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Characterization of bioactive compounds in Luffa Aegptiaca leaf ethanoic extracts using gas chromatography and mass spectrometry

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Abstract: Medicinal plants contain several bioactive compounds or phytochemicals that therapeutically exert a wide spectrum of biological activities, such as hepato-protective, hypolipidemic, analgesic, antioxidant, immune-stimulatory, immune-modulatory, and anticancer. These bioactive compounds are effective, non-toxic, and environmentally friendly. Here, we determined the bioactive compounds in Luffa aegptiaca ethanolic leaf extract using gas chromatography and mass spectrometry (GC-MS). Biochemical analysis revealed 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, 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 that perform multiple physiological functions (Singh et al., 2021). They have globally gained interest due to their efficacy, safety, and environmental friendliness, with fewer side effects (Adewale et al., 2021; Singh et al., 2021). In developing countries, eighty percent of the world population uses medicinal plants for primary health care, and it has been estimated that nearly 75 % of plants contain several bioactive compounds of therapeutic properties (Alagbe, 2021; Alagbe et al., 2021).

Luffa aegyptiaca is one of the most potent herbs with a long history of medicinal use. The plant belongs to the family Curcubitaceae and order Curcurbitales (Alagbe, 2019a; Farag et al., 2015). It is also known as sponge gourd and is widely grown in Asia, Europe, and many parts of Africa (Muthumani et al., 2010; Parkash et al., 2002). Various parts of the plant (seeds, leaves, root, and stem bark) have been documented as a traditional treatment for fever, gastrointestinal infections, convulsions, skin diseases, diabetes, rheumatism, snake bite, and backache (Azeez et al., 2013; Irshad et al., 2010). 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 (Farag & Wessjohann, 2012; Lawal et al., 2010; Smith et al., 2006). Infused methanolic and aqueous leaf extract from Luffa aegyptiaca leaves are capable of inhibiting the growth of pathogenic bacteria (Gram–negative and Gram-positive) (Roy & 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

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pharmacologically active substances (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 their efficacy against a wider range of pathogenic organisms (Ediriweera et al., 2019; Patel et al., 2013). Here, we determine the bioactive compounds in Luffa aegyptiaca leaf extract.

2. Materials and methods

2.1. Experimental site, plant collection, processing, and phytochemical analysis

Fresh Luffa aegyptiaca leaves were collected from Punsari village, Gujarat, and transferred to Sumitra Research Institute, where it was authenticated by a certified taxonomist. 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 an Erlenmeyer 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 prevent the solvent from escaping the mixture. It was later set on a water bath at 70 oC for 10 minutes to recover the extract for further laboratory examination. Quantitative phytochemical analysis was carried out using standard laboratory procedures. Tannins were 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., 2009).

2.2. Analysis of secondary metabolites in Luffa aegyptiaca leaves using GC-MS

Determination of secondary metabolites in ethanolic Luffa aegyptiaca leaf extract was done with LABTRON gas chromatography mass spectrometry (GC-MS-879) by following the standard protocol.

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
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
4-Acetoxy-3-methoxystyrene	40.10	0.02
2,4,6 – Octarien -1-ol 3,7 dimethyl	42.08	0.05
Lutoelin	45.66	0.03

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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

3.1. Phytochemical composition of Luffa aegptiaca leaf extract

Phytochemicals or secondary metabolites are chemical compounds produced by plants, and they possess medicinal (anti-inflammatory, antidiuretic, immune-stimulant, anticancer, immune-modulatory, cytotoxic, hepato-protective, antiviral, etc.) and also have many applications (pharmaceutical, agrochemicals, and cosmetics) (Alagbe et al., 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, oxalates, saponins, steroids, and 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 a wide range of pharmacological activities (Agubosi et al., 2022; Shittu & Alagbe, 2020; Singh et al., 2021). Glycosides are organic compounds made up of sugars and an aglycone. It can exist in various forms (cardiac glycosides, hydroquinone glycosides, and anthraquinone glycosides) and has antiseptic, expectorant, antihyperlipidemic, and rubefacient (Elekofehinti, 2015; Parama et al., 2020). Flavonoids are a group of phenolic compounds with antibacterial, antiviral effects, and prevention of deoxyribonucleic acid binding (Alagbe et al., 2020; Alagbe & Omokore, 2019). It also aids in enzyme induction and enhances detoxification of organs (Yannai, 2012). Terpenoids therapeutically exert a wide spectrum of properties such as antibacterial, anti-helminthic, antioxidant, analgesic, hypolipidemic, and cytotoxic functions (Alagbe, 2019b; Park et al., 2012). Tannins are naturally occurring complex compounds that possess nitrogen-free polyphenols with several pharmaceutical effects (antimicrobial, cytotoxic, antioxidant, and hepato-protective properties). High concentrations of oxalate in the system of animals could result in kidney stone disease and osteoporosis (Griego et al., 2008).

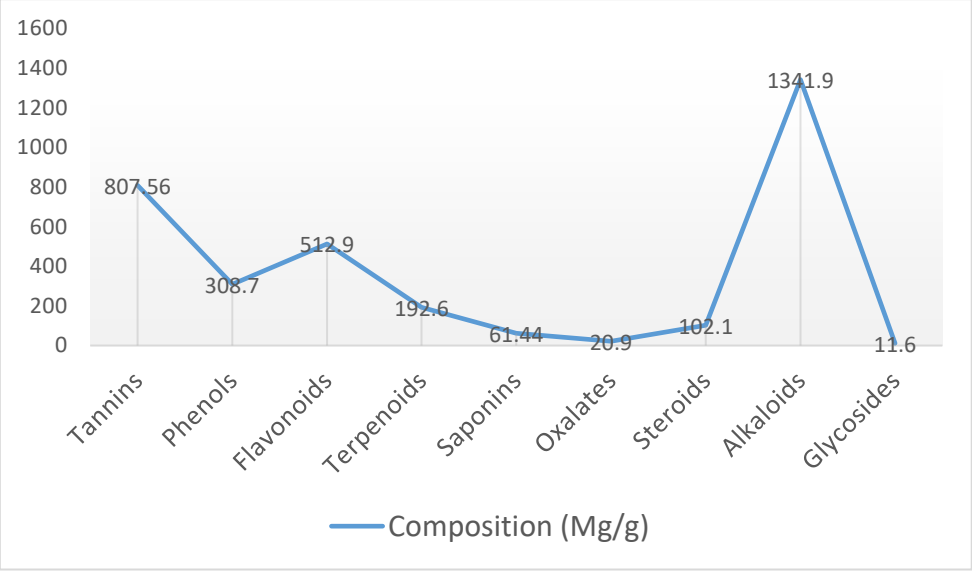

Figure 1: Phytochemical composition of Luffa aegptiaca leaf extract

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

The result reveals the presence of 35 bioactive compounds that are capable of giving a therapeutic effect on the health of humans and animals (Fan & Qian, 2006; Urbanová et al., 2013). 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 have several pharmaceutical or physiological effects on humans and animals (Tanaka et al., 1991; Zhang et al., 2006). The outcome on the GC-MS analysis of Luffa aegptiaca leaf extract agrees with the reports of Yadav et al. (2017), Garai et al. (2018) , 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 & James, 2022; Alagbe & Akintayo-Balogun, 2020).

5. 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 growth of pathogenic bacteria.

CRediT authorship contribution statement

Alagbe, J.O, Experiment and analyze data, Bamigboye Samson, G.C. Nwosu, Data analysis, writing original draft, D.A Agbonika, and Kadiri Mercy Cincinsoko, editing, proofreading.

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Declaration of competing interest

The authors declare no conflict of interest.

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