Email isaac.i62@yahoo.com

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Introduction

Acacia auriculiformis is a straight, medium-sized evergreen tree of the family of Mimosaceae with a height of 15-meter-tall, often multi-stemmed; young growth glaucous. Leaves alternate, simple, reduced to phyllodes (flattened leaf stalks). Flowers in loose, yellow-orange spikes at leaf axils or in clusters of spikes at stem tips; flowers mimosa-like, with numerous free stamens. Fruit a flat, oblong pod, twisted at maturity, splitting to reveal flat black seeds attached by orange, string like arils.[1]

It is a native plant of Australia which was first introduced to India in 1946 in West Bengal. The plant is used as a folk medicine to treat aches, sore eyes, inflammation, malaria, skin diseases like itching, allergy and rashes. The plant also exhibits various pharmacological activities like antioxidant, antimalarial, ant filarial, cestocidal, antimicrobial, spermicidal, wound healing, anti-arthritic, antimutagenic and chemo preventive, hepatoprotective and anti-diabetic activity.[2]

The tree is a colonizer of tropical coastal lowlands. It has the potential to be a pioneer species, but its tendency to spread into the local environment reduces its value as a pioneer outside of its native range, it is commonly found in Malaysia at the coastal lowland and the river bank. The plant has a spreading, superficial and densely matted root system, which makes it suitable for stabilizing eroded land. It has a rapid early growth, even on infertile soil, it also has the ability to fix atmospheric nitrogen and tolerance of both highly acidic and alkaline soils this makes it so popular for stabilizing and revegetating mine spoils.[3] The plantations of the tree also improve the soil physio-chemical properties such as water-holding capacity, organic carbon, nitrogen and potassium through litter fall. The phyllodes provide a good and long-lasting mulch. The dense, dark-green foliage, which remains throughout the dry season, makes it an excellent shade tree. The bark was reported to contains sufficient tannin which are used for commercial exploitation in dye industry in Indonesia and the word suitable for furniture and paper pulp industry.[4]

Phytochemical investigation of *A. auriculiformis* showed the presence of phenolic acids, flavonoids, tannins, alkaloids and terpenes, which were responsible for numerous pharmacological effects, showing hypoglycemic, anti-inflammatory, anti-bacterial, anti-platelet aggregation, anti-hypertensive, analgesic, anticancer and anti-atherosclerotic activities.[5] Amines and relatively simple alkaloids are found abundantly in the flower of *A. auriculiformis*.[6]

However, there are few study concerning the phytochemical of the floral scent of *A. auriculiformis* and to the best of our knowledge there was no study on the antioxidant and antifungal potential of the flora scent. Floral scent composed of a mixture of chemical compounds that bears the properties of volatility such as, low vapour pressure, low polarity and low molecular weight. In general, terpenoids, phenylpropanoids, benzenoids and fatty acid derivatives constitute the diversity of floral scent composition that varies from species to species.[5] The aim of this study was to identify the flora scent composition using GCMS as well as understanding the antioxidant and antifungal potential of the flora scent from *Acacia auriculiformis* flower.

Methods and materials

Collection of floral scent

To obtain headspace samples for thermodesorption (TD), floral volatiles were collected from newly opened flowers as described by Dötterl et al.[7] Single flowers (or in some cases a group of flowers) were enclosed in polyester oven bags for a minimum of 10 min and up to 120 min, depending on the intensity of scent as perceived by the human nose. The accumulated floral volatiles were trapped by pulling air from the bag through small adsorbent tubes for 2 min and up to 30 min using a membrane pump (G12/01 EB, Rietschle Thomas Inc., Puchheim, Germany) at a flow rate of 200ml/min. The adsorbent tubes were made of Chromato Probe quartz microvials of Varian Inc. (length: 15mm, inner diameter: 2mm), from which the closed end was cut off. These tubes were filled with a mixture of 1.5mg Tenax-TA (mesh 60–80) and 1.5mg Carbotrap B (mesh 20–40) (both Supelco, Bellefonte, PA, USA) embedded in glass wool. Additional samples of the surrounding air were collected to distinguish between floral volatiles and volatiles in the ambient air as control samples.

GC-MS analysis

GC-MS (Shimadzu QP2010 Plus) was performed by using non-polar DBX-5 cross-linked column (30m long x 0.25mm ID x 0.25mm film thickness composed of 5% phenyl methyl polysiloxane). The initial temperature was programmed at 50°C and held for two minutes, and then it was increased to 300°C with the rate of 6.5°C/min. The final temperature was held for ten minutes. The temperature of the injector and detector were set up to 280°C and 300°C, respectively. Helium gas was used as a carrier gas. 1µl of the fractions was diluted in 100µl hexane and then injected into the GC-MS. Interpretation of mass-spectrum was conducted using the database of National Institute Standard and Technology (NIST14). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular mass and structure of the components of the test materials were ascertained.

Antioxidant analysis

The free radical scavenging assay of compound 2,2-diphenyl-1-pycryl-hydrazyl (DPPH) was used to evaluate the antioxidant properties of the flora scent from acacia plant. The measurement was based on the method described by Wang et al.[8] with little modification. The sample was prepared by diluting 5mg of the flora scent extract into 5mL of methanol, producing a concentration of 1000µg/mL. The stock solution was sonicated to ensure the homogeneity of the sample. Three other concentrations were prepared at 10, 50 and 100µg/mL, diluted from the 1000µg/mL stock solution.

Approximately 3mL of 0.1 mM solution of 2,2 -diphenyl-1-pycrylhydrazyl (DPPH) in methanol was each added into five series of prepared concentrations (10, 50, 100, and 500µg/mL) of sample solutions (1mL). Analysis was done in triplicate. The solution was mixed vigorously and left to stand at room temperature for 30 minutes in the dark after which its absorbance was measured spectrophotometrically at 517nm using Jasco ultra violet spectrophotometer model V-630. Methanol was used as blank (only methanol) and negative control (1mL methanol mixed with 3mL DPPH), while ascorbic acid (vitamin C) as the standard. The concentration of the sample required to inhibit 50% of the DPPH free radical was calculated as IC_{50} and the value was determined using Log dose inhibition curve which performed by using PRISM version 3.02 software, based on the calculated values of the DPPH scavenging activity (%) of the sample.[9]

DPPH scavenging activity (%) was calculated with formula = $\frac{A_0 - A_1}{A_0} \times 100$

where A_0 was the absorbance of the control, while A_1 was the absorbance in presence of the control.

Antifungal test

Standard: Fluconazole common name Diflucan (Pfizer Inc New York, NY) was used as reference standard for antifungal studies.

Antifungal potential

The antifungal potential of the flora scent of *Acacia auriculiformis* extract was performed by agar disc diffusion method. Dimethyl sulfoxide DMSO was used as a negative control and Fluconazole (Diflucan) was used as a positive control. The plates were incubated at 37°C. The antimicrobial activity was taken on the basis of diameter of zone of inhibition in triplicate, which was measured before and after five days of incubation and the mean of three readings is presented. The presence of inhibition of the treated fungus was calculated using positive control as standard (100% inhibition).[10–12]

Preparation of the florescent

The flora scent extract was dissolved in DMSO, 100% biologically inert substances, with the disc diameter of 6mm. The extracts. This (DMSO) solvent served as reference control for the antifungal study. Potato dextrose agar media was used for the antifungal study. The molten media was then inoculated with 200µl of the inoculums (1×108Cfu) and poured into the sterile Petri plates. The disc was saturated with 20µl of the extracts separately, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated at 28°C and the zone of inhibition was measured every after 24h for five days.

Fungal preparation

As reported by Isaac et al., the fungi were standardized by inoculating sterile normal saline solution with a 48hrs pure culture by adjustment of turbidity to match 0.5Mc Farland standard. Standardization of the microorganisms included harvesting fungal spores from a 7 days old culture on SDA slant. Ten millilitres of sterile normal saline containing 3% w/v Tween 80 was used to disperse the spores with the aid of sterilized glass beads. Standardization of the spore suspension to 1.0×106spores/mL was achieved with a UV spectrophotometer (Spectronic 20D; Milton Roy Company, Pacisa, Madrid, Spain) at 530m (OD at 530) of the suspensions and adjusted to a transmittance of 70-72%. The plates were incubated at 37°C for 24h.[13]

Results

This study gave the phytochemical screening of Flora scent of *A. auriculiformis based* on GCMS analysis on various location (A, B, C). In the study a common compound was noticed among the various location with a concentration higher than the other compound in all the location. Tetradecane Location A, (23.50.80%) with a retention time of 15:749, while location B (17.80%) and location C (7.43%) having the same retention time. However, among the most higher percentage rate in all the three location was found in Location C with Di-n-octyl phthalate with (75.03%) at retention time of 30:233 and location B and A, all have their retention time with 30:200- 30:233 with a percentage peak of 19.16 and 12.20 respectively. other compound is as shown in Table 1.

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Table 1 Chemical composition identified in Acacia *Auriculiformis* Florescent extracts (Location A, B, C)

Location	A	B	C
Compounds			
1,2-Benzenedicarboxylic acid	0.7	1.36	0.39
1-Chloroeicosane	0.5	-	-
1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-	-	0.32	-
1-Hexanol	-	-	2.06
1-Octadecanesulphonyl chloride	-	4.06	-
1-Undecene, 7-methyl-	-	0.57	-
2-(1,1-Dimethylethyl)-5-oxohexanal	-	0.13	-
2,6,10-Trimethyltridecane	-	0.27	-
2,6,11-Trimethyldodecane	1.91	-	-
2-Ethyl-1-hexanol	4.7	6.08	-
2-Nonen-1-ol	1.2	-	-
3,5-Di-tert-butylphenol	-	0.53	0.25
5,5-Diethylheptadecane	-	-	0.25
5-Octadecene	-	0.85	-
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-	-	-	-
9-Octadecenamide	-	1.3	0.2
Adogen	10.71	-	-
Azulene	2.44	2.21	0.69
Cetene	2.42	0.94	-
Cycloheptasiloxane, tetradecamethyl-	-	0.4	0.24
Cyclohexane, 1-ethyl-2-propyl-	-	0.33	0.63
Cyclohexasiloxane, dodecamethyl-	-	0.94	0.14
Cyclooctasiloxane, hexadecamethyl-	-	0.17	-
Cyclopentasiloxane, decamethyl-	-	0.37	-
Cyclotetradecane	0.24	-	-
Dimethyl palmitamine	-	2.19	-
Di-n-octyl phthalate	12.2	19.16	75.03
Dodecane	1.17	1.22	0.31
Dodecyl isopropyl ether	-	0.36	-
Dotriacontane	-	2.25	-
E-14-Hexadecenal	-	-	0.13
Eicosane	6.98	4.03	-
Heneicosane	1.39	1.01	-
Heptacosanoic acid	-	1.88	-
Heptadecane, 2,6,10,15-tetramethyl-	1.06	1.45	-
Hexacosane	-	-	5.83
Hexadecanamide	0.86	-	-
Hexadecane	1.76	1.59	0.21
Hexadecyl octyl ether	-	1.6	-
Hexyl octyl ester	-	0.27	-
Methyl stearate	1.55	0.45	-
Nonadecane	-	0.58	-
Nonanal	-	1.33	0.67
Octadecanal	0.54	0.43	-
Octadecanamide	0.66	-	-
octadecyl 2-pentyl ester	-	-	0.17
Pentadecafluorooctanoic acid, dodecyl est	0.49	-	-
Pentadecane, 8-hexyl-	-	0.54	-
Pentadecyl ester	-	0.87	-
Stearyltrimethylammonium chloride	-	12.84	-
Supraene	1.17	3.76	2.06
Tetradecanal	-	0.21	-
Tetradecane	23.5	17.8	7.43
Tetradecanoic acid	4.87	-	-
Tetrapentacontane	0.43	0.56	-
Tetratetracontane	-	-	1.6
Triacontane	-	0.49	-
Tridecane	1.13	1.55	-
Tridecanol	-	-	-
Undecanal	11.92	-	0.47

Table 2 IC_{50} value of flora scent *Acacia Auriculiformis* extract

Plant parts	Crude Extracts	R^2	IC50 (µg/mL)
Flower	Control	0.9882	10.73
	Florescent	0.9934	16.54

Table 3 Effect of Flora scent of *Acacia auriculiformis* on fungus

Concentration (ppm)	Plant Part (Acacia flower)	*Aspergillus niger*	Candida tropicallis
	Control	2.03±0.05	2.03± 0.08
50	Florescent	0.61±0.09	0.51±0.16[c]
100	Florescent	0.54±0.03[c]	0.53±0.17
200	Florescent	0.69±0.23	0.62±0.13
300	Florescent	0.71±0.11	0.64±0.13
500	Florescent	0.74±0.13	0.75±0.12
1000	Florescent	0.86±0.04[a]	0.88±0.16[ab]

Values are Mean±SD for three determinations
[a]Significantly ($p< 0.05$) higher compared to different concentration in each column.
[b]Significantly ($p< 0.05$) higher compared to same extract at different concentration in each row.
[c]Significantly ($p<0.05$) lower compared to the control.

The antioxidant activity of the flora scent obtained from the *Acacia auriculiformis* plant was determined at 517nm wavelength using UV spectrophotometer. The result obtained are shown in Table 2. However, the activity of the scent as well against the mycelial growth of *Aspergillus niger* and *Candida tropicalis* presented in Table 3. It was observed that the test showed a reasonable inhibition effect against the mycelial growth of the selected pathogen with increase in concentration with 1000ppm having the maximum activity of 0.86±0.04 and 0.88±0.16 respectively for *aspergillus niger* and *Candida tropicallis* Figure 1–3.

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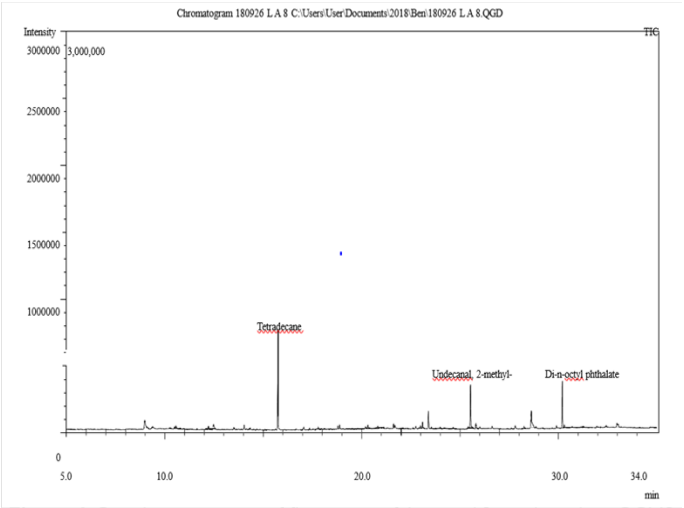

Figure 1 Gas chromatogram of florescent of *A. auriculiformis* based on GCMS analysis location A.

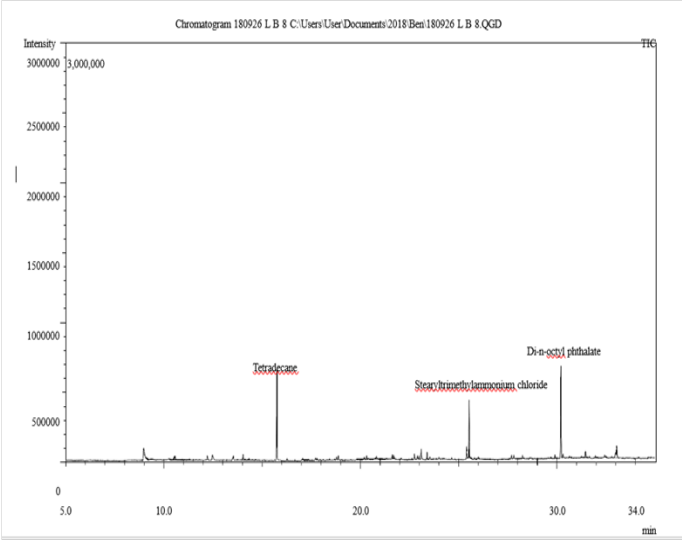

Figure 2 Gas chromatogram of florescent of *A. auriculiformis* based on GCMS analysis location B.

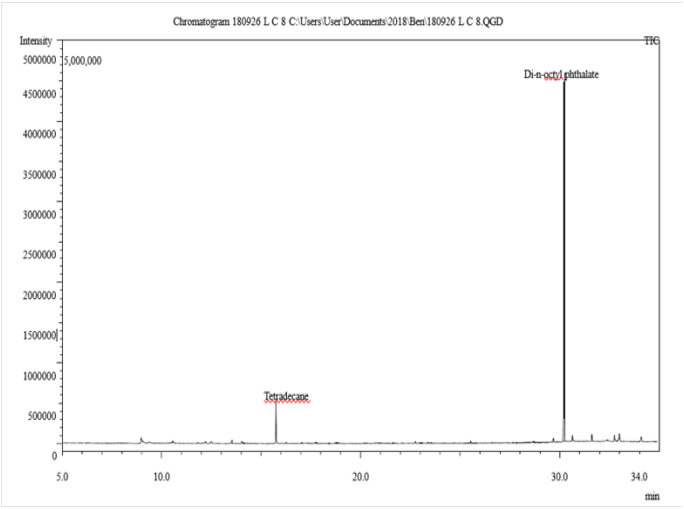

Figure 3 Gas chromatogram of florescent of *A. auriculiformis* based on GCMS analysis location C.

Discussion

Acacia auriculiformis plant have been reported to contain some phytochemical compounds but in this present studies a sample of the flora scent from the flower of the plant *A. Auriculiformis* was analysised using GCMS for its phytochemicals, antioxidant and antifungal activities. The result obtained revealed that the tested flora scent has some bioactive compound among which are Tetradecane, Di-n-ocyl phthalate, Stearyltrimethyl ammonium chloride, and undecanal 2-methyl few among which are promenently mentioned in GCMS Cromatogram above. In all the three location A, B and C, there was some differences in the chemical composition in the scent of the *Acacia auriculiformis*.thus indicating different composition of compound based on location and enviroment.

The antioxidant activy of the flora scent sample tested in the presence study was determined by measuring thier free radical scavenging activities (Table 2) thus showing a significant antioxidant activity which increase with increase in concentration. The antifungal activities against *Aspergillus niger* and *Candida tropicallis* showed a significant test against all the mycelial growth. The result showed that the selected pathogens was susceptable to the flora scent at various concentration. The least of the activity was observed at concentration 50 and 100ppm when compared to the rest. Fluconazole which was used as the standard in this test showed activity against the selected pathogen with higher inhibition rate than the test agent.

Conclusion

The study has shown that the flora scent of *Acacia auriculiformis* with its numerous phytochemical and antioxidant potential are very effective in inhibiting the mycelium growth of *Aspergillus niger* and Candida tropicallis. This flora scent could be used in pharmaceutical and cosmetics industry to augment their products as an agent against pathogens.

Ethics

This article is original and contains unpublished material. The corresponding author confirms no conflict of interest and all other authors have read and approved the manuscript.

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Conflict of interest

The author declares that there is no conflict of interest.

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