FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 6 No. 1, March, 2022, pp 7 - 12
DOI: https://doi.org/10.33003/fjs-2022-0601-773

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AND GROWTH RETARD NATURE OF *Barbula lambarenensis* ON BACTERIA

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ABSTRACT

Research studies have shown that mosses contain bioactive substances and are of significant economic importance. This study aimed at analyzing the composition of Barbula lambarenensis and screen for its bioactivity. The moss collected from its natural habitat was air-dried at room temperature in the laboratory, extracted with methanol and the crude extract was utilized for gas chromatography-mass spectrometry (GC-MS) analysis, antibacterial sensitivity testing and generation time elongation assay. The antimicrobial activity was tested using 12 bacterial strains. The result of the analysis showed the presence of 35 compounds with n-Hexadecanoic acid (22.59%), cis-Vaccenic acid (18.52%), 1, 2, 4-Cyclopentanetrione, 3-butyl- (13.84%) and 5-Hydroxymethylfurfural (10.24%) as the most abundant compounds. Extract of Barbula lambarenensis did not inhibit the growth of test organisms in the antibacterial sensitivity test. Generation time elongation assay showed a reduction in the growth rate of Bacillus cereus and Morganella morganii. The study indicated that Barbula lambarenensis contain numerous compounds with varying bioactivities which can be exploited by the pharmaceutical industry especially in the production of novel medicine.

Keywords: Mosses, Analysis, Extract, Biological activity

INTRODUCTION

Bryophytes are non-vascular, non-flowering, seedless plants that consist of mosses, liverworts and hornworts (Hedges, 2002). Bryophytes are the second largest group of land plants after flowering plants (Marko et al., 2001). Most species of bryophytes lack xylem which differentiates them from vascular plants, while some species have poorly developed conducting tissues (Schimper, 1879). *Barbula lambarenensis* (family Pottiaceae) is a bright, cushion-like moss that grows in tropical climatic conditions. Fatoba (1998) opined that the plant reproduces sexually but relies mostly on asexual propagules or gemmae for growth and expansion. The shoots of *Barbula spp* grow up to a length of more than 2.5 cm.

Cordell (2006) posited that humans are largely dependent on plants for food, medicine, clothing and shelter. According to Solecki (1975), plants have been utilized in medicine as early as 60,000 years ago. Reports regarding medicinal plants date back to almost 5000 years ago in India and at least 2500 years in Greece and Central Asia (Ang-Lee et al., 2001). Plants with medicinal potentials are used in health care because they have certain properties including synergistic actions. Gopalakrishnan and Udayakumar (2017) reported that plants consist of different types of phytochemicals, also known as secondary metabolites which play active roles in pharmaceutical industries for the development of novel drugs and therapeutic agents. Mosses and Liverworts have been reported to have a large variety of bioactive compounds (Fu et al., 2012). Singh et al., (2006) documented that bryophytes have antiviral, antibacterial, antifungal, antioxidant, antiplatelet, antitumor and insecticidal activities. Mosses have known pharmacological applications in the treatment of burns, wounds and bacterial infections (Saboljevic et al., 2010).

Burris et al. (2012) noted that factors such as habitat, plant species, extraction method, the solvent of extraction affect the composition and activities of plant extracts. Experimental work on the analysis of plant extracts is a method of finding out the biologically active components of plants (Gopalakrishnan and Udayakumar, 2014). Even though bryophytes have been reported to be rich sources of bioactive compounds (Edeoga and Eriata, 2001), few studies regarding the biologically active substances of moss species have been documented (Klavina et al., 2015; Isa et al., 2021; Fu et al., 2012). This study aimed to analyze the bioactive compounds of *Barbula lambarenensis* by GC-MS.

MATERIALS AND METHODS

Plant material

The plant material *Barbula lambarenensis* P. de la Varde, was collected from sandcrete block wall at Osun State University, Osogbo (Latitude 7º 28' 14.71'' N and Longitude 4º 53' 11.60'' E). The collected sample was carefully separated from dirt and washed with tap water in the laboratory (G.23) at the Obafemi Awolowo University, Ile- Ife. The sample was air-dried at room temperature (25℃) and kept for further laboratory work.

Crude Extract Preparation

Extract of the moss plant was prepared according to the method of Akinpelu et al., (2017). *Barbula lambarenensis* (80 g) was soaked in 600 ml of methanol for 72 hours in the laboratory. The resulting suspension after 72 hours, was decanted through cheesecloth into a 500 ml conical flask to remove the debris. The solution obtained was filtered using the Whatman No. 1 filter paper. The filtrate was evaporated using the rotary vacuum evaporator at 55℃ to obtain the crude extract, which is used for the antimicrobial sensitivity test and Gas chromatography-mass spectrometry (GCMS) analysis.

Crude Extract Yield

The yield of the crude extract from the preparation was calculated using the formula;

Percentage yield = Crude extract weight/weight of soaked *B. lambarenensis* ×100

Test Organisms

The test organisms used in this study were, *Bacillus cereus* (NCIB6349), *Proteus vulgaris* (NCIB67), *Morganella morganii* (LIO), *Pseudomonas aeruginosa* (NCIB950), *Candida albicans* and *Staphylococcus aureus* (NCIB8588), *Serratia marcescens* (NCIB I377), *Bacillus subtilis* (LIO), *Bacillus stearothermophilus* (NCIB8222), *Micrococcus luteus* (NCIB196), *Klebsiella pneumoniae* (NCIB418), *Clostridium sporogenes* (NCIB 532).

Sterilization Technique

All the glassware was thoroughly washed, rinsed with distilled water and dried. The pipettes were plugged with cotton wool and packed into the pipette container while the glass Petri dishes were packed in the cannister and were placed in the hot air oven for sterilization. The test tubes and glassware with media dispensed into them were sterilized by autoclaving at 121℃, 15 PSI (1 Kg/cm) pressure for 15 minutes. The inoculating needles and wire loop were sterilized by flaming in Bunsen burner till a red hot color was obtained and then allowed to cool before use.

Antibacterial sensitivity test

A sensitivity test of the bacterial strains to *B. lambarenensis* crude extract was carried out following a modified bioassay method of Betoni et al., (2006). *B. lambarenensis* extract (0.2 g/ml) was prepared using 5% methanol which was also used as a control. Sterile Mueller-Hinton agar plates were seeded with indicator bacterial strains (10^6 CFU/mL) and allowed to stand at room temperature for 3 hours. Using a sterile cork borer, wells were made on the seeded plates and these were filled separately with *B. lambarenensis* extract (1 ml). The set of plates was incubated at 37 ℃ for 24 h after which the plates were observed for bacterial growth inhibition.

Generation Time Elongation Assay

The cell doubling time elongation potential of the extract was carried out on *Bacillus cereus* and *Morganella morganii* according to a modified method of Štumpf et al., (2020). 30 mg of *B. lambarenensis* crude extract was constituted in Mueller-Hinton broth and 100 µl of bacterial solution that had been standardized to McFarland standard was added. The same setup without extract was prepared to serve as the control. The experiment and control were incubated at 37°C for 24 h. 100 µl from the experimental and control set up were separately cultured on fresh sterile nutrient agar plates in duplicates. The plates were incubated at 37°C for 24 h. The bacterial growth on experimental plates and control plates was compared to confirm the generation time elongation potential of the extract.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *B. lambarenesis* methanol extract was carried out at Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria, on GCMS-QP2010SE Gas Chromatograph Mass Spectrometer employing the following conditions: plunger speed (suction): high, viscosity comp. Time: 0.2 sec, plunger speed (injection): high, syringe insertion speed: high, injection mode: normal, inj. Port dwell time: 0.3 sec, plunger washing speed: high, washing volume: 8ul, syringe suction position: 0.0 mm, syringe injection position: 0.0 mm, column oven temperature: 60.0°C, injection temperature: 250.00°C, injection mode: splitless, sampling time: 2.00 minutes, flow control mode: linear velocity, pressure: 144.4 kpa, total flow: 38.7 ml/min, column flow: 3.22 ml/min, linear velocity: 46.3 cm/sec, purge flow: 3.0 ml/min, split ratio: 10.1, oven temp. Program rate temperature(°C) hold time (minutes): 60.0, 1.00, 12.00, 240.0, 2.00, 12.00, 300.0, 2.00, equilibrium time :1.0 min, ion source temperature: 230.00°C, interface temperature: 250.00°C, solvent cut time: 4.00 minutes, detector gain: 1.29 KV + 0.00 KV, threshold: 2200. For the mass spectrometry (MS) Table, the start Time: 5.50 minutes, End Time: 24.90 min. ACQ Mode: Scan. Event time: 0.50 sec, scan speed: 1428, start m/z: 45.00, end m/z: 700.00. The total GC running time was 44.5 minutes.

Identification of Compounds

The interpretation of spectra of the components on Mass-Spectrum for the GC-MS was conducted using the database of National Institute Standard and Technology 11 Library (NIST11.L) to ascertain and identify the phytocompounds in the moss extracts. The spectra of the unknown components were compared (head to tail) with those of known components stored in the database of NIST11 Library. The name and molecular structure of the components of the test materials were also ascertained using the fragmentation patterns they exhibited and the information available in the Library.

RESULTS

Crude Extract Yield

The yield of the crude extract from 80 g *B. lambarenensis* extracted with 600 ml methanol was 2.87 g which is equivalent to 3.59% of the soaked plant sample.

Antibacterial Sensitivity Test

The results of the antibacterial test of *B. lambarenensis* crude extract (0.2 g/ml) showed no bactericidal or growth inhibition effect on the tested bacteria.

Generation Time Elongation Assay

The result of the generation time elongation assay showed that *B. lambarenensis* methanol extract exhibited no bactericidal effect on the bacteria. However, a reduction in the growth rate of bacteria was observed when compared with that of the control.

Bioactive compounds

The compounds identified from the methanol extract of *Barbula lambarenensis* using GC-MS analysis are presented in Table 1. The GC-MS analysis showed the presence of organic acids, phenols, ketones, steroids and esters. The extracts revealed the presence of 35 compounds of which n-Hexadecanoic acid (22.59%), cis-Vaccenic acid (18.52%), 1,2,4-Cyclopentanetrione, 3-butyl- (13.84%) and 5-Hydroxymethyl furfural (10.24%) were the most abundant compounds and constituted 65.19% of the extract. The first compound in the extract of *Barbula lambarenensis* with least retention time (RT= 5.533) was Valeric acid, 2-ethoxyethyl ester; while Oct-5-en-2-ol, 8-(1,4,4a, 5, 6, 7, 8, 8a-octahydro-2,5,5,8a-tetramethylnaphth-1-yl)-6-methyl was the last compound which took the longest retention time (RT= 24.593). A compound, named Gingerol had 4 different retention times.

Table 1: Phytoconstituents identified in the methanol extract of *B. lambarenensis*

Peak#	R.Time	Composition%	Name
1	5.533	0.1	Valeric acid, 2-ethoxyethyl ester
2	5.636	0.12	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
3	6.748	0.35	2,4(1H,3H)-Pyrimidinedione, 5-hydroxy-
4	6.874	0.59	Phenol, 2-methoxy-
5	7.74	1.66	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
6	8.383	0.36	5-Acetoxymethyl-2-furaldehyde
7	8.542	0.28	Benzofuran-2,3-dione, 4,7-dimethyl-2,3-dihydro-
8	9	10.24	5-Hydroxymethylfurfural
9	9.095	13.84	1,2,4-Cyclopentanetrione, 3-butyl-
10	9.517	2.04	4-Hydroxy-3-methylacetophenone
11	9.876	0.43	Phenol, 2,6-dimethoxy-
12	10	0.26	3-Allyl-6-methoxyphenol
13	11.923	0.74	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-
14	12.185	1.93	3',5'-Dimethoxyacetophenone
15	12.306	0.53	Dodecanoic acid
16	12.481	2.28	Piperidin-2-one-5-carboxylic acid
17	13.121	0.6	2-Propanone, 1-(2-methoxyphenyl)-, oxime
18	13.564	0.87	1H-3a,7-Methanoazulen-5-ol, octahydro-3,8,8-trimethyl-6-methylene
19	13.837	2.83	Bicyclo[3.2.0]heptan-2-one, 6-hydroxy-5-(ethoxycarbonylmethyl)
20	14.168	0.41	Tetradecanoic acid
21	14.538	0.59	Bis[3,3,4,7-tetramethyl-1,3-2H-benzofuran-1-yl] ether
22	14.674	1.25	4,7-Dimethoxy-2-methylindan-1-one
23	15.982	22.59	n-Hexadecanoic acid
24	17.697	18.52	cis-Vaccenic acid
25	17.828	1.48	Octadecanoic acid
26	18.825	1.27	Gingerol
27	19.711	0.39	Gingerol
28	20.471	0.57	Nonivamide
29	20.708	0.24	Gingerol
30	20.826	1.2	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
31	21.188	3.68	Capsaicin
32	21.393	2.36	Dihydrocapsaicin
33	22.261	3.08	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
34	22.425	0.83	Gingerol
35	22.92	0.56	.beta.-Sitosterol
36	23.589	0.2	B-Homo-A-norcholestan-6-one, (5.alpha.)-
37	23.958	0.27	Cholestane, 4,5-epoxy-, (4.alpha.,5.alpha.)-
38	24.593	0.46	Oct-5-en-2-ol, 8-(1,4,4a,5,6,7,8,8a-octahydro-2, 5, 5, 8a-tetramethylnaphth-1-yl)-6-methyl-

DISCUSSION

The compounds identified have unique structures with varying properties which are characteristics of medicinal plants (Theresa et al., 2016). The moss species, extraction procedure and the solvent used play key roles in the composition and concentration of bioactive compounds. Organic acids are widely used as antimicrobial substances in food products especially for food preservation as stated by

Hauser et al., (2016). Phenols, ketones, esters, organic acids and steroids have been reported to have anti-inflammatory, antimicrobial, antitumor, antioxidant, anti-androgenic and diuretic properties (Lalitha et al., 2015). Sturtevant (1954) stated that *Barbula* spp. are boiled as a tea for treating flu, fever and body aches which suggest the medicinal potential of the compounds extracted from this moss species. Ande et al., (2010) reported that the extracts of *Barbula lambarenensis* made into aqueous solutions were effective against the stem borers of maize plants and thus useful in the field of agriculture.

The most abundant compound, n-Hexadecanoic acid (22.59%) was also reported to have the highest composition in the methanol extracts of young and mature *Archidium ohioense* (26.6%, 51.25% respectively) and *Philonotis hastata* (22.46%, 51.84% respectively*)* (Isa et al., 2021). n-Hexadecanoic acid also had the highest percentage composition in flowering plants such as *Psychotria nilgiriensis* (25.08%) and *Guiera senegalensis* (49.6%) (Lalitha et al., 2015; Shettima et al., 2013). n-Hexadecanoic acid is a fatty acid reported to have anti-inflammatory, antibacterial, antioxidant, antitumor, hemolytic, anti-androgenic, pesticide, nematicide, larvicidal and hypocholesterolemic properties (Aparna et al., 2012; Kumar et al., 2010; Rahuman et al., 2000; Lalitha et al., 2015).

Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester is a palmitic acid ester with pharmaceutical applications similar to n-Hexadecanoic acid (Lalitha et al., 2015). According to Lalitha et al., (2015), it had a 3.01% composition in *Psychotria nilgiriensis.*

Kumar et al., (2014) reported that Octadecanoic acid and other bioactive compounds of *Sesuvium portulacastrum L.*, an halophyte have antimicrobial, antioxidant and antitumor activities. Octadecanoic acid is an important component of the GC-MS analysis of several moss species carried out by Klavina et al., (2015).

Beta-Sitosterol was found in a study conducted by Klavina et al., (2015) on several moss species such as *Aulacomnium palustre, Sphagnum magellanicum* and *Polytrichum juniperum*. Lalitha et al., (2015) recorded 7.52% beta-Sitosterol in the extracts of *Psychotria nilgiriensis.* It is a steroid reported to have anti-inflammatory, antimicrobial, antiasthma, anticancer, antiarthritic and diuretic activities (Lalitha et al., 2015).

Tetradecanoic acid was documented as a bioactive constituent of the chloroform extract of moss species studied by Klavina et al., (2015). According to Sivakumar et al., (2011), tetradecanoic acid has larvicidal and repellant activities.

Capsaicin is a bioactive compound that contains phenol, amide, ether and alkene functional groups. It is found in chili peppers that belong to the genus *Capsicum.* Capsaicin is used in food products in form of spices. It causes a burning sensation of the eyes, skin and sensitive body parts of mammals (Rollyson et al., 2014). It is used as an analgesic in the form of dermal patches and ointments (Fattori et al., 2016). It has found applications in the treatment of psoriasis (Ellis et al., 1993) and hypercholesterolemia (Kelavia et al., 2021). It is used as a pesticide and to deter rodents (Jensen et al., 2003).

Nonivamide is also called pelargonic acid vanillylamide and is found in chili peppers (Constant et al., 1996). It is more heat-stable than capsaicin. Nonivamide is used as food additive due to its pungency. It is an active ingredient of pepper spray used as a form of chemical weapon (Haar et al., 2017). Nonivamide has been reported to be harmful to human health and can lead to death (Haar et al., 2017).

Gingerol is a phenol compound that has demonstrated antioxidant, anti-inflammatory, antifungal, anticancer, gastroprotective and diabetic properties (Baliga et al., 2011). Salehi et al., (2019) carried out a meta-analysis using mice and reported that gingerol caused apoptosis of cancer cells by acting on the mitochondrial membrane. However, gingerol shown at four different retention times in the analysis could be attributed to the presence of four different types of gingerol in *Barbula lambarenesis* extract. This is similar to the work of Sanwal et al. (2010) who reported 6-gingerol, 8-gingerol and 10-gingerol at varying concentrations in each of the eighteen different ginger genotypes from India.

Cis-vaccenic acid is a stereoisomer of vaccenic acid. Vaccenic acid is a naturally occurring fatty acid found in human milk and dairy products (Precht and Molkentin, 1999). Vaccenic acid has been found in association with patients who had bipolar disorder and schizophrenia (McNamara et al., 2008). A study by the department of agriculture in the United States showed that vaccenic acid raised both high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol (Baer, 2010). Arora and Saini (2017) documented 9.25% cis-vaccenic acid in a flowering plant, *Corbichonia decumbens.*

2 methoxy-Phenol is otherwise referred to as Guaiacol. It is used as a precursor for green fuels according to Majid et al., (2014). It contributes to the flavor of food items such as coffee (Dorfner et al., 2003).

5-hydroxymethyl furfural (5-HMF) has a 10.24% composition of the plant extract. A study carried out using transgenic sickle mice showed that oral administration of 5-HMF inhibits the formation of sickled cells in the blood, making 5-HMF a potential for treating sickle cell disease (Abdulmalik et al., 2005).

The extracts of *Barbula lambarenensis* did not inhibit the growth of the test organisms which is contrary to the study carried out by Klavina et al. (2015) wherein the moss species analyzed showed certain antibacterial activities. However, the moss extract showed bacteriostatic activity which could be relevant for further research.

CONCLUSION

The results suggest that *Barbula lambarenensis* has several chemical compounds of great medicinal value. The extract showed no bactericidal activity, however reduced the growth rate of the test organisms. The moss species has a high potential for the production of novel medicine in the pharmaceutical industry. Further studies are needed to know the specific mechanism of action of individual compounds present in *Barbula lambarenensis.* There is a need for toxicity studies using human cell lines to in order to determine the potency and safety of *Barbula lambarenensis*.

Competing Interests

The authors declare that there are no competing interests.

Funding

The authors did not receive any funding for this work.

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