JEDIC Journal of Ethics and Diversity in International Communication

| e-ISSN: 2792-4017 | www.openaccessjournals.eu | Volume: 1 Issue: 4

Preliminary Study on GC-MS Analysis of Prosopis Africana Seed (African Mesquite) Oil

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ABSTRACT: A preliminary studywas carried out to examine the various bioactive compounds in *Prosopisafricana* seed (*African mesquite*)oil usinggas chromatography-mass spectrometry (GC/MS).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) 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 *Prosopisafricana* 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: Prosopisafricana, seed, oil, phytochemicals, 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). Seeds from plants of medicinal origin contains essential oils, which have been proven to be rich in phytochemicals (tannins, flavonoids, phenols, saponins, alkaloids, terpenoidsetc), 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 (Musbau and Asiru, 2020).

Prosopisafricana (*African mesquite*) is a tropical leguminous plant belonging 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 *Prosopisafricana*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).

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).

This experiment was designed to examine the secondary metabolites in *Prosopisafricana* seed oil using GC-MS. It will further 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 $8^{o}57^{1}$ and $8^{o}55^{1}$N and longitude $7^{o}05^{1}$ and $7^{o}06^{1}$E.

ISSN 2792-4017 (online), Published under Volume:1 Issue: 4 in September-2021

Extraction of *Prosopisafricana* seed

Mature seeds from *Prosopisafricana*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 *Prosopisafricana* seed meal and stored in a clean well labeled container for analysis. Prior to the commencement of the analysis, laboratory equipmentswere serviced and all the necessary reagents were purchased.

Extraction of PASO was done using a cold press machine (Model: YZYX168, China). The grinded *Prosopisafricana* (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.

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

The GC-MS result of *Prosopisafricana* 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) 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 (Harzallahand Jannet, 2005; Alagbe and Motunrade, 2019; Alagbe, 2019). 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 *Prosopisafricana* 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 *Prosopisafricana* 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 *Prosopisafricana* seed oil varies according to plant species, geographical origin, season, environmental factors, processing techniques and storage conditions (Gadde*et al*., 2017).

ISSN 2792-4017 (online), Published under Volume:1 Issue: 4 in September-2021

JEDIC Journal of Ethics and Diversity in International Communication

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Table 1 Major chemical compounds of *Prosopisafricana* 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

Conclusion

Prosopisafricana 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 *Prosopisafricana* seed oil are prosogerins and their compositionand concentration vary according to the plant, parts ofthe plant, geographical origin, harvesting season, environmentalfactors, storage conditions, and processingtechniques.

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ISSN 2792-4017 (online), Published under Volume:1 Issue: 4 in September-2021