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Research Article

Evaluation of Bioactive compounds and antimicrobial analysis of the essential oil from the leaves of *Sauropus androgynous* in Rajasthan India

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Abstract:

Sauropus androgynous essential oil contains unique bioactive compounds with therapeutic potentials and it delivers powerful antioxidant, anti-inflammatory, antidiabetic, cytotoxic, gastro-protective, immune-protective, cardio-protective, antimicrobial, antidiarrheal, anti-helminthic amongst others. GC-MS analysis of *Sauropus androgynous* essential oil revealed the presence of 33 bioactive compounds were recorded representing 96.27 % composition dominated by Squalene (19.63 %), Caryophyllene (13.92 %), Nonane, 4-methyl (13.82 %) and n-hexadecanoic acid (10.45 %). Other bioactive compounds examined had a concentration less than 3 %. A synergy between the major and minor compounds are working together to protect, heal, restore and improve the overall vitality of the body. The result obtained on the antimicrobial activity of *Sauropus androgynous* essential oil revealed a high percentage inhibition 77.81 %, 72.00 %, 80.09 %, 73.73 % and 76.26 % against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexineri* and *Salmonella typhi* respectively. The results compare well with that of the standard drug (Ciproflaxacin) (65.00 -71.00 %). It was concluded that *Sauropus androgynous* essential oil contains measurable concentration of compounds that can disrupt the cell membrane of pathogens and will also help to address the increasing cases of antimicrobial resistance.

Key words: bioactive; compounds; antimicrobials; free radicals; oxidative stress

Introduction

Sauropus androgynous, an evergreen, multipurpose herb belongs to the Phyllanthaceae plant family which is found in the tropical rain forests of West Africa, although it is also widely distributed in parts of Asia, South America and Australia (Purba and Paengkoum, 2012; Bao et al., 2020). Inside its leasves are bioactive compounds that go far beyond nutrition (Juxian and Xian, 2022; Hernandez and Alagbe, 2025a). The leaves are rich in minerals like calcium, magnesium and iron supporting bone strength and electrolyte balance (Singh et al., 2011; Singh et al., 2021), vitamins (B-complex and C) and protein between 6 – 10 % (Lin et al., 1999; Basker, 2012). For centuries, Traditional healers have used its leaves to soothe infection, skin disorder, pyrexia, bronchitis, diarrhea and relief from acute and chronic infections because its tissues are filled with therapeutic potentials (Samad et al., 2013; John, 2024b).

The plant contains a unique profile of flavonoids and phenolic acids, nature's compounds known to neutralize the activities of free radicals, reinforces the cardiovascular system by strengthening blood vessel, acts as liver protectors, metabolic regulators, reducing inflammation and restoring enzyme balance (Alagbe, 2024; Singh et al., 2022). They also contains alkaloids, tannins, steroids and saponins which possess several pharmacological functions which includes, cytotoxic, antimicrobial, antitumor, antidiabetic, anti-cancer, antifungal, immune-modulatory, gastro-protective, derma-protective, anti-helminthic, antidiarrheal, anti-allergic, antihistaminic, antipyretic, antiseptic, antispasmodic, antiulcer and antiviral (Naemsuvan, 2013; Zhang et al., 2022). Methanolic extract from *Sauropus androgynous* leaves and stems show high radical scavenging free activity (Gonzalez et al., 2022; Purba et al., 2022), measurable compounds inhibit the activities of bacteria cells in microbial assays (Caldeira et al., 2004; Chatrou et al., 2012). Its poultices can be used for skins and wounds (Gao et al., 2014; Musa et al., 2020). Aqueous extract suppresses inflammatory cytokines, reducing airway infections and regulating sugar absorption in the body (Adewale et al., 2021; John, 2024a).

Previous studies by Rajeswari et al. (2018) have shown that *Sauropus androgynous* leaf extract contained 9,12,15-Octadecatrienoic acid at 12.90 %, Ethyl 9,12,15-Octadecatrienoate (15.93 %), Ethyl (9Z,12Z)-9,12-Octadecadienoate (11.28 %), Hexadecanoic acid (13.76 %), l-(+)-Ascorbic acid 2,6-Dihexadecanoate (27.81 %) as the prominent bioactive compounds during GC-MS analysis. Senthamarai and Anusha (2012) also reported that GC-MS profiling in the leaves of Sauropus Androgynus showed that it contained 2(1H) Naphthalenone (41.17 %), Azulene (36.20 %), Pyrene, hexadecahydro (9.07 %), squalene (8.06 %) and 1, 14-Tetradecanediol (2.82 %) as major bioactive compounds. Previous report

has shown that that for every newly developed antibiotic, bacterial strains rapidly develop resistance mechanisms against it. Therefore, there is an urgent need for alternative ways to treat bacterial infections and to combat the problem of antimicrobial resistance (Carranza et al. 2015; Hernandez and Alagbe, 2025b). One of the alternatives that can be utilised for combating antimicrobial resistance is traditional herbal remedies from plants. Evaluating the bioactive compounds in *Sauropus androgynous* oil will further help to unveil their pharmacological and therapeutic properties.

Materials and methods

Location of the experiment, plant collection and extraction of Sauropus androgynous oil

This study was carried out at the Microbiology Department Gandhi College of Agriculture, Rajasthan India between October to December 2025. The Institution is situated between 21°35'N 26°09'E East India. *Sauropus androgynous* fresh leaves were harvested from Rajasthan India and sent to the department of botany, Gandhi College of Agriculture for identification before it was registered under voucher number HU09/2025C/008. The identified leaves were sorted and shade dried for 13 days until a constant weight was achieved. Dried Sauropus androgynous leaves were pulverized using mechanical grinder. Extraction of oil was done by hydrodistillation with a Clevenger-type apparatus according to the procedures outlined by Singh et al. (2021). 250 g of the pulverized *Sauropus androgynous* was added to 1000 mL of water heated in a glass flask at 60 ℃ for 20 minutes, steam passes via the condenser and when cooled it was collected in a beaker. The oil collected by decantation at the end of the distillation was filtered, dried over column of anhydrous sodium sulfate, and introduced into glass bottles and stored in a refrigerator at 4 °C.

Gas chromatography – mass spectrometry analysis of *Sauropus androgynous* essential oil

GC/MS analysis of *Sauropus androgynous* essential oil was done using Claudus 5006 GC-MS Auto Sampler (China) equipped with two silica capillary columns, interfaced with a quadrupole detector (single quadrupole acquisition Method-MS parameters report), source temperature 230°C, Quadrupole temperature 150 °C; the temperature program was 60 °C for 2 min, 60-240 °C at 3 °C/min, then kept at 240 °C during 8 min; injector temperature, 240 °C. The mass spectrometry transfer line temperature, 250 °C; carrier gas, helium at a flow rate of 0.7 ml/min; injection type, split, 20:1; ionization voltage, 70 eV; electron multiplier 1000 eV; scan range 33-400 amu; scan rate, 1.56 scan/s.

Identification of components Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST, 2001) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Antimicrobial assay

The antimicrobial activity of essential oil of *Sauropus androgynous* essential oil was screened against five standard strains of stock bacteria from the Microbiology department, Gandhi College of Agriculture, Rajasthan. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Shigella flexineri* and *Salmonella typhi*. Microplate Alamar Blue Assay was used to determine susceptibility or resistance of the essential oils to all the selected bacteria strains. Organisms were grown in Mueller Hinton broth and inoculums were adjusted to 0.5 McFarland standard. Essential oil (20 µg/mL) was added in the wells; control wells do not contain essential oil. The volume of 96-well plate was made up to 200 µL. Finally, 5 x10^6 cells were added in all wells including both control and test. The plate was sealed with parafilm and incubated for 18 - 20 hours. Alamar Blue Dye was dispensed in each well and shaken at about 80 revolution per minute in a shaking incubator for 2 – 3 hours. Plates were covered with foil in shaking incubator. Change in color of Alamar Blue dye from blue to pink indicated the growth in bacterial strains. Ciproflaxacin was used as the reference drug.

Bioactive compounds in *Sauropus androgynous* essential oil by GC-MS analysis is presented in Table 1. A total of 33 bioactive compounds were recorded representing 96.27 % composition dominated by Squalene (19.63 %), Caryophyllene (13.92 %), Nonane, 4-methyl (13.82 %) and n-hexadecanoic acid (10.45 %). These compounds have been previously associated with anti-inflammatory (Sinha and Munshi, 2011; Namkeleja et al., 2014), antioxidant (Omokore and Alagbe, 2019; Oluwafemi et al., 2021), cytotoxic and immuno-stimulatory properties (Alagbe, 2022; Muritala et al., 2022). According to Shittu and Alagbe (2020), Squalene has the capability to neutralize free radicals' activities in the body, support blood vessels integrity and protects the heart, lungs and nervous systems. Caryophyllene have been reported to be a proven powerhouse, kills pathogens and inhibits fungal growth (Tiwari et al., 2011; Ojediran et al., 2024). Hexadecanoic acid excels in lowering blood sugar and supports liver detoxification (Sharma, 2012; Jing-Chung et al., 2007; Lima et al., 2010). Nonane, 4-methyl can interfere with mitochondrion energy production in cancer cells, fights infection and calm the nervous system (Choi et al., 2013). All the 33 bioactive compounds have numerous pharmacological properties making them useful in traditional medicine for the treatment of acute and chronic inflammation, cough, fever, gastrointestinal disorders, skin infection, eye problems, sexually transmitted infections, stomach ulcers, pain, snake bite, tooth ache amongst others (Alagbe et al., 2022; Agubosi et al., 2022). A previous study by Senthamarai and Anusha (2012) on the GC-MS analysis of *Sauropus androgynous* leaf reveals that squalene and phytol contained a concentration of 8.06 % and 0.88 % respectively. Lee et al. (2011) also recorded a lower concentration of 0.12 % and 3.07 % for phytol and hexadecanoic acid. Rajeswari et al. (2018); Basker (2012) reported that *Sauropus androgynous* leaf extract contained a higher concentration of 13.80 % hexadecanoic acid. Samad et al. (2018) reported 10.40 % and 0.04 % for hexadecanoic acid and 9, 17-Octadecadienal, and these values were almost similar to the outcome in this experiment. However, geographical location, age of plant, specie, storage method, harvesting technique, processing methods have been identified as factors that influence the concentration of bioactive compounds in medicinal plants (Awa et al., 2012). A synergy between the minor and major components in essential oil from *Sauropus androgynous* in this study forms a rich network of phytochemicals that reduces inflammation by suppressing pro-inflammatory cytokines (Momza et al., 2012; Alagbe et al., 2022), produces bronchodilator effect by opening respiratory airway thereby making breathing easier (Zubairu et al., 2025), improve cardio-vascular health and electrolyte balance thus improving the overall health and vitality of the body (Alagbe, 2025).

The result obtained on the antimicrobial activity of *Sauropus androgynous* essential oil revealed a high percentage inhibition 77.81 %, 72.00 %, 80.09 %, 73.73 % and 76.26 % against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexineri* and *Salmonella typhi* respectively. The results compare well with that of the standard drug (Ciproflaxacin) (65.00 -71.00 %). The presence of squalene, the prominent bioactive compounds in the oil might have been responsible for their antimicrobial activity. Squalene contains measurable compounds that inhibits the activities of some bacteria's (Ajiboye et al., 2013). Phyto-components like hexadecanoic acid, caryophyllene, cyclopentaneundecanoic acid, 8-octadecenoic acid, humulene, gamma. -Terpinene-3-Carene, 9, 17-Octadecadienal, Undecane, 2,10-dimethyl, 2-methyl-Glutaric acid, 2-Ethylhexyl mercaptoacetate and Linoleic acid ethyl ester have been confirmed to show antibacterial activities (Ajayi et al., 2011). This suggests that dietary supplementation of *Sauropus androgynous* essential oil can help to reduce the increasing cases of antimicrobial resistance and promote food safety (Ajayi et al., 2011). Paul et al. (2011), essential oil from *Sauropus androgynous* can defend against infection, disrupts cell membrane of pathogens and inhibited

Staphylococcus spp and *Salmonella spp* in antimicrobial assay. Preveen et al. (2011) reported that *Sauropus androgynous* oil is potent in controlling bacterial growth in biofilms of *Streptococcus spp* and *Shigella spp*.

Compounds	R.T (min)	Molecular formula	Molecular weight (g/mol)	% Area
n-hexadecanoic acid	16.73	$C_{16}H_{32}O_2$	256.424	10.45
Dipyrimadole	18.52	$C_{24}H_{40}N_8O_4$	504.626	2.31
Octadec -9-enoic acid	18.90	$C_{18}H_{34}O_2$	282.47	0.94
N-3-methylButyl acetamide	18.92	$C_7H_{15}NO$	129.20	1.46
Benzene,1,4-dichloro	21.13	$C_6H_4Cl_2$	147.00	0.01
8-octadecenoic acid	22.46	$C_{19}H_{36}O_2$	296.487	0.56
Humulene	22.78	$C_{15}H_{24}$	204.35	0.18
Undecane, 2,10-dimethyl	22.98	$C_{13}H_{28}$	184.36	0.46
Oxalic acid	24.06	$C_{15}H_{28}O_4$	272.38	2.77
Nonane, 4-methyl	25.67	$C_{10}H_{22}$	142.28	13.82
2-Ethylhexyl mercaptoacetate	25.75	$C_{10}H_{20}O_2S$	247.34	1.81
gamma. -Terpinene-3-Carene	26.31	$C_{10}H_{16}$	136.23	2.28
Hexatriacontane	28.94	$C_{36}H_{74}$	507.00	2.15
3-Isothiazolone	29.07	C_3H_3NOS	101.13	1.26
Nerolidol	29.35	$C_{15}H_{26}O$	222.37	0.14
10-Methylnonadecane	29.94	$C_{20}H_{42}$	282.5	1.33
Cyclopropane	30.05	C_3H_6	42.08	2.09
Ethyl palmitate	31.24	$C_{18}H_{36}O_2$	284.5	0.71
2-methyl-Glutaric acid	31.88	$C_6H_{10}O_4$	146.14	0.20
Linoleic acid ethyl ester	31.90	$C_{18}H_{32}O_2$	308.5	0.38
9,17-Octadecadienal	33.25	$C_{18}H_{32}O$	264.4	0.05
4-Trifluoroacetoxytetradecane	33.57	$C_{16}H_{29}F_3O_2$	424.4	0.57
Hexacosanoic acid	33.89	$C_{26}H_{52}O_2$	396.7	1.10
Beta. -Famesene	34.21	$C_{15}H_{24}$	204.35	1.59
Cyclopentaneundecanoic acid	34.57	$C_{16}H_{30}O_2$	254.41	1.87
Pentadecane	37.21	$C_{15}H_{32}$	212.41	0.01
Caryophyllene	37.67	$C_{15}H_{24}$	204.351	13.92
Trans-Nerolidol	38.58	$C_{15}H_{26}O$	222.4	1.12
Methyl stearate	38.71	$C_{19}H_{38}O_2$	298.5	1.07
Ethyl ricinoleate	39.12	$C_{20}H_{38}O_3$	326.5	0.08
Squalene	39.65	$C_{30}H_{50}$	410.7	19.63
Hexahydrofarnesyl acetone	40.19	$C_{18}H_{36}O$	268.5	1.24
Phytol	41.25	$C_{22}H_{42}O_2$	296.5	8.71
Total				96.27
Number of compounds				33.00

Table 1: Bioactive compounds in *Sauropus androgynous* essential oil by GC-MS

Microorganisms	Ciproflaxacin	% Inhibition
Staphylococcus aureus	71	77.81
Escherichia coli	65	72.00
Pseudomonas aeruginosa	75	80.09
Shigella flexineri	68	73.73
Salmonella typhi	70	76.26

Table 2: Antimicrobial activity of *Sauropus androgynous* essential oil

Conclusion

It was concluded that *Sauropus androgynous* essential oil contains several unique bioactive compounds with therapeutic potentials and has been traditionally utilized for the treatment of cold, fever, inflammation, acute and chronic infections. *In vitro* studies have also shown that these compounds also have antimicrobial properties and was able to inhibit the activities of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexineri* and *Salmonella typhi*. Together they form a weapon against infection, inflammation and degeneration.

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