

GC-MS Profiles and Antimicrobial efficacy of *Sarcocephalus latifolia* Methanol Stem Extract and Fractions against Selected Oral Pathogens

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ABSTRACT

Sarcocephalus latifolia is a widely used medicinal plant in West African ethnomedicine. This study evaluated the antimicrobial potential and chemical constituents of methanol stem extract fractions against three oral pathogens: *Streptococcus mutans*, *Staphylococcus aureus*, and *Lactobacillus casei*. Extracts were fractionated through column chromatography and subjected to quantitative physicochemical analysis and agar well diffusion assays against the three oral pathogens. Six (6) key phytochemicals, phenols, tannins, flavonoids, terpenoids, steroids, and alkaloids were analysed. Terpenoid and flavonoids revealed the highest phytochemical (65.63; 12.72 mg/100g), respectively. All fractions showed concentration-dependent antibacterial activity. FA95 and FA24 (What are their identities?) had the most significant zones of inhibition, particularly against *S. mutans* 34.5 ± 0.00 mm and 27.5 ± 0.00 mm, respectively, at 100 mg/ml. The GC-MS analysis identified diverse phytochemicals with established antimicrobial, antioxidant, and anti-inflammatory properties. Among 19 bioactive compounds detected, Octadecanoic acid, Dimethyl sebacate, and Normelicopicine were the most prominent, with proven pharmacological relevance. FA95 and FA24 fractions exhibited strong dose-dependent inhibition against *S. mutans* and *S. aureus*, with zones of inhibition comparable to chloramphenicol. These findings suggest the therapeutic potential of *Sarcocephalus latifolia* as an alternative oral antimicrobial agent.

Keywords: *Sarcocephalus latifolia*, GC-MS, Phytochemicals, Antioxidant, Antimicrobial Activity, Oral Pathogens.

Introduction

Dental caries remains a prevalent global health issue, largely driven by bacterial biofilms dominated by *Streptococcus mutans* and *Lactobacillus* spp (Balhadda et al., 2021). These organisms metabolize dietary sugars to produce acids that demineralize tooth enamel. The rise in antibiotic resistance and side effects of synthetic oral care agents has prompted the search for alternative remedies, particularly from plant-based sources with proven antimicrobial, anti-inflammatory, and antioxidative properties.

Various medicinal plants have demonstrated promising anti-cariogenic potential, owing to their rich phytochemical content such as flavonoids, alkaloids, tannins, and essential oils. For example, *Azadirachta indica* (neem) exhibits broad-spectrum antibacterial activity and has been incorporated into oral rinses and toothpastes for plaque control and caries prevention (Rai et al., 2014).

Syzygium aromaticum (clove) is widely recognized for its eugenol content, which provides analgesic and antibacterial effects against cariogenic bacteria (Raut et al., 2012). *Sarcocephalus latifolia* is also traditionally used to treat oral and gastrointestinal infections and contains numerous secondary metabolites with potential pharmacological benefits (Ahoyo et al., 2019).

Rising resistance to conventional antibiotics underscores the need for alternative therapeutics, especially from natural sources.

This study aimed to profile the chemical constituents of *Sarcocephalus latifolia* stem extract using gas chromatography-mass spectrometry (GC-MS) and evaluate the antimicrobial efficacy of its chromatographic fractions against selected oral pathogens.

Materials and Methods

Collection / Identification of the plant

Fresh Stem bark of the *Sarcocephallus latifolia* tree was collected in the month of September 2023, from Ilai, Mopamuro Local Government Area, Kogi State, Nigeria. The plant was identified at the National Institute for Pharmaceutical Research and Development with the Identification number 7374. The stem bark was washed with distilled water to remove dust particles and dried at room temperature to constant weight, then ground into powder form and stored until required for use.

Preparation of the Extract

Methanol was used for the extraction. A 250 g of the pulverized plant material was taken in a conical flask containing 1500 ml of Methanol and soaked separately for 48 hours with regular shaking at intervals, after which the mixture was filtered using Whatman No. 1 filter paper and it was re-filtered. The filtrate was evaporated to dryness at room temperature to remove the solvent. The dried filtrate (crude extract) was then appropriately labelled and stored in the refrigerator until needed.

Quantitative phytochemical screening

Phytochemical screening of the extract was carried out according to the methods described by (Harbon, 1998; Ezeonu and Ejikeme, 2016; Ibrahim *et al.*, 2025) and is indicated below.

Test for Alkaloids

A 0.5 g of the leaf extract was dissolved in 96% ethanol- 20% tetraoxosulphate(vi) acid (1:1). 1 ml of the filtrate was added to 5 ml of 60% tetraoxosulphate (vi) acid and allowed to stand for 5 minutes. Then 2 ml of 0.5% formaldehyde was added and allowed to stand for 3 hrs. The reading was taken at an absorbance of 565 nm.

Test for flavonoids

The determination of flavonoids was done by acid hydrolysis the spectrophotometric method. 0.5 g of processed leaves extract was mixed with 5 ml of dilute hydrochloric acid and boiled for 10 minutes. The boiled extract was allowed to cool, and 1 ml of the filtrate was added to 5 ml of ethyl acetate and 5ml of 1% ammonium hydroxide. This was the scan from 420-520 nm for the absorbance.

Test for Saponin

A 0.5g of the leaf extract was added to 20 ml of 1 M NHCl and was boiled for 4 hours. After cooling, it was filtered, and 50 ml of pet ether was added to the filtrate for the ether layer and evaporated to dryness. 5 ml of acetone-ethanol was added to the residue. 0.4 ml of each was taken into three different test tubes, 6 ml of ferric sulphate reagent was added to them, followed by 2 ml conc. Tetraoxosulphate (vi) acid. It was thoroughly mixed after 10 minutes, and the absorbance was taken at 490 nm.

Test for Phenols

The quantity of phenol is determined using the spectrophotometer method. 1 g of the leaf extract was boiled with 50 ml of pet spirit for 15 minutes. 5 ml of the boiled sample is then pipette into a 50 ml flask, and 5 ml of distilled water is added. After the addition of distilled water, 2 ml of ammonium hydroxide solution and 5ml of butanol is added to the mixture. The leaves sample was made up to mark and left for 30 minutes to react for colour development and measured at 505 nm wavelength using a spectrophotometer.

Test for Tannins

An aliquot of 0.5 mL of the extract was mixed with 3 mL of 4% vanillin in methanol, and then 1.5 mL of concentrated HCl was added and incubated for 20 minutes at room temperature. After that, the absorbance was measured at 500 nm, and catechin was used as a standard for calibration

Test Terpenoids

For terpenoids, the standard method involves extraction with petroleum ether followed by reaction with chloroform and concentrated sulfuric acid. A fixed volume of extract is mixed with chloroform, and concentrated sulfuric acid is added carefully to form a layer. A reddish-brown interface indicates the presence of terpenoids. Absorbance was measured at 538 nm, and linalool was used as a standard.

Test for Steroid

Steroid quantification was carried out using the Liebermann Burchard reaction. The extract is treated with chloroform, followed by acetic anhydride and concentrated sulfuric acid. After a colour change (green to blue), absorbance was measured at 620 nm.

Test Microorganisms

Standard reference strains *Streptococcus mutans*, *Lactobacillus casei* and *Staphylococcus aureus* were used throughout the study. The inoculum used were standardised to a 0.5 McFarland standard.

Standardization of the Plant Extracts

Solutions of the plant extracts were prepared by dissolving 2 g in 4ml of Dimethylsulfoxide (DMSO) to make initial concentrations of 500 mg/mL, which were subsequently doubly diluted into concentrations of 250mg/mL, 125 mg/mL and 62.5 mg/mL

Antimicrobial Assays

Agar Well Diffusion Method

The agar diffusion method was used to test the antimicrobial activity of the *S. latifolia* methanolic fraction extracts against the test bacteria. The test bacteria were standardized to a 0.5McFarland standard in distilled water and then inoculated onto Mueller-Hinton agar plates (Oxoid, CM0405). Holes were bored onto the seeded plates using a sterile 6mm cork borer according to the extract concentrations prepared, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml and 0.1ml of the extracts was dispensed into the appropriate holes and incubated. After inoculation, using a meter rule, the diameters of zone inhibition of growth were measured and recorded to the nearest millimetre (Qsaem and Abblan, 2016; Abubakar et al., 2025).

Results

Table 1 presents the concentrations (in mg/100 g) of six key phytochemicals, namely: phenols, tannins, flavonoids, terpenoids, steroids, and alkaloids extracted from the stem of *Sarcocephalus latifolia* using methanol as a solvent.

Table 1: Quantitative Phytochemical analysis of *Sarcocephalus latifolia* stems Extracts

S/no	Parameter	Stem
1	Phenol(mg/100g)	4.02
2	Tannins	9.50
3	Flavonoids	12.72
4	Terpenoid	65.63
5	Steriods	2.88
6	Alkaloids	0.93

The stem extract of *Sarcocephalus latifolia* contains several bioactive compounds. Terpenoids were the most abundant (65.63 mg/100 g), followed by flavonoids (12.72 mg/100 g) and tannins (9.50 mg/100 g). Moderate amounts of phenols (4.02 mg/100 g) and steroids (2.88 mg/100 g) were detected, while alkaloids were present in the lowest concentration (0.93 mg/100 g). The high terpenoid and flavonoid content suggests strong potential for antimicrobial and antioxidant activities.

Table 2: Fractions from Column Chromatography of Methanolic Stem extracts of *Sarcocephalus latifolia* plant

Fraction	Solvent used	Ratio (v/v)%	Fractions pooled
MSE ₁	Ethyl-acetate	100	1-74
MSE ₂	Ethyl-acetate: methanol	90:10	75-79
MSE ₃	Ethyl-acetate: methanol	80:20	80-100
MSE ₄	Ethyl-acetate: methanol	50:50	101-118
MSE ₅	Methanol	100	119-130

Key: Hex= Hexane, EA= Ethyl Acetate, Me= Methanol, MRE= Methanolic Stem Extract (fraction 1-130)

The methanolic stem extract was successfully separated into five major fractions (MSE1–MSE5) using increasing polarity solvent systems. Early fractions (MSE1) were eluted with 100% ethyl acetate, while later fractions (MSE5) required 100% methanol, indicating the presence of compounds with varying polarity. This shows effective separation of phytochemicals based on polarity.

Table 3: Re-fractionated Methanol Stem Extracts from primary extract fractions 71-94

Fraction	Solvent used	Ratio (v/v)%	Fractions pooled	Colours
FA3	Ethyl-acetate	100	1	Green
FA20	Ethyl-acetate: methanol	90:10	10-20	Light-green
FA24	Ethyl-acetate: methanol	80:20	21-24	Reddish-Brown
FA95	Ethyl-acetate: methanol	50:50	71-95	Reddish-Brown
FA100	Methanol	100	96-100	Reddish-Brown

Further purification of pooled fractions (71–94) produced five sub-fractions (FA3–FA100). The color variations (green to reddish-brown) indicate differences in chemical composition. Fractions eluted with higher methanol proportions (FA95 and FA100) likely contain more polar bioactive compounds.

Table 4: Biochemical Characteristics of the Bacterial Isolates (Test Organisms)

Biochemical Characteristics	<i>Strep. mutans</i>	<i>Staph. aureus</i>	<i>Lactobacillus casei</i>
Capsule	+ve	-ve	+ve
Gram Staining	+ve	+ve	+ve
Catalase	-ve	+ve	-ve
Shape	Cocci	Cocci	Rod
Motility	-ve	-ve	-ve
Spore	-ve	-ve	-ve
Urease	-ve	+ve	-ve
Oxidase	-ve	-ve	-ve
Glucose	+ve	+ve	+ve
Lactose	+ve	-ve	+ve
Maltose	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve

Key: +ve = positive (present), -ve = negative (absent)

All isolates were Gram-positive bacteria. *Staphylococcus aureus* was catalase and urease positive, distinguishing it from *Streptococcus mutans* and *Lactobacillus casei*, which were catalase negative. The sugar fermentation profiles further confirm the identity of the organisms. These characteristics validate the test organisms used for antibacterial screening.

The re-fractionated stem extracts exhibited concentration-dependent antibacterial activity against *S. aureus*, *S. mutans*, and *L. casei*.; At lower concentrations (12.5 mg/ml), activity was minimal or absent in some fractions. At higher concentrations (50–100 mg/ml), inhibition zones increased significantly. FA95 and FA100 generally showed stronger activity, particularly against *S. mutans* and *L. casei*.

However, the positive control (chloramphenicol) consistently produced larger inhibition zones than the plant fractions, indicating superior antibacterial potency compared to the extracts.

Table 5: Antibacterial Activity of Re-fractionated Stem Extract ‘fractions pooled 71-94’ against the test organism

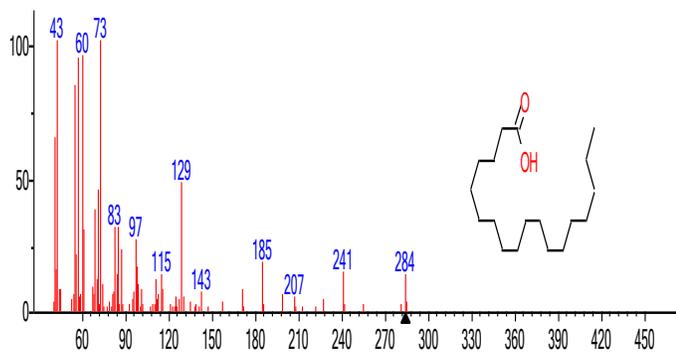
Extracts mg/ml	FA3	FA20	FA24	FA95	FA100	PC.CHR
<i>S. aureus</i>						
12.5	0.00±0.00	7.00±1.00	5.00±1.00	4.00±1.00	0.00±0.00	29.30±1.00
25	4.00±0.00	11.10±1.00	8.00±2.00	11.00±1.00	10.00±0.00	30.10±1.00
50	7.50±1.00	18.00±0.00	15.00±0.00	18.20±2.00	14.20±1.00	31.40±1.00
100	16.00±1.00	22.10±0.00	18.00±0.00	20.30±1.00	25.50±1.00	30.20±1.00
<i>S. mutans</i>						
12.5	5.00±0.00	10.00±0.00	12.00±0.00	2.00±0.00	6.00±0.00	32.00±2.00
25	6.00±0.00	22.20±0.00	20.20±2.00	11.00±1.00	11.00±1.00	30.00±0.00
50	12.50±0.00	27.50±0.00	23.20±1.00	19.30±1.00	30.30±1.00	30.50±0.00
100	25.40±1.00	34.50±0.00	32.50±0.00	30.20±0.00	30.90±0.00	35.20±1.00
<i>L. casei</i>						
12.5	3.00±0.00	4.00±0.00	3.50±1.00	6.00±0.00	5.00±0.00	30.60±0.00
25	4.20±0.00	8.10±1.00	7.40±1.00	14.00±0.00	8.00±0.00	32.00±0.00
50	12.00±1.00	11.10±0.00	9.20±1.00	16.30±1.00	18.70±1.00	30.00±0.00
100	16.50±0.00	15.20±0.00	14.70±0.00	25.50±1.00	26.00±0.00	33.50±0.00

Key: FA, Re-fractionated extract, PC, Positive Control, CHL-Chloramphenicol.

Values of zone diameter of inhibition are measured in (mm) of three replicates ±standard deviation (SD) = 0.05

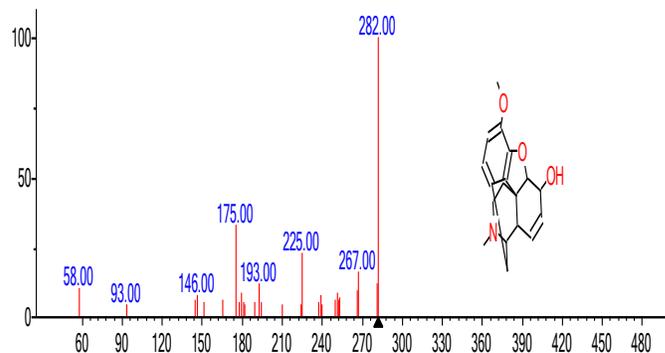
Table 6: Bioactive components identified in the extract by GC-MS analysis of the extract

Peak	Compounds	RT	Area	Area %	Area Sum %	Height	Symmetry
1	erythro-9,10-Dibromopentacosane	10	295534.16	1.51	0.62	153231.58	1.03
2	Cetene	10.2	295539.94	1.51	0.62	162468.37	1.59
3	3,5-Dimethoxycinnamic acid	12.4	377668.81	1.93	0.8	202319.32	1.63
4	Phytol	12.4	421040.42	2.15	0.89	218856.42	1.26
5	Octadecanoic acid	13.9	6082155.21	31.12	12.85	354180.75	2.08
6	Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl)-	14.3	1478041.91	7.56	3.12	381554.57	0.78
7	1-Tetradecene	14.5	1020272.68	5.22	2.16	314162.83	1.08
8	Dimethyl sebacate	15.8	4682958.68	23.96	9.9	594809.7	1.71
9	Octadecanoic acid	16.1	4136790.18	21.17	8.74	1036205.95	0.94
10	d-Mannitol, 1-O-(16-hydroxyhexadecyl)-	16.3	483081.1	2.47	1.02	241990.79	1.03
11	Trp-Leu-Lys	17.2	380208.14	1.95	0.8	58202.88	2.35
12	Adipic acid, 2-octyl undecyl ester	18.2	19544125.31	100	41.31	6985143.03	0.39
13	Yangambin	19.1	634388.69	3.25	1.34	98991.93	1.4
14	5-Iodo-histidine	19.4	374155.56	1.91	0.79	58992.87	0.88
15	Normelicopicine	20.4	3951520.2	20.22	8.35	647324.05	3.17
16	Pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis(oxy)]-, cyclic 3-(1,2-ethanediyl acetal)	20.7	484273.45	2.48	1.02	80798.5	1.67
17	Ethyl 4-([(E)-(2-nitrophenyl)methylidene]amino)benzoate	23.7	530512.71	2.71	1.12	64898.5	1.62
18	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	24.5	256008.36	1.31	0.54	35716.45	0.79
19	5-Bromo-4-nitroimidazole-2-[2-thioacetic acid]	24.7	1886076.63	9.65	3.99	135547.66	1.49



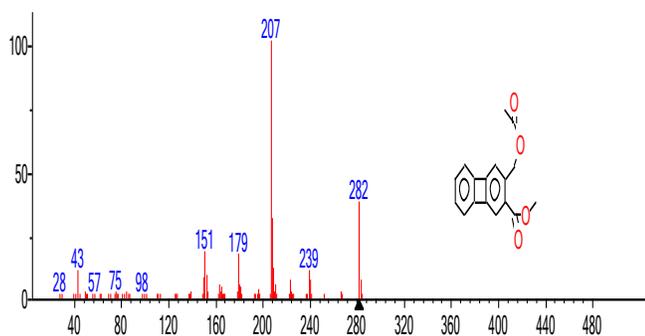
MW: 284 CAS# 57-11-4 C₁₈H₃₆O₂ (replib) Octadecanoic acid

Fig. 1A: Octadecanoic acid



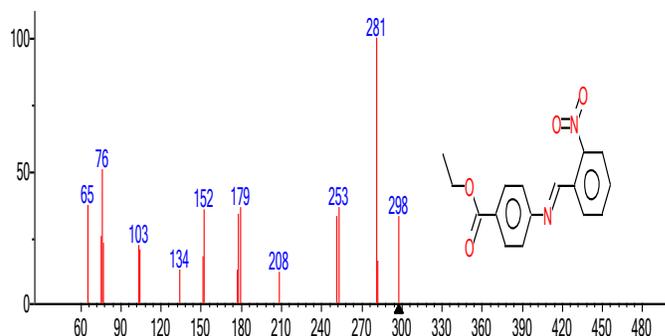
MW: 299 CAS# 76-57-3 C₁₈H₂₁NO₃ (nist_msms) Codene [M+H+H₂O]⁺ QQQ 20V P=282

Fig. 1B: 5-Bromo-4-nitroimidazole-2-[2-thioacetic acid



MW: 282 CAS# 93103-70-9 C₁₇H₁₄O₄ (mainlib) 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene

Fig. 1 C: 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene.



MW: 298 CAS# 57707-09-2 C₁₆H₁₄N₂O₄ (mainlib) Ethyl 4-[(E)-(2-nitrophenyl)methylidene]amino benzoate #

Fig. 1 D: Ethyl 4-[(E)-(2-nitrophenyl) methylidene] amino benzoate.

Figure 1: A cross-section of a Chromatogram of some GC-MS identified Compounds

Discussion

The quantitative phytochemical analysis of the methanolic stem extract of *Sarcocephalus latifolia* revealed the presence of six major secondary metabolites: phenols, tannins, flavonoids, terpenoids, steroids, and alkaloids. Although alkaloids were detected in relatively low amounts (0.93 mg/100 g), they are recognized as highly bioactive compounds with established antimicrobial, antimalarial, and analgesic properties, and even minimal concentrations may exert significant pharmacological effects (Harborne, 1998). The phenolic content (4.02 mg/100 g), though moderate, is noteworthy due to the well-documented antioxidant potential of phenolic compounds, which act by donating hydrogen atoms or electrons to neutralize free radicals (Singleton & Rossi, 1965).

This antioxidant capacity may contribute to the plant's protective role against oxidative stress-related disorders. Tannins (9.50 mg/100 g) were present in appreciable quantities and are known to exhibit antimicrobial and anti-inflammatory activities through protein precipitation and enzyme inhibition mechanisms (Price *et al.*, 1978). Similarly, flavonoids (12.72 mg/100 g) are associated with broad-spectrum bioactivities, including antibacterial and antiviral effects (Chang *et al.*, 2002). Terpenoids, which constituted the highest proportion (65.63 mg/100 g), are widely reported to disrupt microbial membranes and interfere with essential metabolic processes (Ghorai *et al.*, 2012). Their predominance in the extract may therefore explain the strong antimicrobial potential and the plant's traditional use in the management of wounds and infections.

Steroids (2.88 mg/100 g), though present in smaller amounts, may further contribute anti-inflammatory and analgesic effects (Edeoga *et al.*, 2005). Fractionation of the methanolic extract using gradient mixtures of ethyl acetate and methanol enabled effective separation of phytochemicals according to polarity. The pooled fractions (MSE1–MSE5) and subsequent re-fractionated samples (FA3–FA100) demonstrated distinct color variations, suggesting differences in chemical composition and polarity. The gradual increase in methanol concentration facilitated the isolation of more polar constituents in later fractions, particularly FA95 and FA100, which likely contain higher concentrations of polyphenolic and alkaloidal compounds. In contrast, earlier fractions such as FA3 and FA20 may be enriched with less polar constituents, including terpenoids. This polarity-based separation provides a rational framework for subsequent antimicrobial and instrumental analyses.

The biochemical characterization confirmed that all test organisms were Gram-positive bacteria relevant to oral infections. *Streptococcus mutans* and *Lactobacillus casei* were catalase-negative, whereas *Staphylococcus aureus* was catalase-positive, consistent with standard taxonomic descriptions (Tortora *et al.*, 2012). These profiles validate the suitability of the selected organisms for evaluating antibacterial efficacy against oral pathogens.

The antibacterial assay demonstrated concentration-dependent inhibitory activity across all fractions. Notably, FA95 and FA24 exhibited the highest zones of inhibition, particularly against *S. mutans*, indicating enhanced bioactivity at higher concentrations. FA20 also showed remarkable activity at moderate concentrations, suggesting possible synergistic interactions among its constituents. Overall, *S. mutans* appeared to be the most susceptible organism, potentially due to the vulnerability of its extracellular polysaccharide matrix to phenolic and fatty acid components. In contrast, *L. casei* displayed comparatively lower sensitivity, especially at reduced concentrations.

GC–MS analysis identified nineteen major bioactive compounds, several of which possess established antimicrobial properties. Octadecanoic acid, the predominant compound, is known to disrupt bacterial lipid membranes (Rawani *et al.*, 2011).

Dimethyl sebacate interferes with microbial enzymatic systems (Yunus *et al.*, 2010), while Normelicopicine, an alkaloid, may inhibit bacterial replication through DNA interaction (Mahomoodally, 2013). The presence of phenolic derivatives, lignans such as Yangambin, and nitroimidazole-related heterocycles further strengthens the antimicrobial profile of the extract (Kaboré *et al.*, 2014; Napimoga *et al.*, 2019; Nwozo *et al.*, 2023). The pronounced activity observed in FA95 and FA24 correlates with the abundance of lipophilic bioactive compounds, whose membrane-penetrating properties likely enhance antibacterial efficacy (Jansen *et al.*, 2021). Collectively, these findings support the ethnomedicinal use of *Sarcocephalus latifolia* stem and demonstrate that its antimicrobial activity is attributable to a diverse array of phytochemicals acting individually and synergistically.

Conclusion

This study validates the antimicrobial efficacy of *Sarcocephalus latifolia* methanolic stem extract fractions against key oral pathogens. The GC-MS profiling revealed several bioactive compounds with demonstrated antimicrobial potential. The abundance of bioactive secondary metabolites supports the plant's traditional use in ethnomedicine and suggests its potential development as a source of natural therapeutic agents. The synergistic interaction among these compounds may account for the broad-spectrum antimicrobial and anti-inflammatory properties observed in biological studies. The fractions FA95 and FA24, in particular, demonstrated remarkable activity against *Streptococcus mutans*. The presence of Octadecanoic acid, Dimethyl sebacate, and Normelicopicine, Ethyl 4-[(E)-(2 nitrophenyl) methylidene] amino) benzoate is likely responsible for the potent effects observed. These findings support the traditional use of *S. latifolia* and provide a scientific basis for its development into oral care formulations.

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