Trop J Nat Prod Res, August 2024; 8(8): 8202-8211 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In vitro Antioxidant, Mineral Analysis and Antimicrobial Activities of Extract and Fractions from the Aerial Part of *Heterotis rotundifolia* (Sm.) Jacq. Fel

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ARTICLE INFO

Article history:
Received 12 June 2024
Revised 26 June 2024
Accepted 14 August 2024
Published online 01 September 2024

ABSTRACT

Heterotis rountidifolia (Sm.) Jacq. Fel, is employed in Nigeria traditional medicine for the treatment of various diseases. The study investigated the antioxidant, mineral composition and antimicrobial activities of aerial part of *Heterotis rotundifolia* using standard procedures. The proximate composition on dry matter basis showed high carbohydrate (48.41%) and low lipid (0.77%) contents while mineral content revealed that sodium (5.68 mg/100 g) and zinc (4.64 mg/100 g) were the highest. The total caloric value per 100 g was 260.93 kcal. Methanol (ME) fraction exhibited the highest radical scavenging (EC_{50} = 59.36 µg/mL) and reducing (EC_{50} = 76.54 µg/mL) activity. Contents of total flavonoids and phenolics were highest in ME and ethyl acetate (EAE) fractions (39.2 mg GAE/g and 183.5 mg RE/g, respectively), while hexane (HE) fraction showed the lowest radical scavenging, ferric reducing and nitric oxide assay (EC_{50} = 268.56, 87.86, and 98.30 µg/mL, respectively). The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) against various bacterial and fungal strains using tube dilution method showed strong activity at MIC of 50 mg/mL depicted by n-HE fraction, though lower MBC of 37.5 mg/mL by ME fraction made the ME fraction of better potentials. Gas chromatography-mass spectrum (GC-MS) analysis of the ME fraction revealed 5-Hydroxymethylfurfural (16.87%), Methyl 6-*O*-[1-methylpropyl]-*β*-D-galactopyranoside (16.07%), 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy- (8.28%), 6-methyl-*β*-D-glucopyranose, 1,6-anhydro- (6.56%), 2-heptanol (6.82%), stigmastan-3,5-diene (4.32%) amongst others. This research demonstrates that extract and fractions from aerial part of *H. rotundifolia* possesses antioxidant, antimicrobial and nutritive potentials, albeit with generally weak antifungal activity and, may be attributed to the presence of its phytochemical constituents.

Keywords: *Heterotis rotundifolia*, Antimicrobials, Antioxidant assay, Phenolics, flavonoids, Gas chromatography-mass spectrophotometry.

Introduction

Plants offer vast therapeutic benefits through their rich array of medicinal substances, serving as antioxidants, anti-inflammatories, anticancer agents, and antimicrobials.[1] Through extensive research, potent phytochemicals have been identified, isolated, and transformed into active pharmaceuticals and nutritional supplements.[1,2] These natural compounds, along with essential nutrients and minerals, play crucial roles in energy production, structural development, and metabolic processes within the body. Studies have highlighted that deficiencies in these vital components can lead to serious health issues like cancer and cardiovascular problems.[3] Phytochemical components such as phenolics and flavonoids act as antioxidants, effectively scavenging free radicals and preventing lipid peroxidation.

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Citation: Enin GN, Adegoke AA, Ita BN, Udosen CI, Inyang VF, Onuaha EC, Antia BS. *In vitro* Antioxidant, Mineral Analysis and Antimicrobial Activities of Extract and Fractions from Aerial Part of *Heterotis rotundifolia* (Sm.) Jacq. Fel. Trop J Nat Prod Res. 2024; 8(8):8202-8211. https://doi.org/10.26538/tjnpr/v8i8.42

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The search for new sources of antimicrobials has repeatedly been solicited by the World Health Organization[5,6] because of the threat posed by various pathogens as well as the critical level of threat occasioned by antimicrobial resistance.[7] The WHO reports stated that medicinal plants are crucial resources for most rural dwellers in Africa, with nearly 80 percent utilizing them for primary healthcare needs.[8] These metabolites display anticarcinogenic, antimutagenic, cardioprotective, and other health-remediating effects with a large number reportedly housed in some species of *Melastomataceae*.[9]

H. rotundifolia (Sm.) Jac. Fel. is a short-lived perennial plant of the *Melastomataceae* family which is commonly found in the wild. The plant is referred to as "Pink Lady" in English but has local names among the tribes in Nigeria. For example, among the Igbos - Nkpisi-nku, among the Yorubas-Awede[10] and Nyin Ndan among the Ibibio people of Southern Nigeria. The ethnopharmacology of the plant indicates that the plant is used to treat gastrointestinal disorders, stomach aches, venereal diseases, diarrhoea, dysentery, rheumatism, pneumonia, conjunctivitis, bilharziasis and hookworm infestation in the East and Tropical Africa.[10] Phytochemical studies carried out on the plant depict the presence of terpenes, flavonoids, tannins, alkaloids, and glycosides. Secondary metabolites vitexin (8-*β*-D-glucopyranosyl apigenin), isovitexin (6-*β*-D- orientin (8-*β*-D-glycopyranosyl luteolin), isorientin (6-*β*-D-glycopyranosyl luteolin) and pheophytin A have been isolated.[11-13] Previous research on *H. rotundifolia* predominantly focused on leaves extract and fractions. However, there is growing interest in exploring the aerial parts of plants as potential sources of bioactive compounds and nutraceuticals. These compounds show promising activities against various diseases

and infections. Therefore, this study aims to identify bioactive constituents, mineral elements, and antimicrobial activity in ethanol extract and various fractions from the aerial part of *H. rotundifolia* to elucidate their potential medicinal and nutraceutical benefits.

Materials and Methods

Plant collection and identification

H. rotundifolia matured plant was harvested from a farmland in Itak Ikot Nkap in Ikono L.G.A of Akwa Ibom State, Nigeria in June 2022. The plant identification and authentication were conducted by Professor Margaret E. Bassey of the Department of Botany and Ecological Study, UNIUYO, Uyo, Nigeria. Herbarium specimen (UUH 4320) of the sample was deposited in the Faculty of Science Herbarium.

Extraction

The aerial portions were detached from the root stalk, washed with running water, dried in the air for fourteen days, and ground into powder form with a lab mill. The powdered material (439.895 g) was macerated with hexane (HE), ethyl acetate (EAE), and methanol (ME) successively. The filtrate was concentrated and evaporated to dryness *in vacuo*. Another 120 g was soaked in 50% ethanol, filtered, and the filtrate was concentrated to dryness *in vacuo* to obtain the ethanol crude extract. The extracts were preserved at the refrigerating temperature until use.

Phytochemical screening

Tests for saponins, tannins, flavonoids, alkaloids, cardiac glycosides, terpenoids and anthraquinones were conducted according to approved protocols.[14-16]

Determination of total phenolic content

The overall concentration of phenolic compound was assessed spectrophotometrically in line with the method of Kim *et al*. (2003),[17] with slight modification. Briefly, 0.5 mL (1 mg/mL) of sample was mixed with 2 mL of 10% Folin-ciocalteu reagent, 2 mL of 7% Na_2CO_3, and 2 mL of distilled were added to form a solution. The mixture was left for 15 seconds and then incubated at 40 °C for 30 minutes to develop colour. The absorbance was measured at 765 nm, and the total phenolic content was determined through a gallic acid calibration curve, and the findings were reported accordingly.[17]

Determination of total flavonoid content

The total flavonoid composition was obtained utilizing the protocol of Subhashini *et al*. (2010)[18]. The sample (1 mg/mL) was diluted with 200 µL distilled water followed by 150 µL of 5% sodium nitric solution. This mixture was incubated for 5 minutes and added to 150 µL of 10% $AlCl_3.6H_2O$. Six minutes later NaOH solution (1M, 2 mL) was added. The absorbance was noted at 510 nm and the total flavonoid content was quantified from the rutin calibration graph. Results were expressed as rutin equivalents per 100 g of dry weight (mg RE/ 100 g).

Proximate analysis

Proximate analysis to determine moisture, ash, lipid, protein, and crude fibre contents was performed following the standard procedures of the Association of Official Analytical Chemists.[19] The caloric content of the samples was determined by applying the factors 4 for crude protein, 9 for lipids, and 4 for carbohydrates to their respective values and then adding the totals together. Selected antinutritive compounds were estimated using a well-established methodology.[19]

Evaluation of antioxidant activity

The antioxidant evaluation was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric ion reducing antioxidant power (FRAP) and Nitric oxide assays.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH activity was assessed following the technique described by Shekhar and Anju (2024).[20] A 1 mL aliquot of DPPH (0.1 M) was mixed with 3 mL of the solution containing the extract, fractions, as well as vitamin C, and the mixture was mixed for one min. Each sample was then left in the dark for half an hour before measuring the absorbance at 517 nm. A reduced absorbance in the mixture signified increased activity of scavenging free radicals. The percentage of DPPH scavenging activity was determined according to equation 1 below:

$$\text{DPPH percentage scavenging effect} = \frac{[(A0 - As)]}{[A0]} \times 10 \quad ----- \; equation\ 1$$

Where A0 is the absorbance of the control reaction, and As is the absorbance of the standard.

Ferric reducing antioxidant power (FRAP) Assay

FRAP activity of the plant was determined using a standard procedure.[21] Different concentrations (20, 40, 60, 80, 100 µg/mL) of the sample (2 mL) were combined with a solution bearing 2 mL of 0.2 M Na_3 PO_4 buffer (pH 6.6) with 2 mL of 1% w/v potassium hexacyanoferrate (III), $K_3[Fe(CN)_6]$. The resulting mixture was then incubated at 50ºC for 0.33 h. Afterward, 2 mL of 10% w/v trichloroacetic acid was introduced. Then, the solution was spun at 650 rpm for 0.17 h before measuring the absorbance.

Nitric oxide radical scavenging ability (NO)

Sodium nitroprusside, SNS (10 mM, pH = 7.4) was introduced to 1.0 mL extract and incubated for 60 minutes at ambient temperature. Thereafter, 1.0 mL of fresh Griess reagent was added. Absorbance data (Techmel & Techmel, USA) was obtained at 540 nm and percentage NO scavenging activity was calculated using equation 2 below.[21]

$$\text{Percentage inhibition of NO} = \frac{[(\text{Absorbance control} - \text{Absorbance Sample})]}{[\text{Absorbance control}]} \; X\ 100 \quad --- \; equation\ 2$$

A control reaction mixture lacking the extract was employed.

Mineral analysis

Mineral elements were analysed using established methods.[19,22] Fe, Zn, Na, K, Ca, and Mg were measured following acid digestion with an Atomic Absorption Spectrophotometer, and the concentration of each elemental composition was computed with a recourse to the dry matter.

Evaluation of antimicrobial activity

The test isolates were clinical isolates previously typed using molecular methods in other studies and stored in culture collection of the Department of Microbiology, University of Uyo. *Staphylococcus aureus* NCTC 6751 (Gram-positive), *Escherichia coli* NCTC 10418 (Gram-negative) and *Candida albicans* ATCC 10231 were used as control organisms for the antimicrobial susceptibility testing (AST). The viability tests for all the test isolates were conducted by preparing a suspension of each isolate, streaking each of them on agar, incubating the plates, examining them for colony (growth) to confirm viability by comparing with other isolates with known viability (positive control) and sterile saline (negative control). The test isolates that formed colonies like the positive control were ascertained to be viable and used for AST. To assess the antimicrobial susceptibility of the test organisms, the agar-well diffusion method was used, with minor modifications.[13,23] After incubating for 24 h, the test organisms were seeded into the agar using the pour plate method, with each plate containing one seeded test organism, done in triplicates. The extract was diluted to different concentrations. Using a 5 mm cork borer, wells were created in the inoculated agar, and the various concentrations of the prepared extracts were added. Gentamycin and nystatin were utilized as reference drugs for bacteria and fungi, respectively. All plates were placed in a 37 °C incubator for 24 hours. The zone of inhibition (clearance) was determined by deducting the 5 mm diameter of the cork from the overall diameter of clearance. An activity index (A.I.) was calculated for each fraction by dividing the zone of inhibition of the extract by the zone of inhibition of the reference antibiotic. An A.I. of 1 or greater indicates that the extract has equal or greater activity than the reference antibiotic.[13]

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentrations (MBC and MFC) of HR

The tube dilution method was used.[23] The n-hexane, ethyl acetate, and methanol extracts were diluted into various concentrations of 25 - 250 mg/mL. The interference control was implemented using the solvents that were utilized to prepare various concentrations. A 0.2 mL Mueller-Hinton broth culture of the test organisms was added to each dilution. After 24 hours of incubation at 37°C, the tubes were measured for turbidity using a turbidimeter. Inoculated broth without extract served as the positive control, whereas extracts added to the broth without inoculum functioned as the negative control. The least concentrations that killed the organism with proof of no regrowth when inhibited cultures were subcultured were reported as MBF/MFC, whilst the minimum concentrations that prevented the growth of organisms were noted as MIC.[23]

GC-MS analysis

A GC-MS-QP2010SE (SHIMADZU model, Japan with a column length of 30 m; thickness, 0.25 m; and diameter, 0.25 mm was employed to analyse the samples. Helium gas was the carrier gas at 1 mL/min and a sample injection volume of 1µL was at split ratio (10:1). The oven temperature was taken from 60 °C, with an increase of 5 °C/min, to 180 °C and subsequently, a ramp of 20˚C/min to 250˚C. The ion source temperature was adjusted to 230°C, and the ionization voltage was set at 70 eV. The GC-MS data interpretation utilized the National Institute of Standards and Technology (NIST).[24]

Statistical analysis

Data obtained from this work were analyzed statistically using one – way ANOVA.

Results and Discussion

Phytochemical screening of the aerial part of *H. rotundifolia* reveals the presence of alkaloids, tannins, saponins, steroids, flavonoids, cardiac glycosides, and terpenoids. However, the presence of anthraquinones was not detected (Table 1). Results of proximate estimation reveal that the plant contains an appreciable amount of carbohydrate (48.41%); crude fibre (13.62%); ash (11.48%); moisture (10.62%) and crude protein (14.40%) while the crude lipid content was 0.77%. Quantitatively, the phytochemical constituents were recorded (mg/100 g) thus: Glycosides (11.02±0.01); saponin (28.15±0.01); vitamin C (0.35±0.00); *β*-carotenoid (0.16±0.00) mg/100 g dry matter respectively (Table 2). The outcomes of the mineral element constituents of *H. rotundifolia* are shown in Table 3. The result shows the availability of Na (5.68%), K (3.25%), Ca (0.11%), Mg (0.04%), Fe (1.42%) and Zn (4.64%) revealing that Na was the most abundant inorganic elements, followed by Zn and K. Mineral ratios estimation also indicated that Na: K was 1.75:1, Ca: Mg was 2.90:1 and K: Ca was 27.99:1 whereas K: (Ca+Mg) was calculated as 20:83:1.

The phenolic content in the extracts ranged from 16.60 to 39.20 mg GAE/g. HE has the lowest phenolics content (16.6 mg GAE/g) while ME extract has the highest phenolic content (38.20 mg GAE/g). Also, the total flavonoid contents of the extracts ranged from 43.5 to 183.5 mg QUE/g. EAE was found to have the highest flavonoids content (183.5 mg QUE/g), while the least flavonoids content (47.5 mg QUE/g) was found in HE (Table 4).

The extracts inhibited the DPPH, FRAP and NO radical activity in concentration-dependent manner (Figure 1, 2 and 3). For the DPPH, FRAP and NO activities, ME exhibited the highest radical scavenging (EC_{50} = 59.36 µg/mL), reducing (EC_{50} = 76.54 µg/mL) and NO (EC_{50} = 37.49 µg/mL) activity while HE showed the lowest radical scavenging (EC_{50} = 268.56 µg/mL), ferric reducing (EC_{50} = 87.86 µg/mL) and nitric oxide activities (EC_{50} = 98.30 µg/mL). These results suggest that ME fraction demonstrated the highest antioxidant activity. All (100 %) the clinical isolates collected from the culture collection centre were viable. The results of the antimicrobial efficacy of different fractions (HE, EAE, and ME) of *H. rotundifolia* as evaluated against a spectrum of bacterial and fungal pathogens were presented in Table 6.

Table 1: Phytochemical constituents of *H. rotundifolia*

Phytochemicals	Result
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Anthraquinones	–
Cardiac Glycosides	+

Key: + = Present; – = absent.

Table 2: Proximate and Phytochemical composition of *H. rotundifolia*

Parameters	Percentage values (Dry weight)
Moisture	10.62±0.00
Ash	11.48±0.00
Lipid	0.77±0.00
Crude Protein	14.40±0.01
Crude Fibre	13.62±0.01
Carbohydrate	48.41±0.80
Energy (kcal/100 g)	260.93±0.02
Carbohydrate: Fibre Ratio	3.554:1
Glycosides	11.02±0.01
Vitamin C	0.35±0.00
Saponin	28.13±0.01
β-Carotenoid	0.16±0.00

As depicted in Table 5, the HE fractions exhibited notable antimicrobial activity against various pathogens, with the largest zone of inhibition observed at a concentration of 250 mg/mL against *Escherichia coli* (30 mm, A.I. = 0.83). Significant inhibition was also seen against *Klebsiella pneumoniae* (22 mm, A.I.= 0.85) and *Staphylococcus aureus* (21 mm, A.I. = 0.66). At the lowest concentration (50 mg/mL), moderate activity was observed, with the highest inhibition against *Escherichia coli* (14 mm, A.I. = 0.39). No activity was detected against *Candida albicans* at this concentration. The ME fraction displayed broad-spectrum antimicrobial activity, with the highest inhibition zone recorded at 250 mg/mL against *Escherichia coli* (27 mm, A.I. = 0.75) and moderate activity against *Staphylococcus aureus* (19 mm, A.I. = 0.59). At 50 mg/mL, the fraction showed minimal to no activity against several pathogens, including *Bacillus subtilis* and *Aspergillus niger*. Gentamycin and Nystatin were used as reference antibiotics for bacteria and fungi, respectively. Gentamycin exhibited superior inhibition zones against all bacterial strains, with the highest against *Escherichia coli* (41 mm). Nystatin showed substantial antifungal activity with a zone of inhibition of 30 mm against *Candida albicans* and 14 mm against *Aspergillus niger*. The antimicrobial activity of HE, EAE, and ME fractions of *H. rotundifolia* was quantified by MIC and MBC/MFC against various strains (Table 6).

Table 3: Mineral composition of *H. rotundifolia*

Minerals	Values ±SD
Na	5.68±0.00
K	3.25±0.00
Ca	0.11±0.00
Mg	0.04±0.00
Fe	1.42±0.01
Zn	4.64±0.01
Na:K	1.75:1
Ca:Mg	2.90:1
K:Ca	27.99:1
K:(Ca+Mg)	20.83:1

Table 4: EC_{50} values, TFC and TFC of *H. rotundifolia* extracts

	Concentration in µg/mL			
Assay	**HE**	**EAE**	**ME**	**AA**
DPPH EC_{50}	268.56	151.76	59.36	12.23
FRAP EC_{50}	87.86	84.19	76.54	25.01
NO EC_{50}	98.30	66.76	37.49	20.78
TFC	Concentration in mg RE/g			
	16.6±0.02	19.0±0.0	39.2±0.002	–
TPC	Concentration in mg RE/g			
	47.5±0.001	183.5±0.001	112.0±0.002	–

TFC = Total flavonoid content, TPC = Total phenolic content

The HE fractions showed strong activity, with the lowest MIC of 25 mg/mL against *Escherichia coli* and an MBC of 50 mg/mL. *Staphylococcus epidermidis* and *Staphylococcus aureus* had MIC and MBC values of 50 mg/mL and 75 mg/mL, respectively. The fraction was less effective against *Candida albicans* and *Aspergillus niger*, with MIC values of 150 mg/mL and 100 mg/mL. The EAE fraction demonstrated significant activity, notably against *Enterobacter aerogenes*, with an MIC of 25 mg/mL and an MBC of 50 mg/mL, indicating high potency. In addition to its activity against *Enterobacter aerogenes*, the EAE fraction also exhibited appreciable antimicrobial properties against other common bacterial strains such as *Escherichia coli* and *Staphylococcus aureus*. For these bacteria, the MIC was 50 mg/mL, and the MBC was 75 mg/mL. These values indicate that while the EAE fraction is effective against these bacteria, it requires slightly higher concentrations compared to *Enterobacter aerogenes*. Nonetheless, these results are promising, as both *Escherichia coli* and *Staphylococcus aureus* are significant pathogens that can cause various infections in humans, and finding effective treatments against them is crucial. However, the efficacy of the EAE fraction was notably reduced when tested against *Bacillus subtilis* and certain fungal strains, such as

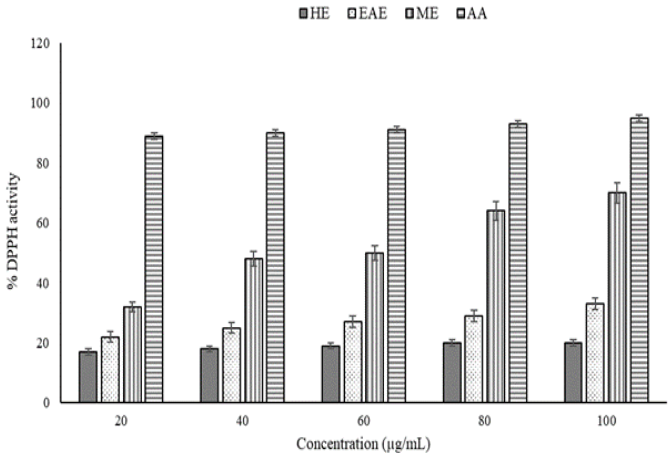

Figure 1: DPPH activity of *H. rotundifolia* extracts

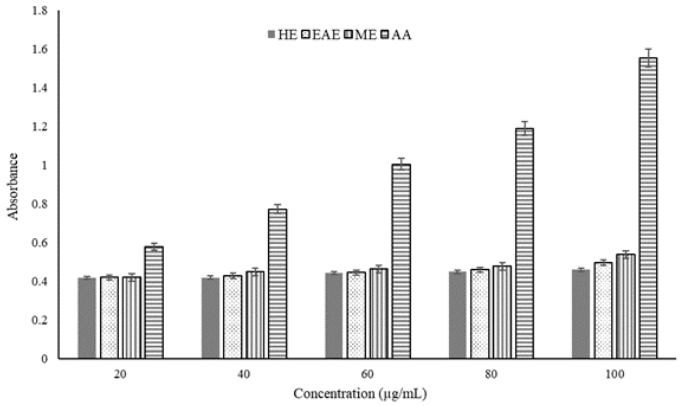

Figure 2: Ferric reducing antioxidant power of *H. rotundifolia* extracts

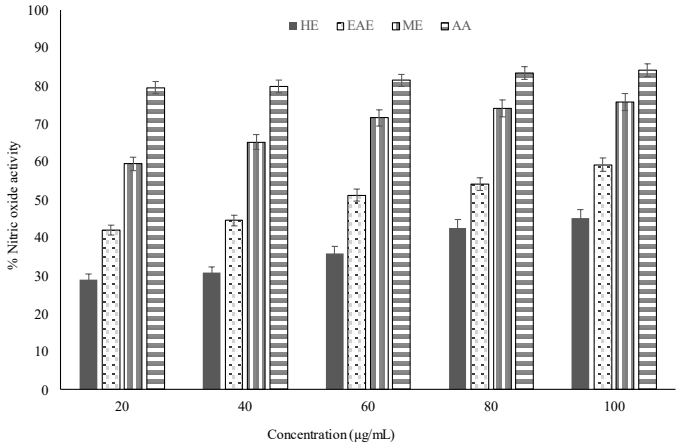

Figure 3: Nitric oxide activity of *H. rotundifolia* extracts

Candida albicans and *Aspergillus niger*. The MIC values for these organisms were significantly higher, ranging from 200 to 250 mg/mL. These higher MIC values indicate that much larger concentrations of the EAE fraction are required to inhibit the growth of *Bacillus subtilis* and the fungal strains, suggesting that the antimicrobial properties of the EAE fraction are less effective against these particular organisms. The ME fraction demonstrated the lowest MIC of 25 mg/mL against *Escherichia coli*, with an MBC of 37.5 mg/mL, indicating strong antibacterial activity.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 5: Antimicrobial activity of *H. rotundifolia* extracts on pathogens

	Zones of Inhibition (mm)																	
Strain/Fractions (mg/mL)	**Sa**		**Bs**		**Se**		**Ec**		**Pa**		**Ea**		**Kp**		**Ca**		**An**	
Hexane (n-H)	n-H	A.I	n-H	A.I	n-H	A.I	n-H	A.I	n-H	A.I	n-H	A.I	n-H	A.I	n-H	A.I	n-H	A.I
250	21	0.66	17	0.77	19	0.51	30	0.83	20	0.49	15	0.45	22	0.85	14	0.47	17	1.21
200	18	0.56	14	0.64	15	0.41	25	0.69	17	0.41	12	0.36	18	0.69	11	0.37	14	1.00
150	14	0.44	12	0.55	11	0.30	21	0.58	14	0.34	9	0.27	14	0.54	8	0.27	11	0.79
100	11	0.34	9	0.41	8	0.22	18	0.50	10	0.24	7	0.21	11	0.42	0	0.00	8	0.57
50	8	0.25	7	0.32	6	0.16	14	0.39	7	0.17	0	0.00	8	0.31	0	0.00	0	0.00
Ethyl acetate (EA)	EA	A.I	EA	A.I	EA	A.I	EA	A.I	EA	A.I	EA	A.I	EA	A.I	EA	A.I	EA	A.I
250	19	0.59	10	0.45	15	0.41	21	0.58	14	0.34	25	0.76	12	0.46	9	0.30	8	0.57
200	16	0.50	7	0.32	13	0.35	18	0.50	10	0.24	22	0.67	9	0.35	7	0.23	6	0.43
150	13	0.41	0	0.00	11	0.30	15	0.42	8	0.20	18	0.55	8	0.31	0	0.00	0	0.00
100	11	0.34	0	0.00	8	0.22	11	0.31	0	0.00	15	0.45	0	0.00	0	0.00	0	0.00
50	8	0.25	0	0.00	7	0.19	8	0.22	0	0.00	11	0.33	0	0.00	0	0.00	0	0.00
Methanol (ME)	ME	A.I	ME	A.I	ME	A.I	ME	A.I	ME	A.I	ME	A.I	ME	A.I	ME	A.I	ME	A.I
250	19	0.59	13	0.59	15	0.41	27	0.75	14	0.34	20	0.61	10	0.38	12	0.40	10	0.71
200	15	0.47	11	0.50	12	0.32	24	0.67	11	0.27	17	0.52	8	0.31	9	0.30	7	0.50
150	11	0.34	9	0.41	9	0.24	18	0.50	8	0.20	14	0.42	0	0.00	7	0.23	0	0.00
100	9	0.28	7	0.32	6	0.16	14	0.39	0	0.00	11	0.33	0	0.00	0	0.00	0	0.00
50	6	0.19	0	0.00	0	0.00	11	0.31	0	0.00	9	0.27	0	0.00	0	0.00	0	0.00
Gentamycin (30 µg)	32		22		37		36		41		33		26					
Nystatin (100 mg/mL)															30		14	

Note: Sa = *Staphylococcus aureus;* Bs = *Bacillus subtilis;* Se = *Staphylococcus epidermidis;* Ec = *Escherichia coli*; Pa = *Pseudomonas aeruginosa;* Ea = *Enterobacter aerogenes;* Kp = *Klebsiella pneumoniae; C*a = *Candida albicans*; An = *Aspergillus niger*. AI = Activity index; n-H = Hexane; EA = Ethyl acetate; ME = Methanol

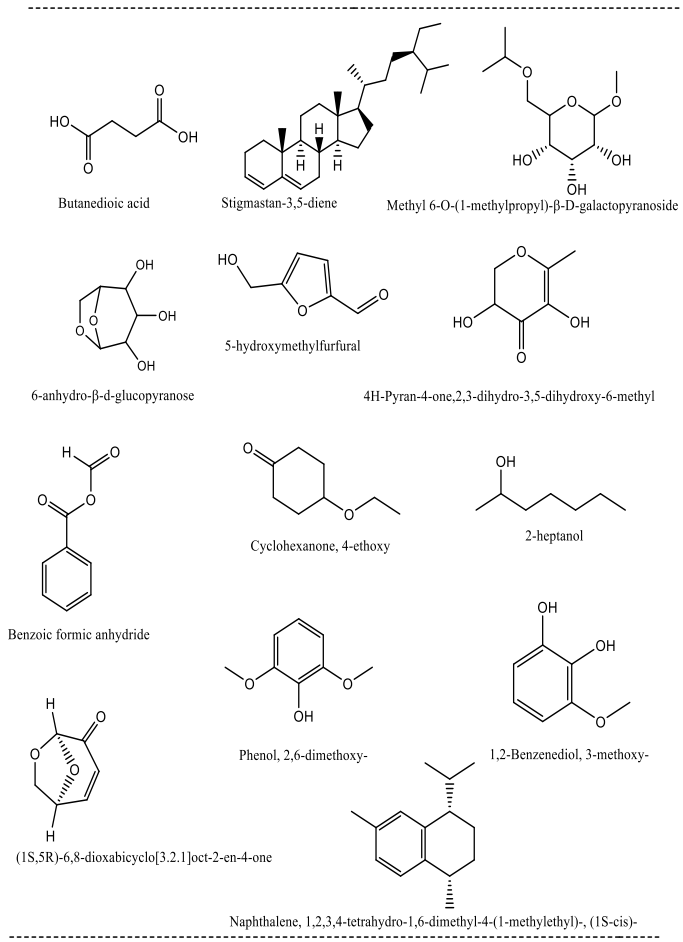

Figure 4: Compounds from GC-MS analysis of *H. rotundifolia* aerial part

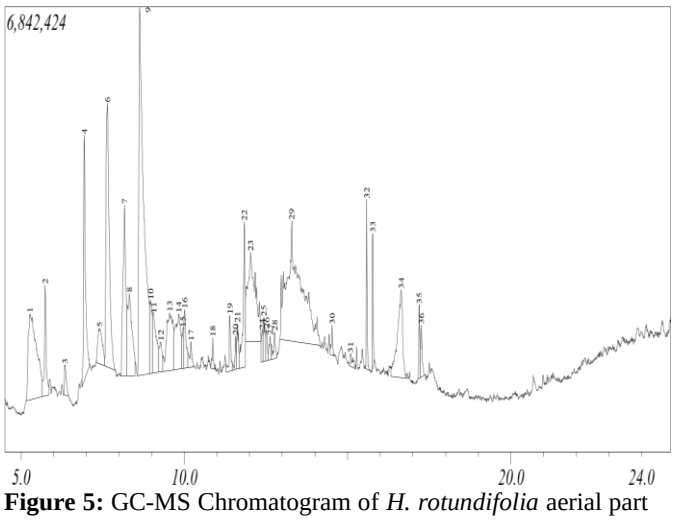

Figure 5: GC-MS Chromatogram of *H. rotundifolia* aerial part ME fraction. Peaks were matched to their respective chromatogram, compared to a database of known spectra at NIST and the results listed in Table 7.

This fraction also showed moderate activity against *Staphylococcus epidermidis* and *Enterobacter aerogenes* with MICs of 100 mg/mL and 50 mg/mL, respectively. However, similar to the ethyl acetate fraction, the methanol fraction was less effective against fungal strains, requiring higher concentrations to inhibit and kill *Candida albicans* and *Aspergillus niger*. The result of the GC-MS analysis of the ME fraction is presented, in Table 7. Peak area (%) values were enclosed in a bracket. The results showed that terpenoids, phenols, glycosides, alcohols, carboxylic acids and ketones were the major classes of compounds. The prominent peaks when matched to their respective compounds in the chromatogram (Figure 5) revealed: 5-Hydroxymethylfurfural (16.87%), Methyl 6-*O*-[1-methylpropyl]-*β*-D-galactopyranoside (16.07%), Butanedioic acid (9.94%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy- (8.28%), 6-methyl-*β*-D-Glucopyranose, 1,6-anhydro- (6.56%), 2-Heptanol (6.82%), stigmastan-3,5-diene (4.32%) amongst other compounds (Figure 4 & 5). Previous studies on the leaf extracts have reported the availability of flavonoids, and many other phytochemicals.[25,26] Vitexin (8-*β*-D-glucopyranosyl apigenin), isovitexin (6-*β*-D-glucopyranosyl apigenin), orientin (8-*β*-D-glycopyranosyl luteolin), isoorientin (6-*β*-D-glycopyranosyl luteolin) and Pheophytin A glycosides were reported from the leaves extracts.[11-13] Phenols, polyphenols and flavonoids are known anti-inflammatory, anti-infective and antioxidant players.[25] Results of proximate estimation reveal that the plant contains an appreciable amount of carbohydrate (48.41%) and crude fibre (13.61%), the ratio of carbohydrate-to-dietary fibre contents (carbohydrate: fibre ratio) was calculated to be, 3.554:1 (Table 2). Dietary fiber in foods improves digestion, supports efficient waste elimination, and can reduce serum cholesterol and the risk of heart disease, including hypertension.[27] A low carbohydrate-to-fibre ratio diet enhances optimal body composition change at a given Body Mass Index (BMI).[28] Protein content was 14.40%, while the lipid content was 0.77%. Lipids are targeted energy sources. It insulates and protects internal tissues and aids important cell functions. Low lipid content food reduces the risk of atherosclerosis, cancer, and aging.[29] The calculated overall energy value in *H. rotundifolia* per 100 g was 261.00 kcal/ 100 grams of the dried sample (Table 2). This value is higher when compared to the reported value for *Myrianthus arboreus* (133.34 kcal/100 g),[30] lower compared to our recent report on *Uvaria chamae* leaves (375.87 kcal/100 g).[31] This value is, however, low compared to the daily energy requirement of 2500-3000 kcal for adults.[22] Globally, overweight and obesity have posed serious concerns leading to many ailments. The inclusion of moderate caloric value, *H. rotundifolia* in meals could reduce overweight and obesity. Mineral composition and their ratios which identify the optimal levels of minerals that closely collaborate in the body were presented in Table 3. Low concentrations of calcium (0.11%) and magnesium (0.04%) were observed in *H. rotundifolia*; however, moderate concentrations of Zn, K and Na were commendable. Sodium and potassium are essential for metabolic energy production,[32] pH equilibrium, tissues,[33] control of muscle contraction and conduction of nerve impulses.[34] Deficiency in potassium and sodium results to functional and structural issues such as those compromised neuromuscular roles of skeletal, paralysis, and mental confusion.[35] Calcium and magnesium serve as building blocks for bones and teeth, regulate nerve and muscle function, and participate in numerous enzymatic reactions.[36] Zinc supports immune health, protein synthesis, and Zn-dependent enzymes play roles in macronutrient metabolic activity and cell replication.[37] In this report the HE, EAE, and ME fractions of *H. rotundifolia* exhibited significant antimicrobial activity against various pathogens, with varying degrees of effectiveness. The findings of the current study showed that the ME fraction of *H. rotundifolia* exhibited broad-spectrum activity, particularly against *Escherichia coli* and *Staphylococcus aureus.* These findings corroborate the report of Abere *et al.* (2010)[17] that reported the inhibition of *E. coli, P. aeruginosa, S. aureus,* and *S. typhi* by alcohol-based extracts of *Dissotis rotundifolia* (Sm.) (synonyms, *Heterotis rotundifolia*).[38]

A study by Singh *et al.* (2021)[39] demonstrated the high effectiveness of ME extracts against Gram+ve and Gram-ve organisms. The activities exhibited by other fractions of *H. rotundifolia* indicate that the extract is a reliable repository of essential bioactive compounds with significant therapeutic potentials.

T**able 6**: Minimum Inhibitory (MIC) and Minimum Bactericidal or Fungicidal Concentration (MBC and MFC) of *H. rotundifolia* fractions

Strains/ Fractions	**n-H**		**EAE**		**ME**	
	(mg/mL)					
	MIC	**MBC/MFC**	**MIC**	**MBC/MFC**	**MIC**	**MBC/MFC**
Bacillus subtilis	50 ± 0.00	75± 0.00	200	225 ± 0.00	100 ± 0.00	125
Staphylococcus epidermidis	50 ± 0.00	75± 0.00	50	75	100 ± 0.00	125
Escherichia coli	25 ± 0.00	50± 0.00	50	75	25 ± 0.00	37.5 ± 0.50
Pseudomonas aeruginosa	50 ± 0.00	75± 0.00	150	175 ± 0.00	150± 0.00	175 ± 0.00
Enterobacter aerogenes	100 ± 0.00	125± 0.00	25	50± 0.00	50	75 ± 0.00
Klebsiella pneumonia	50 ± 0.00	75	150	200 ± 0.00	200± 0.00	225 ± 0.00
Staphylococcus aureus	50 ± 0.00	75	50	75 ± 0.00	50	75 ± 0.00
Candida albicans	150 ± 0.00	200 ± 0.00	200	250 ± 0.00	150 ± 0.00	175 ± 0.00
Aspergillus niger	100 ± 0.00	150 ± 0.00	200	250 ± 0.00	200 ± 0.00	250 ± 0.00

n/d means not determine. AI = Activity index; n-H = Hexane; ME = Methanol; EA = Ethyl acetate

The HE fraction of *H. rotundifolia* showed potent activity, especially against *Escherichia coli* and *Klebsiella pneumoniae*. These studies support the idea that non-polar solvents like hexane are effective in extracting bioactive compounds with significant antimicrobial properties. Research by Ali *et al.* (2020)[40] found that hexane extracts of various plants exhibited strong antimicrobial activity. In another study, Nwabor *et al.* (2020)[41] evaluated the antimicrobial effect of EAE extract of medicinal plants and reported notable activity against *Enterobacter* species. This finding is comparable to the current study, where the EAE fraction of *H. rotundifolia* showed significant activity against *Enterobacter aerogenes*. The appreciable activity showed by the extract against *E. coli* might be considered to rationalize their traditional use for gastrointestinal infections.[42] Their strong activity was observed as a low MIC of 50 mg/mL depicted by HE fractions, though lower MBC of 37.5 mg/mL by ME extract as against 50 mg/mL by HE made the methanolic extract of better potentials. Their activities to many of these test bacteria also rationalize their traditional use to treat multiple conditions including diarrhea, dysentery, conjunctivitis, sexually transmitted infections, cough, and stomach ache symptoms. The aetiological agents of these infections are close to humans,[43] hence the need for sure therapy. These observed activities justify their traditional utilizations to treat gastrointestinal disorders, stomach aches, venereal diseases, diarrhoea, dysentery, rheumatism, pneumonia, conjunctivitis, bilharziasis, and hookworm infestation in the East and Tropical Africa.[10]The extracts of *H. rotundifolia* showed generally weak antifungal activity. Higher doses which may be toxic or prolong the application of the extract may make a difference if such weak antimicrobials are to be considered. The observed antimicrobial activity of the ME fraction of the *H. rotundifolia* aerial part could be mainly attributable to glycosides, flavonoids, and phenolic compounds available in the extract.[13,44-45] 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (PDDM), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (DMF) and Levoglucosenone derivatives have shown antimicrobial activities.[46-48] Glycosides, flavonoids, and phenolic compounds are well-documented for their antimicrobial properties. These compounds can disrupt microbial cell walls, inhibit nucleic acid synthesis, and interfere with microbial metabolism. Glycosides are known to hydrolyse and produce aglycones and sugars, which can penetrate bacterial cell walls and disrupt cellular functions. Flavonoids, with their phenolic structures, can form complexes with extracellular and soluble proteins, as well as bacterial cell walls, thus disrupting microbial activity. Phenolic compounds, owing to their hydroxyl groups, can denature proteins and inactivate enzymes in microbial cells, leading to cell death. For instance, studies have shown that the methanol extract of various plants containing these compounds exhibits strong antibacterial activity. Al-Tamimi et al. (2020)[44] reported the antibacterial activity of the ME fraction of baltic amber against pathogenic bacteria, attributing the activity to the presence of phenolic compounds and flavonoids. Similarly, Syed et al. (2014)[45] demonstrated the antibacterial properties of *Piper sarmentosum* extracts, which are rich in flavonoids and phenolic compounds, against plant pathogenic bacteria. The PDDM is a derivative of pyran, a six-membered oxygen-containing heterocycle. Compounds containing the pyran ring are known for their biological activities, including antimicrobial effects. The 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDM) derivative has been specifically noted for its ability to inhibit the growth of various bacterial strains by disrupting cell wall synthesis and function. 2,4-DMF: This furan derivative has demonstrated antimicrobial properties through mechanisms such as inhibition of bacterial DNA gyrase amongst others. Sung et al. (2007)[44] found that this compound exhibited strong antimicrobial activity against nosocomial pathogens, causing cell cycle arrest and leading to bacterial cell death. Levoglucosenone is a chiral building block derived from biomass and has been utilized in the synthesis of various bioactive compounds. Its derivatives have shown potent antimicrobial activities. For example, Sharipov et al. (2019)[48] synthesized methylsulfanylmethyl ether derivatives of levoglucosenone and evaluated their fungicidal activity, demonstrating significant antimicrobial effects against fungal pathogens. The antimicrobial mechanisms of these compounds involve multiple biochemical and molecular interactions within microbial cells.

Table 7: Major Phytochemicals Identified in H. rotundifolia aerial part of ME fraction

Peak	Phytochemical	RT (min)	MW (g/mol)	Peak Area (%)
1.	2-Heptanol	5.27	116.20	6.82
2.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	5.74	144.12	1.89
4.	Levoglucosenone	6.93	126.11	3.98
5.	Butanedioic acid, monomethyl ester	7.41	132.11	1.57
6.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.64	144.12	8.28
7.	Methanol, oxo-, benzoate	8.17	150.13	4.48
8.	Cyclohexanone, 4-ethoxy-	8.32	144.21	3.60
9.	5-Hydroxymethylfurfural	8.63	126.11	16.87
10.	Benzeneacetic acid	8.97	136.14	1.49
11.	1,2-Benzenediol, 3-methoxy-	9.06	140.13	2.14
13.	Butanedioic acid	9.55	118.08	9.94
14.	Phenol, 2,6-dimethoxy-	9.82	154.16	2.82
22	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	11.83	202.33	2.65
23	*β*-D-Glucopyranose, 1,6-anhydro-	12.03	162.14	6.58
29.	Methyl 6-*O*-[1-methylpropyl]-*β*-D-galactopyranoside	13.29	250.29	16.07
34.	Stigmastan-3,5-diene	16.64	396.70	4.32

RT - retention time; MW – molecular weight

Glycosides can bind to microbial cell walls and membranes, causing leakage of cellular contents and eventual cell death. Flavonoids and phenolic compounds can chelate metal ions, which are essential cofactors for microbial enzymes, thereby inhibiting enzyme activity and microbial growth. The specific compounds identified, such as 4H-Pyran-4-one and 2,4-DMF, often interfere with key bacterial processes. For example, pyran derivatives can inhibit cell wall biosynthesis, leading to weakened cell walls and cell lysis. Furan derivatives may interfere with DNA replication and repair mechanisms, thereby halting bacterial proliferation and causing cell death. Levoglucosenone derivatives, due to their structural uniqueness, can integrate into microbial cell membranes and disrupt membrane integrity. This disruption can lead to increased membrane permeability, loss of vital cellular components, and cell death. Additionally, these derivatives may inhibit specific enzymes critical for fungal cell wall synthesis, providing a dual mechanism of action against microbial pathogens. The demonstrated antimicrobial activity of the ME fraction of the *H. rotundifolia* aerial part highlights the potential of these natural compounds as alternative or complementary treatments to conventional antibiotics. With the rising challenge of antibiotic resistance, exploring such natural products provides a valuable avenue for developing new antimicrobial agents. Future research should focus on isolating and characterizing these bioactive compounds in greater detail. Understanding their precise mechanisms of action at the molecular level will aid in the design and synthesis of more potent derivatives with enhanced antimicrobial efficacy. Additionally, investigating the synergistic effects of these compounds when used in combination with existing antibiotics could provide insights into new treatment strategies for combating resistant bacterial strains. Moreover, *in vivo* studies and clinical trials are necessary to evaluate the safety and efficacy of these compounds in real-world settings. Assessing the pharmacokinetics and pharmacodynamics of these bioactive compounds will be crucial in determining their potential as therapeutic agents.

Conclusion

The findings of this study show that the aerial parts of *H. rotundifolia* exhibit notable antioxidant and antimicrobial properties, attributable to their phytochemical constituents. Additionally, significant amounts of nutrients and mineral elements were detected, indicating that *H. rotundifolia* aerial part contains valuable ingredients which could serve as a good source of nutraceuticals.

Conflict-of-Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors are grateful to the University of Uyo, Uyo for providing the laboratory space, and to Professor (Mrs.) Margaret E. Bassey (Chemotaxonomist) for identifying and authenticating plant samples.

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