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Physicochemical And Preliminary Study on Gc-MS Analysis of Prosopis Africana Seed (African Mesquite) Oil

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Abstract: A preliminary study was carried out to examine the various bioactive compounds and physicochemical properties of *Prosopis africana* seed oil (*African mesquite*). The extracted oil was yellow in colour with a yield of 10.73 % at liquid state, acid value, saponification, iodine, peroxide values ranged between 15.18 (mg NaOH/g of oil), 112.7 (mg/KOH/g of oil), 150.4 (gI_2/100g of oil) and 27.75 (meq/kg) respectively. A total number of 24 bioactive compounds was identified using GC-MS this includes: 2-phenyl propane (3.09 %), 1,3,5-trimethyl benzene (4.80 %), prosogerin A (12.67 %), prosogerin B (10.06 %), prosogerin C (7.11 %), prosogerin D (4.05 %), prosogerin E (2.89 %), luteolin 7-O-β-D-glucoside (0.67 %), quercetin 3-O-rutinoside (4.88 %), isoprosopilosine (4.28 %), tryptamine (1.66 %), β-phenethylamine (5.63 %), gallic acid (0.57 %), 4-hydroxy benzoic acid (1.41 %), 2,4-bis (1-phenylethyl) phenol (9.60 %), 2,4,6-tris(1-phenylethyl) phenol (7.83 %), rhodoxanthin (1.67 %), ferulic acid (1.20 %), pheophytin A (1.54 %), pheophytin B (3.31 %), campsterol (2.80 %) and β-sitosterol (1.51 %). Prosogerins are the most abundant compounds in the sample and are known to posses antimicrobial, anti-inflammatory and capable of scavenging free radicals thus preventing diseases in animals. It was concluded that *Prosopis africana* seed oil can be used as potential alternative to antibiotics and can be used to bridge the gap between food safety and livestock production.

Key words: Prosopis africana, seed, oil, phytochemicals, antimicrobials, antioxidants, free radicals

Introduction

Seeds from plants are potential reservoirs for bioactive compounds or secondary metabolites, proteins, carbohydrates, amino acid and fats. Recently, there has been increasing awareness on food safety and the use of plants as potential alternatives to antibiotics due to antimicrobial resistance, toxic residues of synthetic drugs in livestock products, increase in different ailments and death (Singh *et al*., 2021). Plants of medicinal origin contains essential oils, which have been proven to be rich in phytochemicals (tannins, flavonoids, phenols, saponins, alkaloids, terpenoids etc), effective and generally regarded as safe (Adewale *et al*., 2021). The high cost of animal protein has directed interest towards several seed protein as potential sources of vegetable protein for human food due to its nutrient compositions (Musbau and Asiru, 2020).

Prosopis africana (*African mesquite*) is a tropical leguminous plant. It belongs to the family Fabaceae. The tree reaches about 4-20 m in height; has an open crown and slightly rounded buttresses; bark is very dark, scaly, slash, orange to red-brown with white streaks (Aremu *et al*., 2007). Seeds from *Prosopis africana* are used to prepare traditional fermented soup condiment or as flavor enhancers in Northern parts of Nigeria (Tajudeen *et al*., 2011). The seeds are rich in several bioactive compounds which makes a medicinal plant (Oloyede, 2005). The ripe pods of *P. africana* are harvested by shaking off the ripe pod from the tree branches. According to Musbau and Asiru (2020) proximate composition of fermented *P. africana* seeds and pods revealed the presence of crude protein (27.75 %, 7.62 %), carbohydrate (27.09 %, 72.72 %), ether extracts (10.52 %, 6.86 %), fibre (6.76 %, 3.28 %), moisture (0.03 %, 5.62 %) and ash (6.04 %, 3.90 %). Oluwafemi *et al*. (2021) also reported that *P. africana* seed oil contains crude protein, crude fibre, ether extract, ash and energy at 30.71 %, 6.47 %, 3.66 %, 5.08 % and 383.26 (Kcal/kg).

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The pods are useful in the feeding of ruminant animals due to their high nutritive value and can also be used traditionally for the treatment of tooth ache and other skin infections (Olorunmaiye *et al*., 2019). Physicochemical property is an index used to evaluate the edibility as well as industrial application of oil.

This experiment was designed to examine the physicochemical properties as well as the secondary metabolites in *Prosopis africana* seed oil using GC-MS. This research will give a clue on its medicinal properties as well as the physiological actions of the oil in the body of animals

Materials and methods

Experimental site

This study was carried out at the department of Animal Science Research Laboratory, University of Abuja, along airport road, Gwagwalada, Abuja, Nigeria. Gwagwalada is located between latitudes 8^o57^1 and 8^o55^1N and longitude 7^o05^1 and 7^o06^1E.

Extraction of *Prosopis africana* seed

Mature seeds from *Prosopis africana* were harvested from different trees at the University of Abuja, Teaching and Research Farm. It was identified and authenticated by a certified taxonomist at the Department of Biological Sciences, University of Abuja, Gwagwalada with a voucher specimen number ANS/08F/2020. The seeds were sorted to remove the bad ones and washed with running water to remove dirt's and air dried for 18 days to maintain the bioactive chemicals in the seeds, mechanically separated from their pods with knife to obtain the seeds. Dried seeds was grinded using a laboratory grinder (Panasonic: model AS/309F) to obtain *Prosopis africana* seed meal and stored in a clean well labeled container for analysis. Prior to the commencement of the analysis, laboratory equipments were serviced and all the necessary reagents were purchased.

Extraction of PASO was done using a cold press machine (Model: YZYX168, China). The grinded *Prosopis africana* (2000 g) was poured at into the feeder of the machine which works at a low temperature and PASO was collected via the squeeze cage.

Gas chromatography – Mass spectrometry analysis (GC-MS)

GC-MS analysis were performed on a GC – 2010 Shimadzu capillary gas chromatography directly coupled to the mass spectrometer system (GC-MS – model QP 2010; S/N column (70464300019 SA; Shimadzu) DB – 5ms non polar fused silica capillary column (30m X 0.25mm, 0.25µm film thickness) was used under following conditions: oven temperature program isotherm 2 min at 70ºC, 3ºC/min gradient to 200ºC and final temperature kept for 35 min; injection temperature 200ºC carrier gas is helium with flow rate 1.51ml/min; linear velocity 45.1 cm/sec. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with ionization energy 70eV, in the electronic ionization mode and ion source temperature is 200ºC. The solvent cut time 3 min. The sector mass analyzer was set to scan from 40 to 1000m/z with interface temperature of 240ºC. The components of the essential oil were identified on the basis of comparison of their relative indices and mass spectra by computer matching with National Institute of Standards and Technology (NIST08) libraries.

Physicochemical analysis of *Prosopis africana* oil

Determination of acid value, saponification value, iodine value and acid values were determined according to the methods outlined by AOAC (2005).

Gas chromatography mass spectrophotometer (GC-MS) composition of *Prosopis africana* oil

The GC-MS result of *Prosopis africana* seed oil revealed many bioactive compounds. These compounds with their molecular weight, molecular formulae, retention time and their composition (%) determined from their peak areas is presented in Table 1. A total number of 24 bioactive compounds was identified including: 2-phenyl propane (3.09 %), 1,3,5-trimethyl benzene (4.80 %), prosogerin A (12.67 %), prosogerin B (10.06 %), prosogerin C (7.11 %), prosogerin D (4.05 %), prosogerin E (2.89 %), luteolin 7-O-β-D-glucoside (0.67 %), quercetin 3-O-rutinoside (4.88 %), isoprosopilosine (4.28 %), tryptamine (1.66 %), β-phenethylamine (5.63 %), gallic acid (0.57 %), 4-hydroxy benzoic acid (1.41 %), 2,4-bis (1-phenylethyl)

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phenol (9.60 %), 2,4,6-tris(1-phenylethyl) phenol (7.83 %), rhodoxanthin (1.67 %), ferulic acid (1.20 %), pheophytin A (1.54 %), pheophytin B (3.31 %), campsterol (2.80 %) and β-sitosterol (1.51 %). The result obtained in this study agrees with the report of Peter *et al*. (2009); Ferguson *et al*. (2005); Bhardwaj *et al*. (1979, 1981); Simpson and Solbrig (1977). Bioactive compounds in plants are also regarded as phytochemicals which have been proven to be relatively cheap, safe effective and recently projected as suitable alternative to antibiotics (Adewale *et al*., 2021; Musa *et al*., 2020). For instance, prosogerins are group of flavonoids which performs multiple pharmacological functions such as: antibacterial, anti-inflammatory, antiviral, antitumor, hypolipidaemic, antiprotozoal, cytoprotective, antioxidant, antiplatelet and so on (Harzallah and Jannet, 2005; Alagbe and Motunrade, 2019; Alagbe, 2019, 2018). Prosogerins (A, B, C, D and E) are the most abundant compounds in this study; this confirms the earlier findings of Valli *et al*. (2014). Isoprosopilosine, tryptamine and β-phenethylamine are group of alkaloids which have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity and many others (Aneela *et al*., 2014; Ukani *at al*., 2000). Alkaloids are also capable of working as an antimicrobial, antispasmodic and scavenging free radicals to prevent disease in the body of animals (Alagbe and Motunrade, 2019; Latif *et al*., 2003). Studies have also reported the beneficial effects of phenol as an antioxidant, therefore the presence of 2,4-bis (1-phenylethyl) phenol and 2,4,6-tris(1-phenylethyl) phenols is a clear indication that *Prosopis africana* seed oil can function as an antibacterial (Kasolo *et al*., 2010; Singh *et al*., 2021) and can be traditionally used to treat skin diseases, wounds and other ailments.

Various bioactive chemicals in *Prosopis africana* seed oil exhibit a wide spectrum of antibacterial activities against pathogenic organisms thus influencing voluntary intake in animals and bile secretion (Zeng *et al*., 2015). Composition of *Prosopis africana* seed oil varies according to plant species, geographical origin, season, environmental factors, processing techniques and storage conditions (Gadde *et al*., 2017).

Table 1 Major chemical compounds of *Prosopis africana* oil as detected by gas chromatography mass spectrophotometer (GC-MS)

Chemical compounds	RI	% Area	MW(g/mol)	MF	Mass peak
2-phenyl propane	796	3.09	136.2	$C_9H_{12}O$	27, 42, 54, 79
1,3,5-trimethyl benzene	951	4.80	120.2	C_9H_{12}	27, 55, 101, 134
Prosogerin A	1224	12.67	312.3	$C_{17}H_{12}O_6$	27, 87, 102, 141
Prosogerin B	1009	10.06	330.8	$C_{18}H_{14}0_7$	27, 45, 58, 105
Prosogerin C	806	7.11	372.4	$C_{20}H_{20}O_7$	27, 62, 71, 90, 166
Prosogerin D	1205	4.05	358.3	$C_{19}H_{18}O_7$	27, 41, 48, 54, 71
Prosogerin E	1104	2.89	344.3	C18H16O_7	27, 38, 51, 74, 89
Luteolin 7-O-β-D-glucoside	1967	0.67	447.4	$C_{21}H_{19}O_{11}$	27, 38, 77, 96, 155
Quercetin 3-O-rutinoside	2196	4.88	610.51	$C_{27}H_{30}O_{16}$	27, 63, 89, 94, 106
Apigenin-8-glucoside	1663	3.31s	564.5	$C_{26}H_{28}O_{14}$	27, 47, 69, 76, 93
Quercetin-3-glucoside	1907	5.43	464.09	$C_{21}H_{20}O_{12}$	27, 85, 89, 94, 97
Isoprosopilosine	1800	4.28	933.4	$C_{57}H_{104}O_9$	27, 41, 55, 81, 95
Tryptamine	2506	1.66	160.2	$C_{10}H_{12}N_2$	27, 41, 57, 93. 121
β-phenethylamine	1002	5.63	121.18	$C_8H_{11}N$	27, 67, 80, 89, 93
Gallic acid	2707	0.57	170.12	$C_7H_6O_5$	27, 88, 89, 97, 109
4-hydroxy benzoic acid	779	1.41	138.12	$C_7H_6O_3$	27, 40, 43, 86, 92
2,4-bis (1-phenylethyl) phenol	1117	9.60	330.5	$C_{24}H_{26}O$	27, 45, 57, 66, 72
2,4,6-tris(1-phenylethyl) phenol	1251	7.83	406.6	$C_{30}H_{30}O$	27, 48, 53, 67

Rhodoxanthin	2265	1.67	562.9	$C_{40}H_{50}O_2$	27, 40, 43, 48, 60
Ferulic acid	1307	1.20	194.18	$C_{10}H_{10}O_4$	27, 38, 40, 54
Pheophytin A	922	1.54	871.2	$C_{55}H_{74}N_4O_5$	27, 42, 47, 76, 78
Pheophytin B	1006	3.31	885.2	$C_{55}H_{72}N_4O_6$	27, 34, 37, 38
Campsterol	1227	2.80	400.7	$C_{28}H_{48}O$	27, 32, 43, 47
β-sitosterol	2093	1.51	414.71	$C_{29}H_{50}O$	27, 45, 30, 45, 52

RI: retention index; MW: molecular weight; MF: molecular formula

Physicochemical composition of *Prosopis africana* seed oil

Physicochemical composition of *Prosopis africana* seed oil is presented in Table 2. The extracted oil was yellow in colour with a yield of 10.73 % at liquid state. Acid, saponification, iodine, peroxide values ranged between 15.18 (mg NaOH/g of oil), 112.7 (mg/KOH/g of oil), 150.4 (gI_2/100g of oil) and 27.75 (meq/kg) respectively. The physical evaluation of *Prosopis africana* seed oil shows that the colour of the oil was light yellow and it has no offensive odour. However, the oil yield was lower than 18 % reported for soya bean seed and 43 % for groundnut seed (Franz *et al*., 2011). They are however higher than 1.42 % reported for the seeds of *Piliostigma thonningii* (Deepak *et al*., 2012). Refractive index is the ratio of the velocity of light in vacuum to the velocity of light in the medium is an indication of the level of saturation of the oil (Isman *et al*., 2007). Iodine value is a measure of degree of unsaturation and it is an identity characteristic of seed oils, making it an excellent raw materials for soaps and cosmetic industries (Joulang and Konig, 1998). Acid value gives an indication of the quantity of free fatty acids in oil.

Saponification value is a measure of oxidation during storage and also indicates deterioration of the oils (Hedges and Lister, 2006). Peroxide value is a measure of peroxides contained in the oil and used in determining the degree of spoilage. The standard peroxide value for edible oils which have not undergone rancidity must be well below10 meq/kg (Olayemi *et al*., 2018).

Table 2 Physicochemical composition of *Prosopis africana* seed oil

Parameter	Composition	*WHO (edible oil)
Colour	Yellow	NS
Odour	Agreeable	NS
State	Liquid	-
% Yield	10.73	NS
Acid value (mg NaOH/g of oil)	15.18	3.80 – 4.00
Saponification value (mg/KOH/g of oil)	112.7	170.0 – 181.0
Iodine value (gI_2/100g of oil)	150.4	100.0 – 150.0
Peroxide value (meq/Kg)	27.75	10.0
Refractive index at (20^{o}C)	2.01	1.47 – 1.51
Specific gravity at (25^{o}C)	1.02	0.80 – 0.84
% Free fatty acid	14.10	5.78 -7.28

*WHO (2007)

Conclusion

Prosopis africana seed oil contains chemical compounds with recognized medicinal value/therapeutics, or which are precursors for chemo-pharmaceutical semi-synthesis. The medicinal value of this oil lies in some chemical substances (phytochemicals or bioactive chemicals) that produce a definite physiological action in the body of animals. The major bioactive compounds in *Prosopis africana* seed oil are prosogerins and their composition and concentration vary according to the plant, parts of the plant, geographical origin, harvesting season, environmental factors, storage conditions, and processing techniques.

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