DRUG DISCOVERY

Characterization of bioactive compounds in *Luffa aegptiaca* leaf ethanolic extracts using gas chromatography and mass spectrometry

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ABSTRACT

Medicinal plants contain several bioactive compounds or phytochemicals that therapeutically exert wide spectrum of biological activities such as; antimicrobial, antifungal, antiviral, anti-inflammatory, hepato-protective, hypolipidemic, analgesics, antioxidant, immune-stimulatory, immune-modulatory and anticancer. These bioactive compounds are effective, non-toxic and environmental friendly. The aim of this experiment was to determine the bioactive compounds in *Luffa aegptiaca* ethanolic leaf extract using gas chromatography and mass spectrometry (GC-MS). Phytochemical analysis of the sample reveals the presence of alkaloids (1314.9 mg/g), glycosides (11.60 mg/g) while tannins, flavonoids, terpenoids, steroids, saponins and oxalates were present in 807.56 mg/g, 512.9 mg/g, 308.7 mg/g, 192.6 mg/g, 102.1 mg/g, 61.44 mg/g and 20.90 mg/g respectively. GC-MS analysis of *Luffa aegptiaca* ethanolic leaf extract shows that it contains 35 bioactive compounds with marked pharmaceutical properties. 1,11-Bicyclopropyl, 2-octanoic acid had the highest concentration (19.06 %) while β-methylnaphthalene had the lowest concentration (0.01%). It was concluded that the presence of these phytochemicals will inhibit some pathogenic bacteria's thus promoting a healthy gut in animals.

Keywords: *Luffa aegptiaca* extracts, medicinal plants, phytochemicals, gas chromatography, mass spectrometry

1. INTRODUCTION

Medicinal plants have been generally recognized to contain phytochemicals or secondary metabolites which performs multiple physiological functions and hence they are believed to have better compatibility with the body (Alagbe, 2023; Singh et al., 2022). They have globally gained interest due to their efficacy, safety and environmental friendly with lesser side effects (Adewale et al., 2021; Singh et al., 2021). According to WHO, (1996), 80% of the world's population use medicinal plants for primary health care in developing countries and it has been

estimated that nearly 75% of plants contain several bioactive compounds of therapeutic properties (Shittu et al., 2022; Alagbe, 2021).

Luffa aegyptiaca is one of the most potent herbs with long history of medicinal use. The plant belongs to the family Cucurbitaceae and order Cucurbitales (Alagbe, 2019; Farag et al., 2015). It is also known as sponge gourd and widely grown in Asia, Europe and many parts of Africa (Parkash and Tso, 2002; Muthumani et al., 2010). Parts of the plant (seeds, leaves, root and stem bark) have been documented as a traditional treatment for fever, gastro intestinal infections, convulsion, skin diseases, diabetes, rheumatism, snake bite and back ache (Irshad et al., 2010; Azeez et al., 2013). It can also display anti-inflammatory (Kao et al., 2012), immuno-modulatory, immune-stimulants, antiviral (Ng et al., 2011), antifungal, hypoglycemic, cytotoxic and antioxidant properties due to the presence of some secondary metabolites (Lawal et al., 2010; Farag et al., 2012; Smith et al., 2006).

Infused methanolic and aqueous leaf extract from *Luffa aegyptiaca* leaves are capable of inhibiting the growth of pathogenic bacteria's (Gram –ve and Gram +ve) (Roy and Lingampeta, 2014; Wildman, 2001). Isolates from the seeds and aqueous stem bark extract (Luffacylin and dihydroxy spinasterol) have been reported to exhibit antifungal and antimicrobial activities due to the presence of pharmacologically active substances (Koledoye et al., 2021; Amin et al., 2009). Characterization of *Luffa aegyptiaca* leaves to ascertain their phytoconstituents using gas chromatography and mass spectrometry will help to develop novel drugs and its efficacy against wider range of pathogenic organisms (Patel et al., 2013; Ediriweera et al., 2019). This research was designed to determine the bioactive compounds in *Luffa aegyptiaca* leaf extract.

2. MATERIALS AND METHODS

Experimental site, plant collection, processing and phytochemical analysis

The experiment was carried out at the Department of Microbiology, Sumitra Research Institute Gujarat, India (23°13′N 72°41′E) in the month of November to December 2022.

Fresh *Luffa aegyptiaca* leaves were collected from Punsari village, Gujarat and transferred to Sumitra Research Institute where it was authenticated by a certified taxonomist. The leaves were washed under running tap water to remove dirt and air dried on a metallic slate at room temperature for 12 days. Dried leaves were blended into powder using an electric blender to allow easy penetration of solvent (ethanol). 200 grams of the blended sample was transferred into Erlenmeyer's flask followed by the addition of 1000 liters of 95% ethyl alcohol, stirred occasionally at intervals for 24 hours and filtered into another flask using Whatman's filter paper thereafter it was covered with aluminum foil to avoid the solvent from escaping the mixture. It was later set on a water bath at 70°C for 10 minutes to recover the extract for further laboratory examination.

Quantitative phytochemical analysis was carried out using standard laboratory procedures Tannins was analyzed using methods outlined by Biswas et al., (2020), flavonoids and terpenoids (Surana et al., 2016), phenols, alkaloids and saponins (Madhu et al., 2016), phytates and oxalates (He et al., 2014) and steroids (Adeniyi et al., 2017).

Analysis of secondary metabolites in *Luffa aegyptiaca* leaves using Gas chromatography and mass spectrometry

Identification and quantification of secondary metabolites in ethanolic *Luffa aegyptiaca* leaf extract was carried out with LABTRON gas chromatography mass spectrometry (GC-MS-879) with pre-filter mass analyzer and electron multiplier ensuring high sensitivity. The gas chromatography has the following specifications; flow rate (1.0 -1.5 mL/min), inlet temperature (450°C), pressure range (0˷ 100 psi), heating rate (up to 120°C), split ratio (1000:1) and temperature programming (7 stages / 8 platforms). Mass spectrometry specifications; ion-source temperature (100°C – 350°C), scan rate (up to 1000 amu/s), stability (± 0.10 amu/48 hours), filament emission current (0˷ 350 μA), EI source ionization energy (5 eV – 250 eV), sensitivity (full scan (S/N is ≥ 30:1).

Table 1 Secondary metabolites in *Luffa aegyptiaca* leaves using Gas chromatography and mass spectrometry

Compounds	Reaction time (Sec)	% Area
2-methoxy-4-vinylphenol	3.561	0.95
β-elemenone	4.700	5.67
Erythritol	4.766	8.40
7-methylenebicyclo hepata -3-ene	5.006	3.81
Guanosine	5.882	8.56
α-terpineol	6.881	0.18
Limonene	7.009	1.25
Isorbide dinitrate	8.012	0.09
Heptadec-3-enal	8.116	2.10

Ethyl Oleate	9.301	0.04
9,15-Octadecadienoic acid	10.04	0.06
Dibutyl benzene-1,2 dicarboxylate	10.33	1.85
Diethyl suberate	11.67	0.03
4-Acetoxy-3-methoysterene	11.88	0.46
Camphol	16.09	5.03
β-Linalool	20.06	3.60
6,6-Dimethyl -1,3-heptadien-5-ol	24.09	1.25
Benzaldehyde	28.00	0.73
β-methylnaphthalene	28.82	0.01
3-Octanone	29.04	0.55
Isomyocorene	30.03	0.08
Dihydromyrcenol	31.40	1.45
Glucopyranoside	32.67	4.03
Estra-1, 3.5 (10) –trien-17-ol	33.60	0.57
Spirost-8-en-11-one	35.09	1.22
2-Nonenoic acid	36.02	1.78
Ethyl iso-allocholate	37.01	0.79
Monomethyl pimelate	38.30	2.06
Hexan-3-yl 2-methylpropyl benzene 1,2 carboxylates	39.55	0.10
4-Acetoxy-3-methoxystyrene	40.10	0.02
2,4,6 – Octarien -1-ol 3,7 dimethyl	42.08	0.05
2R, 3S-1- (1,3,4 – Trihydroxy-2-butoxymethyl)	43.10	10.80
Lutoelin	45.66	0.03
1,11-Bicyclopropyl] 2-octanoic acid	46.08	19.06
Apigenin	47.01	6.01
1-methylcyclopropanemethanol	47.66	0.02
Total		92.69

3. RESULTS AND DISCUSSION

Phytochemical composition of *Luffa aegptiaca* leaf extract

Phytochemicals or secondary metabolites are chemical compounds produced by plants and it possess medicinal (anti-inflammatory, antidiuretic, immune-stimulant, anticancer, immune-modulatory, cytotoxic, hepato-protective, antiviral etc.) and also have many applications (pharmaceutical, agrochemicals and cosmetics) (Shittu and Alagbe, 2021). Phytochemical composition of *Luffa aegptiaca* leaf extract shows that Alkaloids had the highest concentration (1314.9 mg/g) while glycosides had the lowest concentration (11.60 mg/g). Tannins, flavonoids, terpenoids, steroids, saponins and oxalates were present in 807.56 mg/g, 512.9 mg/g, 308.7 mg/g, 192.6 mg/g, 102.1 mg/g, 61.44 mg/g and 20.90 mg/g respectively. The higher concentration in alkaloid values is a clear sign that the extract can invoke a bitter taste and can perform wide range of pharmacological activities including antimalarial, antiarrhythmic, analgesics and stimulant activities (Alagbe, 2021; Shittu et al., 2021; Agubosi et al., 2022).

Glycosides are organic compounds made up of sugars and aglycone. It can exist in various forms (cardiac glycosides, hydroquinone glycosides and anthraquinone glycosides) and has antiseptic, expectorant, antihyperlipidemic and rubefacient (Parama et al., 2020; Elekofehinti et al., 2015). Phenolic compounds in diets may provide health benefits associated with reduced risk of cardiovascular diseases (Elumalai et al., 2011; Jones et al., 1994), antioxidant activity, scavenge free radicals and reduce oxidative stress (Zhou et al., 2011; Muritala et al., 2022). Flavonoids are group of phenolic compounds with antibacterial, antiviral effects and prevention of deoxyribonucleic acid binding (Alagbe et al., 2020; Alagbe and Omokore, 2019). It also aids in enzyme induction and enhancing detoxification of organs (Shmuel, 2012). Terpenoids therapeutically exerts wide spectrum of properties such as; antibacterial, anti-helminthic, antioxidant, analgesics, hypolipidemic and cytotoxic functions (Park et al., 2012; Alagbe, 2019). Tannins are naturally occurring complex compounds which possess nitrogen free polyphenols with several pharmaceutical

effects (antimicrobial, cytotoxic, antioxidant and hepato-protective properties) (Tanaka et al., 1991). High concentration of oxalate in the system of animals could result in kidney stone disease and osteoporosis (Griego et al., 2008).

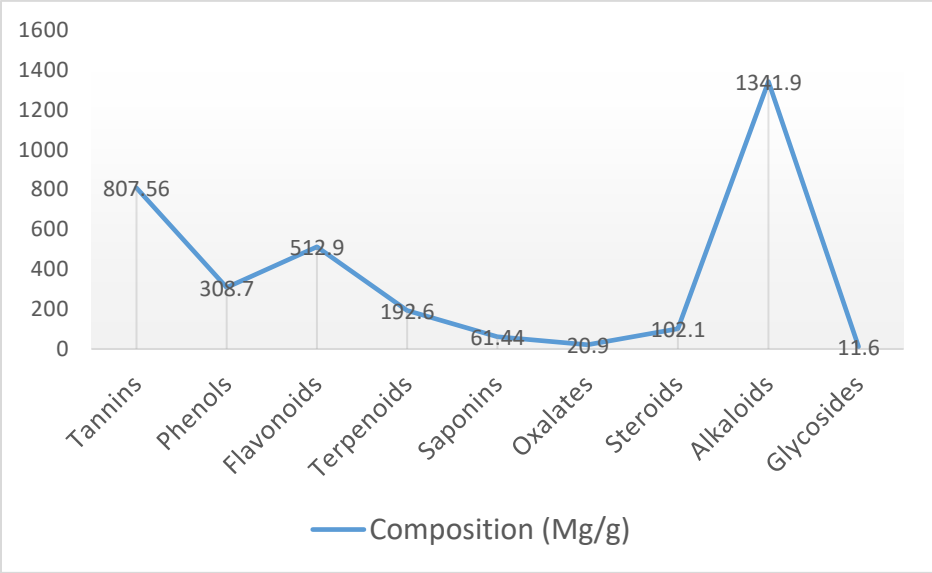

Figure 1 Phytochemical composition of *Luffa aegptiaca* leaf extract

Secondary metabolites in *Luffa aegptiaca* leaf extract using gas chromatography and mass spectrometry

Gas chromatography and mass spectrometry (GC-MS) analysis of Luffa aegptiaca leaf extract is a physical method of separation in which the components to be separate are distributed between two phases (stationary and gaseous phases) quantitatively (Urbanova et al., 2013; Fan and Qian, 2006). The result reveals the presence of 35 bioactive compounds that are capable of giving therapeutic effect on the health of human and animals. These compounds includes: 2-methoxy-4-vinylphenol (0.95%), β-elemenone (5.67%), Erythritol (8.40%), 7-methylenebicyclo hepata -3-ene (3.81%), Guanosine (8.56%), α-Terpineol (0.18%), Limonene (1.25%), Isorbide dinitrate (0.09%), Heptadec-3-enal (2.10%), Ethyl Oleate (0.04%), 9,15-Octadecadienoic acid (0.06%), Dibutyl benzene-1,2 dicarboxylate (1.85%), Diethyl suberate (0.03%), 4-Acetoxy-3-methoysterene (0.46%), Camphol (5.03%), β-Linalool (3.60%), 6,6-Dimethyl -1,3-heptadien-5-ol (1.25%), Benzaldehyde (0.73%), β-methylnaphthalene (0.01%), 3-Octanone (0.55%), Isomyocorene (0.08%), Dihydromyrcenol (1.45%), Glucopyranoside (4.03%), Estra-1,3.5 (10) –trien-17-ol (0.57%), Spirost-8-en-11-one (1.22%), 2-Nonenoic acid (1.78%), Ethyl iso-allocholate (0.79%), Monomethyl pimelate (2.06%), Hexan-3-yl 2-methylpropyl benzene 1,2 carboxylate (0.10%), 4-Acetoxy-3-methoxystyrene (0.02%), 2,4,6 – Octarien -1-ol 3,7 dimethyl (0.05 %), 2R, 3S-1-(1,3,4 – Trihydroxy-2-butoxymethyl (10.80%), Lutoelin (0.03%), 1,11-Bicyclopropyl) 2-octanoic acid (19.06%), Apigenin (6.01%) and 1-methylcyclopropanemethanol (0.02%).

These compounds play a vital role in traditional herbal remedies and also have several pharmaceutical or physiological effect on human and animal (Zhang et al., 2006; Tang, 1992). The outcome on the GC-MS analysis of *Luffa aegptiaca* leaf extract agrees with the reports of Garia et al., (2018); Yadav et al., (2017) but contrary to the findings of Akther et al., (2014). This disparity could be attributed to geographical location, species or parts of plant used (root, seed, stem bark, flower etc.), extraction methods, harvesting procedure and age of plants (Agubosi et al., 2022; Alagbe and Akintayo, 2020).

4. CONCLUSION

The result on the phytochemical composition of *Luffa aegyptica* leaf extract confirms that it contains several bioactive compounds of marked pharmacological activities (antimicrobial, antifungal, antiviral, antioxidant, immune-stimulatory, hepato-protective, cytotoxic, hypolipidemic, immuno-modulatory, analgesics and antihelminthic) and are also capable of inhibiting the activities of pathogenic bacteria, reducing mortality and oxidative stress.

Ethical approval

Luffa aegyptiaca leaves were collected from Punsari village, Gujarat and transferred to Sumitra Research Institute where it was authenticated by a certified taxonomist. The ethical guidelines are followed in the study for sample identification & experimentation.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Adeniyi SA, Orjiekwe CL, Ehiagbonare JE. Determination of alkaloids and oxalates in some selected food samples in Nigeria. Afr J Biotechnol 2009; 8(1):110-2.
2. Adewale AO, Alagbe JO, Adeoye AO. Dietary Supplementation of *Rauvolfia Vomitoria* Root Extract as a Phytogenic Feed Additive in Growing Rabbit Diets: Haematology and serum biochemical indices. Int J Orange Technol 2021; 3(3):1-12.
3. Agubosi OCP, Alexander J, Alagbe JO. Influence of dietary inclusion of Sunflower (*Helianthus annus*) oil on growth performance and oxidative status of broiler chicks. Cent Asian J Med Nat Sci 2022; 2(7):187-195.
4. Agubosi OCP, Imudia FD, Alagbe JO. Evaluation of the nutritional value of air dried and sun-dried sweet potato (*Ipomoea batatas*) peels. Eur J Life Safe Stab 2022; 14(22):43-51.
5. Agubosi OCP, Soliu MB, Alagbe JO. Effect of dietary inclusion levels of *Moringa oleifera* oil on the growth performance and nutrient retention of broiler starter chicks. Central Asian J theor appl sci 2022; 3(3):30-39.
6. Omolere ABM, Alagbe JO. Probiotics and medicinal plants in poultry nutrition: A review. United Int J Res Technol 2020; 2 (1):7-13.
7. Akther F, Rahman A, Proma JJ, Kabir Z, Paul PK, Rahmatullah M. Methanolic extract 0f *Luffa cylindrica* fruits show antihyperglycemic potential in Swiss albino mice. Adv Nat Appl Sci 2014; 8(8):62-65.
8. Alagbe JO, Ramalan SM, Shittu MD, Olagoke OC. Effect of *Trichilia monadelpha* stem bark extract on the fatty acid composition of rabbit's thigh meat. J Environ Issu Clim Chang 2022; 1(1):63-71.
9. Alagbe JO. Proximate, mineral and phytochemical analysis of *Piliostigma thonningii* stems bark and roots. Int J Chem Biol Phy Sci 2019; 1(1):1-7.
10. Alagbe JO. Dietary Supplementation of *Rauvolfia Vomitoria* Root Extract as a Phytogenic Feed Additive in Growing Rabbit Diets: Growth Performance and Caecal Microbial Population. Concepts Dairy Vet Sci 2021; 4(2):2021.
11. Alagbe JO. Use of medicinal plants as a panacea to poultry production and food security: A review. Gospod Innowacje 2022; 22(2022):1-12.
12. Alagbe JO, Akintayo-Balogun OM. Effects of dietary supplementation of *Albizia lebbeck* seed oil (ALO) on the fatty acid composition of weaner rabbits. J Biochem Biotech Res 2020; 8(2):29-33.
13. Alagbe JO, Omokore EA. Effect of replacing soya bean meal with *Indigofera zollingeriana* leaf meal on the performance and carcass characteristics of growing rabbits. Int J Multidiscip Res Dev 2019; 6(5):74-77.
14. Alagbe JO. Haematology, serum biochemistry, relative organ weight and bacteria count of broiler chicken given different levels of *Luffa aegyptiaca* leaf extracts. Int J Adv Biol Biomed Res 2019; 7(4):382-392.
15. Alagbe JO. Effect of dietary supplementation of *Cymbopogon Citratus* oil on The Performance and Carcass characteristics of broiler chicks. European J Biotechnol Biosci 2020; 8(4):39-45.
16. Alagbe JO. *Daniellia oliveri* leaf extracts as an alternative to antibiotic feed additives in broiler chicken diets: Meat Quality and Fatty acid composition. Indones J Innov Appl Sci 2021; 1 (3):177-186.
17. Alagbe JO. *Prosopis africana* stem bark as an alternative to antibiotic feed additives in broiler chicks diets: Performance and Carcass characteristics. J Multidimens Res Rev 2021; 2(1): 64-77.
18. Alagbe JO. Gas chromatography and mass spectroscopy of *Juniperus phoenice* stem bark extract and its influence on the haemato-biochemical values of growing rabbits. British Scientific Periodical 2022; 1(1):18-33.
19. Alagbe JO. *Prosopis africana* (African mesquite) oil as an alternative to antibiotic feed additives on broiler chickens diets: Haematology and serum biochemical indices. Central Asian J theor appl sci 2022; 3(2):19-29.

20. Alagbe JO. Bioactive compounds in ethanolic extract of *Strychnos innocua* root using gas chromatography and mass spectrometry (GC-MS). Drug Discov 2023; 17:e4dd1005.
21. Alagbe JO, Adedeji MO, Habiba Z, Nwosu Gloria, Wyedia Dabara Comfort. Physico-chemical properties of *Indigofera zollingineriana* seed oil. Asian J Adv Med Sci 2021; 3(4):306-308.
22. Alagbe JO, Adeoye A, Oluwatobi OA. Proximate and mineral analysis of *Delonix regia* leaves and roots. Int J Integ Educ 2020; 3(10):144-149.
23. Alagbe JO, Agubosi OCP, Ajagbe AD, Shittu MD, Akintayo BOM. Performance, haematology and serum biochemical parameters of growing grass cutters fed *Phyllantus amarus* and *Piliostigma thonningii* leaf meal mixture as partial replacement for Soya bean meal. United Int J Res Technol 2020; 2(1):14-23.
24. Alagbe JO, Agubosi OCP, Oluwafemi RA, Gabriel Zakara. Efficacy of *Trichilia monadelpha* stem bark on the growth performance of growing rabbits. British J Innov Sci Res Dev 2022; 1(2):10-19.
25. Alagbe JO, Shittu MD, Eunice Abidemi Ojo. Prospect of leaf extracts on the performance and blood profile of monogastric – A review. Int J Integ Educ 2020; 3(7):122-127.
26. Alagbe JO, Shittu MD, Tanimomo, Babatunde K. Influence of *Anogeissusleio carpus* stem bark on the fatty acid composition in meat of broiler chickens. Euro J Life Safe Stab 2022; 14(22):1 3-22.
27. Alagbe JO, Shittu MD, Ushie FT. GC-MS analysis of methanolic stem bark extract of *Zollingeriana indigofera*. Asian J Adv Res 2021; 11(4):144-146.
28. Alagbe JO, Shittu MD, Bamigboye SO, Oluwatobi AO. Proximate and mineral composition of *Pentadiplandra brazzeana* stems bark. Elect Res J Eng Comp Appl Sci 2020; 1 (2009):91-99.
29. Alagbe JO, Shittu MD, Ramalan SN, Tanimomo KB, Adekunle DA. Growth performance, semen quality characteristics and hormonal profile of male rabbit bucks fed *Rubia cordifolia* root extracts. Int J Agric Biol Eng 2022; 1(1):1-13.
30. Alagbe JO, Zubairu Habiba, Adedeji OM, Bamigboye S, Dora Agbonika. Influence of Juniperus thurifera root extract on the nutrient digestibility and caecal microbial count of growing rabbits. Web of Synergy: Int Interdiscip Res J 2022; 1(1):5-17.
31. Al-Fatlawi AAY, Al-Salih ARH, Yassen MAR. βsitosterol protects against cisplatin-induced nephrotoxicity through amelioration of oxidative stress in rats. Muthanna Med J 2017; 4(2):60-74.
32. Amin A, Gali-Muhtasib H, Ocker M, Schneider-Stock R. Overview of major classes of plant-derived anticancer drugs. Int J Biomed Sci 2009; 5(1):1-11.
33. Azeez MA, Bello OS, Adedeji AO. Traditional and medicinal uses of Luffa cylindrica: A review. J Med Plant 2013; 1(5):102–11.
34. Cárdeno A, Aparicio-Soto M, Montserrat-de la Paz S, Bermudez B, Muriana FJ, Alarcón-de-la-Lastra C. Squalene targets pro-and anti-inflammatory mediators and pathways to modulate over-activation of neutrophils, monocytes and macrophages. J Funct. Foods 2015; 14:779-790.
35. Ediriweera MK, Tennekoon KH, Samarakoon SR. In vitro assays and techniques utilized in anticancer drug discovery. J Appl Toxicol 2019; 39(1):38- 71.
36. Elekofehinti OO. Saponins: Anti- diabetic principles from medicinal plants - A review. Pathophysiology 2015; 22:95-103. doi: 10.1016/j.pathophys.2015.02.001
37. Elumalai A, Chinna Eswaraiah MA. Pharmacological Review on Garcinia indica. International journal of universal pharmacy and Life Sciences, 2011; 1(3):57-60.
38. Fan W, Qian MC. Identification of aroma compounds in Chinese 'Yang he Daqu'liquor by normal phase chromatography fractionation followed by gas chromatography (sol) olfactometry. Flavour Fragr J 2006; 21 (2):333–42.
39. Farag MA, Rasheed DM, Kamal IM. Volatiles and primary metabolites profiling in two Hibiscus sabdariffa (roselle) cultivars via headspace SPME-GC-MS and chemometrics. Int Food Res J 2015; 78:327–35.
40. Farag MA, Wessjohann LA. Volatiles profiling in medicinal licorice roots using steam distillation and solid-phase microextraction (SPME) coupled to chemometrics. J Food Sci 2012; 77(11):C1179–84.
41. Garai S, Ghosh R, Bandopadhyay PP, Mandal NC, Chattopadhyay A. Anti-microbial and anti-cancer properties of echincystic acid extracted from Luffa cylindrica. J Food Process Technol 2018; 9:2. doi: 10.4172/57-7110.1000717
42. Griego FY, Bogen KT, Price PS, Weed DL. Exposure, epidemiology and human cancer incidence of naphthalene. Regul Toxicol Pharmaceuticals 2008; 51(2):22–6.
43. He J, Wu ZY, Zhang S, Zhou Y, Zhao F, Peng ZQ, Hu ZW. Optimisation of microwave-assisted extraction of tea saponin and its application on cleaning of historic silks. J Surfactants Deterg 2014; 17(5):919-28.
44. Hung PV, Maeda T, Miyatake K, Morita N. Total Phenolic Compounds and Antioxidant Capacity of Wheat Graded

Flours by Polishing Method. Food Res Int 2009; 42(1):185-190.

45. Irshad IAM, Goel HC, Moshahid M, Rizvi A. Phytochemical screening and high-performance TLC analysis of some cucurbits. Res J Phytochemicals 2010; 4(4):242–7.
46. Jisika M, Ohigashi H, Nogaka H, Tada T, Hirota M. Bitter steroid glycosides, Vernon sides A1, A2 and A3 and related B1 from the possible medicinal plant Vernonia amygdalina used by wild Chimpanzees. Tetrahedron 1992; 48:625-630.
47. Jones GA, McAllister TA, Muir AD, Cheng KJ. Effects of safonin (Onobrychis viciifolia scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl Environ Microbiology 1996; 60:1374-1378.
48. Kao T, Huang C, Chen B. Functional components in *Luffa cylindrica* and their effects on anti-inflammation of macrophage cells. Food Chem 2012; 135(2):386–95.
49. Lawal I, Uzokwe N, Igboanugo A, Adio A, Awosan E, Nwogwugwu J. Ethno medicinal information on collation and identification of some medicinal plants in Research Institutes of South-west Nigeria. Afr J Pharm Pharmacol 2010; 4(1):001–7.
50. Muritala Daniel Shittu, Alagbe JO, Ojebiyi OO, Ojediran TK, Rafiu TA. Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of *Polyalthia longifolia* leaf extract in place of conventional antibiotics. Anim Sci Genet 2022; 18(2):5 7-71.
51. Musa B, Alagbe JO, Adegbite Motunrade Betty, Omokore EA. Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. United Int J Res Technol 2020; 2(2):13-21.
52. Muthumani P, Meera R, Subin M, Devi P, Kameswari B, Priya B. Phytochemical screening and anti-inflammatory, bronchodilator and antimicrobial activities of the seeds of *Luffa cylindrica*. Res J Pharmaceut Biol Chem Sci 2010; 1(4):11–22.
53. Narayani M, Johnson M, Sivaraman A, Janakiraman N. Phytochemical and Antibacterial Studies on *Jatropha curcas* L. J Chem Pharm Res 2012; 4(5):2639-2642.
54. Nascimento GGF, Lacatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol 2012; 31(4):886-8 91.
55. Ng Y-M, Yang Y, Sze K-H, Zhang X, Zheng Y-T, Shaw P-C. Structural characterization and anti-HIV-1 activities of arginine/glutamate-rich polypeptide Luffin P1 from the seeds of sponge gourd (*Luffa cylindrica*). J Struct Biol 2011; 174(1):16 4–72.
56. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the roots of *Landolpbia owerience* for antibacterial activity. J Ethnopharmacol 2001; 78(2-3):119-27.
57. Parama D, Boruah M, Kumari Y, Rana V, Banik K, Harsha C. Diosgenin, a steroidal saponin and its analogues: Effective therapies against different chronic diseases. Life sci 2020; 260: 118182.
58. Parekh J, Chands S. In vitro antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. Flower (Lythraceae), Braz J Microbiol 2007; 38:204-7.
59. Park S-N, Lim YK, Freire MO, Cho E, Jin D, Kook J-K. Antimicrobial effect of linalool and a-terpineol against periodontopathic and cariogenic bacteria. Anaerobe 2012; 18 (3):369–72.
60. Parkash A, Ng T, Tso W. Isolation and characterization of luffacylin, a ribosome inactivating peptide with anti-fungal activity from sponge gourd (*Luffa cylindrica*) seeds. Peptides 2002; 23(6):1019–24.
61. Patel K, Gadewar M, Tahilyani V, Patel DK. A review on pharmacological and analytical aspects of diosmetin: A concise report. Chin J Integr Med 2013; 19(10):792-800.
62. Roy S, Lingampeta P. Solid wastes of fruits peels as source of low-cost broad spectrum natural antimicrobial compounds furanone, furfural and benezenetriol. Int Res J Eng Technol 2014; 3(7):273-279.
63. Shittu MD, Alagbe JO. Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. Int J Integ Educ 2020; 3(11):119-124.
64. Shittu MD, Alagbe JO, Adejumo DO, Ademola SG, Abiola AO, Samson BO, Ushie FT. Productive Performance, Caeca Microbial Population and Immune-Modulatory Activity of Broiler Chicks Fed Different Levels *Sida Acuta* Leaf Extract in Replacement of Antibiotics. J Proteom Bioinform 2021; 5(1):00 0143.
65. Shmuel Y. Dictionary of food compounds with CD-ROM, 2nd ed: CRC Press, Taylor and Francis Group 2012; 2346.
66. Singh Sharma, Alagbe Olujimi John, Liu Xing, Sharma Ram and Kumar Amita. Comparative analysis of ethanolic *Juniperus thurifera* leaf, stem bark and root extract using gas chromatography and mass spectroemetry. Int J Agric Ani Produ 2022; 2(6):18-27.
67. Singh AS., Alagbe JO, Sharma S, Oluwafemi RA, Agubosi OCP. Effect of dietary supplementation of melon (*Citrallus linatus*) seed oil on the growth performance and antioxidant status of growing rabbits. J Multidimens Res Rev 2021; 2(1):78-95.

68. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. GCMS: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching and identification. Anal Chem 2006; 78(3):779–87.
69. Surana AR, Kumbhare MR, Wagh RD. Estimation of total phenolic and total flavonoid content and assessment of in vitro antioxidant activity of extracts of Hamelia patens Jacq. stems. Res J Phytochem 2016; 10(2):67- 74.
70. Tanaka S, Uno C, Akimoto M, Tabata M, Honda C, Kamisako W. Anti-allergic effect of bryonolic acid from Luffa cylindrica cell suspension cultures. Planta Med 1991; 57(6):527–30.
71. Tang WGE. *Luffa cylindrica* (L.) Roem. In: Chinese Drugs of Plant Origin: Springer, Berlin, Heidelberg 1992.
72. Urbanová T, Tarkowská D, Novák O, Hedden P, Strnad M. Analysis of gibberellins as free acids by ultra-performance liquid chromatography– tandem mass spectrometry. Talanta 2013; 112:85–94.
73. Wildman REC. Classifying nutraceuticals. In Handbook of nutraceuticals and functional foods (Edn. Wolinsky, Hickson and J.F. Jr., I). CRC press LLC 2001.
74. Yadav R, Yadav BS, Yadav RB. Phenolic profile and antioxidant activity of thermally processed sponge gourd (Luffa cylindrica) as studied by using high performance thin layer chromatography (HPTLC). Int J Food Prop 2017; 20(9):2 096-2112.
75. Zhang Z-Z, Li Y-B, Qi L, Wan X-C. Antifungal activities of major tea leaf volatile constituents toward Colletorichum camelliae Massea. J Agric Food Chem 2006; 54(11):3936–40.
76. Zhou J, Xie G, Yan X. Encyclopedia of Traditional Chinese Medicines - Molecular Structures, Pharmacological Activities, Natural Sources and Applications: Springer-Verlag Berlin Heidelberg 2011; 730.