Food Science & Applied Microbiology Reports
Vol. 2(1),2023; 34-40
ISSN (Online) 2958-0684
https://finessepublishing.com/fsamr

Bioactive Compounds of Prosopis Africana oil (African mesquite) Using Gas Chromatography and Mass Spectrometry (GC-MS) Technique

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Abstract: Phytochemicals (bioactive compounds) are generally regarded as chemicals of plant origin used by plants for growth, defense against pathogens and other competitors. They also have a wide range of pharmacological activities including antiarrhythmic, antioxidant, antimalarial, vasodilatory, antimicrobial, analgesics, hepato-protective, anti-inflammatory, hypo-cholesterolemic, anti-androgenic, antiviral, antifungal and immune-modulatory. This study was carried out to investigate the bioactive compounds of Prosopis africana oil (African mesquite) using gas chromatography–mass spectrometry technique. A total of 73 bioactive compounds (77.16 %) were identified based on their peak areas. The major compounds identified in the oil sample were prospering A (29.90 %), caryophyllene (12.33 %), 2, 4-bis (1-phenylmethyl) phenol (5.80 %), gallic acid (5.22 %), β-cycloidal (3.11 %), β-sitosterol (2.50 %), α-terpinene (2.09 %) and ellagic acid (2.04 %). In conclusion, Prosopis africana oil is loaded with phytochemicals and has several health-promoting properties and it can also be used as a remedy for the treatment of several ailments in human beings and animals.
Keywords: Phytochemicals, Prosopis africana, Pharmacological properties, Gas chromatography

1. Introduction

Prosopis africana (African mesquite) is an evergreen multipurpose leguminous plant belonging to the family Fabaceae. It has about 45 species and it is widely distributed in most of Africa, Asia, and America (Adikwu et al., 2001; Ugwoke et al., 2019). The seeds of Prosopis africana are rich in phyto-constituents such as alkaloids, tannins, flavonoids, terpenoids, steroids, saponins, steroids, glycosides, and phenols with significant therapeutic properties (Alagbe, 2021; Olorumaiye et al., 2019). The seeds have antioxidant, antifungal, antimicrobial, antifungal, angelsics, antipyretic, anti-proliferative, antifungal, anti-inflammatory, hepato-protective, antiprotozoal, anti-androgenic and cytotoxic properties (Harzallah and Jannet, 2005; Singh et al., 2023) and it also contains important minerals such as calcium, potassium, zinc, manganese, magnesium, copper, and sodium (Ferguson et al., 2015; Oluwafemi et al., 2021). These minerals are vital for the activation of key enzymes that provide greater protection from diseases (Alagbe, 2022; Shittu and Alagbe, 2020).

Moreover, Prosopis africana seeds contain amino acids alanine, proline, valine, and isoleucine which are the main structural proteins found in the connective tissues of animals (Peter et al., 2009; Barminas et al., 1998). The medicinal properties of plants depend on their composition of phyto-constituents or bioactive compounds (Ogunshe et al., 2007; Le Houe'rou, 2003). The stem bark and root decoction of Prosopis are used traditionally for the treatment of rheumatism, cough, malaria, bronchitis, skin diseases, and gastrointestinal and sexually transmitted infections (Agubosi et al., 2022). Fermented seeds of the plant can be used in the preparation of soup because of its sweet smell (Gberikon et al., 2015; Platt, 1980).

Preclinical investigations have shown the pharmacological responses of Prosopis africana oil to inhibit the activities of some pathogenic organisms such as Salmonella spp, Candida albicans, Escherichia coli, Bacillus subtilis, Bacillus pumilus, Bacillus licheniformis and Bacillus megaterium (Oguntoyinbo et al., 2010; Alagbe et al., 2023). Aqueous and ethanolic extracts from the leaves and stem bark of Prosopis africana can be used for the treatment of diarrhea and coccidiosis in poultry (Adewale et al., 2021). Identifying the bioactive compounds

[Received] 14 April 2023; Accepted 23 June 2023; Published (online) 27 June 2023]

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DOI: 10.61363/fsamr.v2i1.60

in a medicinal plant could lead to the production of novel drugs (Alagbe, 2020). One key method in identifying unknown compounds, quantification, and characterization is the use of gas chromatography–mass spectrometry (GC-MS) technique.

Investigating the bioactive compounds in a sample will give a clue about its efficacy and safety. Hence, given the immense medicinal properties of Prosopis africana seed. This study was designed to evaluate the phytochemicals or bioactive compounds in Prosopis africana oil using GC-MS technique.

2. Materials and methods

2.1 Experimental site

This study was carried out at the Department of Animal Science Laboratory, University of Abuja main campus along Airport Road, Gwagwalada, Abuja, Nigeria located between latitudes 8o571 and 8o551N and longitude 7o051 and 7o061E (Balogun, 2001).

2.2 Collection, identification processing of Prosopis africana oil

Prosopis africana seeds were purchased from a local market in Gwagwalada, Nigeria. It was identified and authenticated at the Herbarium of the Department of Biological Sciences, University of Abuja, Nigeria with a voucher number ABJ/09A/20. It was shade-dried for 13 days ground into powder using a laboratory blender (Panasonic, Model EDH-023), and stored in a labeled polythene bag for further testing.

2.3 Extraction of Prosopis africana oil using steam distillation method

Prosopis africana oil was extracted using the steam-distillation technique, the entire extraction procedure requires a digital weighing scale, round bottom flask, distilled water, glass yarn heating mantle, measuring cylinder, and separatory funnel. 50 grams of ground Prosopis seed was weighed into a round bottom flask, and mixed with 250 mL of distilled water. The mixture was placed in a glass yarn heating mantle and adjusted to a temperature of 80 ^{C} before setting up the condenser above the round bottom flask. The mixture is boiled vigorously for 15 minutes, and the distillate is collected in a beaker until no more droplets of oil come over followed by the addition of 5 grams of sodium chloride and stirred vigorously. Thereafter, the distillate was transferred into the separatory funnel and evaporated in a steam bath to obtain *Prosopis africana* oil.

2.4 Bioactive compounds of PRAO using GC-MS technique

Bioactive compounds of PRAO were identified using LABTRON GC-MS (GC-MS-879). It is a high-precision gas chromatography-mass spectrometer with a pre-filter mass analyzer and electron multiplier ensuring high sensitivity. Gas chromatography (Model – GC-87) has the following technical specifications; flow rate (1-15 ml/min), maximum flow rate (10 ml/min), in let temperature (450 ^{o}C), pressure range (0 – 100 psi), heating rate (up to 120 ^{o}C / min), room temperature (4o C – 450 ^{o}C), pressure control mode (electronic pressure control), split mode (split / split less), split ratio (1000: 1) and temperature programming (7 stages / 8 platforms) while the mass spectrometry (Model- MS-89): mass range (1.5 – 1000 amu), ion – source temperature (100 ^{o}C – 350 ^{o}C), GC-MS interface temperature (450^{o} C), scan rate (up to 1000 amu/sec), stability (± 0.10 amu/ 48 hours), filament emission current (0 - 350^{o} C μA), EI source ionization energy (5 eV – 250 eV), sensitivity (S/N is ⩾ 30:1) and vacuum (Turbo molecular pump: 67 L/s). Volatile compounds were identified with standard compounds in the National Institute of Standard and Technology mass spectral library using the Chemstation library database. Percentage area was based on the ratio between the peak area of each compound and the sum of the peak areas of all compounds.

Table 1: Bioactive compounds of *Prosopis africana* oil as detected by GC-MS

Peak No	RT (min)	Area (%)	Name of compound
1	2.77	29.90	Prosogerin A (6 Methoxy-7-hydroxyl dioxyflavone)
2	3.93	5.80	2,4-bis (1-phenylmethyl) phenol
3	4.03	0.63	β-phenethylamine
4	4.51	0.14	2,4,6-tris(1-phenylmethyl) phenol
5	4.92	0.16	α-pinene
7	5.22	2.09	α-terpinene
8	5.91	0.46	β-pinene
9	6.07	0.12	β-myrcene
10	6.34	0.74	α-phellandrene

11	6.88	0.11	α-terpinolene
12	7.22	0.35	γ-terpinene
13	8.09	0.03	1-terpineol
14	8.44	0.07	4-terpineol
15	9.57	0.18	Humulene
16	9.88	12.33	Caryophyllene
17	10.12	1.85	Copaene
18	11.55	0.21	Cis-linaclotide
19	11.83	0.30	α-Selinene
20	11.90	0.06	γ-Elemene
21	12.10	0.01	α-Gurjunene
22	12.33	0.42	β-Elemene
23	12.68	3.11	β-Cyclocitral
24	12.75	0.05	3-hexenyl-2-methylbutanoate
25	12.92	0.02	Exo-methyl-camphenilol
26	13.10	0.06	Caryophyllene oxide
27	13.47	0.03	Benzene -1-methoxy-2-methyl
28	13.55	0.18	Napthalene, 1,2 hydro-1,1, 6 –trimethyl
29	13.80	1.55	Cycloheptasiloxane, tetradecamethyl
30	13.87	1.41	Nerolidyl acetate
31	13.91	0.001	1-Cyclohexene-1- butanal, alpha, 2, 6, 6- tetramethyl
32	14.00	0.11	Cyclooctasiloxane, hexadecamethyl
33	14.31	0.60	2,2, 6-Trimethyl-2H, 5Hpyrano(3,2-c) quinoline-5one
34	14.49	0.27	Dibutyl phthalate
35	14.52	0.70	Acetic acid, dodecyl ester
36	14.70	0.64	1,1, 5-Trimethyl-1,2-dihydro naphthalene
37	14.83	0.02	Alloaromedendrene oxide
38	14.92	0.03	Butylated Hydroxytoluene
39	14.95	0.01	Octacosane
40	16.71	0.16	Kaur-16-ene
41	16.37	0.10	1,5,8-p-Menthatriene
42	16.55	0.20	Spicigerine
43	18.28	0.07	Prosophylline
44	18.84	2.04	Ellagic acid
45	18.92	0.55	Apigenin-6-glucoside
46	19.20	1.80	Quercertin-3-rhamnoside
47	19.44	0.01	Isoprosopilosine
48	19.75	1.50	Prosopilosidine
49	19.87	0.02	Trimethyl-tetrahydronaphtalene
50	21.21	0.21	Spathulenol
51	21.40	0.01	1,3-benzodioxole,5-(2,2-dimethyl)
52	21.61	0.22	2-nonen-4-one
53	22.02	0.11	Bicyclo [2.2.1] heptan-3-one
54	25.04	2.50	β-sitosterol
55	25.80	0.32	Cis-vaccenic acid
56	26.11	0.16	3-chloro-N-isochron-1-methyl propionamide
57	26.70	0.03	3,4 – dichloro benzonitrile
58	26.84	0.07	Mannitol, 1,4-di-O-methyl tetraacetate
59	27.02	0.14	β-1-rhamnofuranoside thio-octyl
60	27.18	0.03	Guaia-1(10), 11-diene
61	27.35	0.12	3,4 – dimethyl phenyl heptyl ether
62	27.80	0.09	1,1,5 –trimethyl -1,2 –dihydro naphthalene
63	27.93	0.03	1-cyclohexane-1-propanal

64	28.06	0.04	1-Trimethylsilylpent-1-en-4-one
65	28.93	0.02	β-ocimene
66	28.98	0.05	Trans- β-ocimene
67	33.40	0.18	1-Methyl-6-methylene bicyclo heptane
68	33.73	0.04	1,6,10,14 –Hexadecatetraen-3-ol
69	33.81	0.01	Alloaromadendrene oxide (1)
70	33.92	0.40	1-cyclohexane-1-propanal
71	36.27	0.18	1-Formyl -2,2,6 –trimethyl cyclohexane
72	36.84	5.22	Gallic acid
73	36.93	0.06	6-Isopropenyl-4,8-dimethyl naphthalene-2-ol
74	37.10	0.01	1,6,10-Dodecatrien-3-trimethyl –ol
Total		77.16	

RT: Retention time (min)

3. Results and discussion

3.1 Bioactive compounds of Prosopis africana oil

The bioactive compounds of Prosopis africana oil are presented in Table 1. A total of 73 bioactive compounds (77.16 %) were identified based on their peak areas. The major compounds identified in the oil sample were prospering A (29.90 %), caryophyllene (12.33 %), 2, 4-bis (1-phenylmethyl) phenol (5.80 %), gallic acid (5.22 %), β-cycloidal (3.11 %), β-sitosterol (2.50 %), α-terpinene (2.09 %) and ellagic acid (2.04 %). Other minor compounds includes; β-phenethylamine (0.63 %), 2, 4, 6-tris (1-phenylethyl) phenol (0.14 %), α-pinene (0.16 %), β-myrcene (0.12 %), α-phellandrene (0.74 %), α-terpinolene (0.11 %), γ-terpinene (0.35 %), 1-terpineol (0.03 %), 4-terpineol (0.07 %), humulene (0.18 %), copaene (0.85 %), cis-linaloxide (0.21 %), α-selinene (0.30 %), γ-elemene (0.06 %), α-gurjunene (0.01 %), β-elemene (0.42 %), 3-hexenyl-2-methylbutanoate (0.05 %), exo-methyl-camphenilol (0.02 %), benzene -1-methoxy-2-methyl (0.03 %), napthalene, 1,2 hydro-1,1, 6 –trimethyl (0.18 %), cycloheptasiloxane, tetradecamethyl (1.55 %), nerolidyl acetate (1.41 %), 1-cyclohexene-1- butanal, alpha, 2, 6, 6- tetramethyl (0.001 %), cyclooctasiloxane, hexadecamethyl (0.11 %), 2,2, 6-trimethyl-2H, 5H pyrano (3,2-c) quinolin-5-one (0.60 %), dibutyl phthalate (0.27 %), acetic acid, dodecyl ester (0.70 %), 1,1, 5-trimethyl-1,2-dihydronaphthalene (0.64 %), alloaromedendrene oxide (0.02 %), butylated hydroxytoluene (0.03 %), octacosane (0.01 %), kaur-16-ene (0.16 %), 1,5,8-p-menthatriene (0.10 %), spicigerine (0.20 %), prosophylline (0.07 %), apigenin-6-glucoside (0.55 %), quercertin-3-rhamnoside (1.80 %), isoprosopilosine (0.01 %), prosopilosidine (1.50 %), trimethyl-tetrahydronaphtalene (0.02 %), spathulenol (0.21 %), 1,3-benzodioxole,5-(2,2-dimethyl) (0.01 %), 2-nonen-4-one (0.22 %), bicyclo [2.2.1] heptan-3-one (0.11 %), cis-vaccenic acid (0.32 %), 3-chloro-N-isochroman-1-methyl propionamide (0.16 %), 3,4 – dichlorobenzonitrile (0.03 %), mannitol, 1,4-methyl tetraacetate (0.07 %), β-1-rhamnofuran oside thio-octyl (0.14 %), guaia-1(10), 11-diene (0.03 %), 3,4 – dimethyl phenyl heptyl ether (0.12 %), 1,1,5 –trimethyl -1,2 –dihydronaphthalene (0.09 %), 1-cyclohexane-1-propanal (0.03 %), 1-trimethylsilylpent-1-en-4-yne (0.04 %), β-ocimene (0.02 %), trans- β-ocimene (0.05), -methyl-6-methylenebicycloheptane (0.18 %), 1,6,10,14 –hexadecatetraen-3-ol (0.04 %), alloaromadendrene oxide (1) (0.01 %), 1-cyclohexane-1-propanal (0.40 %), 1-formyl -2,2,6 –trimethyl cyclohexane (0.18 %), 6-isopropenyl-4,8-dimethyl nephthalen-2-ol (0.06 %) and 1,6,10-dodecatrien-3-trimethyl –ol (0.01 %). However, all these compounds have significant pharmacological effects (Singh et al., 2021; Muritala et al., 2022).

Prosogerin A has been reported to possess antimicrobial and anti-inflammatory properties (Agubosi et al., 2021). Caryophyllene occurs naturally in essential oils of several plants including Piper nigrum, Lantana camara leaf oil, and Capaifera spp, and has been reported to function as an antioxidant, thus scavenging free radicals (Adane, 2020; Pagare et al., 2015). 2,4-bis (1-phenylethyl) phenol, α-terpinene, 2,4,6-tris(1-phenylethyl) phenol, β-myrcene, α-terpinolene, and α-phellandrene had been reported by researchers to have anti-inflammatory and hypocholesterolemic properties (Silva et al., 2015; Aguoru et al., 2016). Humulene, β-sitosterol, α-gurjunene, α-selinene, and exo-methyl-camphenilol possess hepato-protective, anti-proliferative, antifungal, antipyretic, and antihelminthic properties (Alagbe, 2023; Oluwafemi et al., 2021 Shibula et al., 2015). Naphthalene, 1,2 hydro-1,1, 6 – trimethyl and benzene -1-methoxy-2-methyl can perform nematicide and anti-androgenic activities (Dua et al., 2010; Borokini and Omotayo, 2012). The other bioactive compounds in less concentration contained in Prosopis Africana oil also have significant therapeutic properties and are used in ayurvedic remedies for a variety of ailments (Ayoola and Coker, 2008). However, the result obtained in this experiment agrees with the findings of Lokesh et al. (2018); Nakano et al. (2001), and Aneela et al. (2014) but contrary to the reports of

Agubosi et al. (2021). These differences are influenced by factors like species, climate, harvest stage, location, storage condition, and method of extraction of the oil (Idris et al., 2009; Agubosi et al., 2022).

4. Conclusion

It can be concluded that Prosopis africana oil possesses several health-promoting properties (antimicrobial, antifungal, antiviral, anti-fibrotic, immune-modulatory, cytotoxic, antipyretic, antitumor, antihelminthic, and hepato-protective) due to the presence of bioactive compounds. These compounds can reduce the rising concern about environmental pollution, antibiotic resistance, and food safety. It could also enhance livestock performance and the production of novel drugs.

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