PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (8) 2383-2391 August 2024

Phytochemical Analysis, Antioxidant and Antimicrobial Properties of Hexane, Ethyl acetate, and Methanol Leaf Extracts of *Ipomoea hildebrandtii* Vatke Shrub Plant

[1]WALIAULA, JN; [2]MOLIK, ZA; [3]KEPIRO, FT; *[1,2]OGBOLE, OO; [1,2]AJAIYEOBA, EO; [3]KARERU, PG

*[1]*Pan African University of Life and Earth (Including Health and Agriculture) Sciences Institute, University of Ibadan, Ibadan, Nigeria.*
*[2]*Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.*
[3]*Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

Corresponding Author Email: nikeoa@yahoo.com
ORCID: https://orcid.org/0000-0002-6487-9494
Tel: +2348056434577

Co-Authors Email: ndalajohn95@gmail.com; patgkareru@gmail.com; leencoln25@gmail.com; tumeleikepiro@gmail.com; edajaiye@gmail.com

ABSTRACT: *Ipomoea hildebrandtii* Vatke have been effectively used to relieve general body pains, treat wounds and cuts in the semi-arid region of Kenya. Hence the objective of this paper was to evaluate the phytochemical analysis, antioxidant and antimicrobial properties of hexane, ethyl acetate and methanol leaf extracts of *Ipomoea hildebrandtii* Vatke shrub plant. The profiling of the phytochemicals was carried out using the Fourier-transform infrared (FTIR) and Gas Chromatography-Mass spectroscopy (GC-MS) analyses. Antimicrobial and antioxidant activities of the methanol, hexane and ethyl acetate extracts of *I. hildebrandtii* were determined using disk diffusion and 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical scavenging assays respectively. Preliminary phytochemical screening revealed that the leaf extracts are enriched with alkaloids, tannins, terpenoids, steroids, glycosides, flavonoids, phenols, quinones, and saponins. With GC-MS, the major compound tetratetracontane (73.14%) was identified in hexane and 9-octadecanamide, (Z)- in ethyl acetate and methanol extracts (30.99%) and (43.09%) respectively. The IR spectroscopy revealed various functional groups such as C=O, C=C, –OH and –NH. The methanol extract showed good antimicrobial activity at 1000 mg/mL against all the microorganisms tested except *Escherichia coli* while the hexane extract was the best antimicrobial activity against *E. coli* at 1000 mg/mL. The ethyl acetate extract showed the best antioxidant activity against DPPH (IC_{50} = 48.70 ± 1.54 µg/mL) compared to the standard ascorbic acid (IC_{50} = 21.24 ± 0.12 µg/mL). The experimental findings showed that all extracts from *I. hildebrandtii* leaves possess significant antimicrobial and antioxidant activities justifying its use in traditional medicine. These biological activities might be due to the presence of the presence of the identified phytochemicals in them.

DOI: https://dx.doi.org/10.4314/jasem.v28i8.15

Cite this Article as: WALIAULA, J. N; MOLIK, Z. A; KEPIRO, F. T; OGBOLE, O, O; AJAIYEOBA, E. O; KARERU, P. G. (2024). Phytochemical Analysis, Antioxidant and Antimicrobial Properties of Hexane, Ethyl acetate, and Methanol Leaf Extracts of *Ipomoea hildebrandtii* Vatke Shrub Plant. *J. Appl. Sci. Environ. Manage.* 28 (8) 2383-2391

Dates: Received: 04 June 2024; Revised: 27 June 2024; Accepted: 11 July 2024 Published: 05 August 2024

Keywords: *Ipomoea hildebrandtii*; Phytochemicals; Antioxidant; Antimicrobial

According to the World Health Organisation, antibiotic resistance is a severe public health issue, particularly in underdeveloped nations where infectious illnesses are still a significant cause of death (da Silva *et al.*, 2016). The development of resistance of pathogens to the existing drugs has led to research in folk medicine to seek new approaches to develop drugs against microbial infections and to examine

*Corresponding Author Email: nikeoa@yahoo.com
*ORCID: https://orcid.org/0000-0002-6487-9494
*Tel: +2348056434577

various medicinal plants for their potential antimicrobial activity (Khatiwora *et al.*, 2012). Antimicrobial agents from plant sources have long been employed to develop essential novel therapies. The phytochemistry of medicinal plants has revealed numerous pure bioactive compounds that have so far been essential tools in the practice of modern medicine (Silva *et al.*, 2012). Plants of the genus *Ipomoea* contain bioactive compounds such as glycosides, having antimicrobial, antitumor, and antifungal properties. The therapeutic esteem of this class ofc compound is remarkable and it is utilized for the treatment of different illnesses such as constipation, loose bowels, diabetes, rheumatism and inflammation. It has also been demonstrated that *Ipomoea* glycosides have antimicrobial, antitumor and antifungal properties (Lautie *et al.*, 2020). As reported, several *Ipomoea* species, including *Ipomoea batatas* and *Ipomoea indica*, all have antimicrobial properties whose activity has been exploited (Meira *et al.*, 2012) except *Ipomoea hildebrandtii*, whose antimicrobial potential is unexplored.

Ipomoea hildebrandtii Vatke, commonly known as morning glory, is an evergreen sub–woody shrub (1–4 m tall) with hairy stems and white or purple flowers of the Convolvulaceae family (Witt and Luke, 2017). This plant species is an annual herb growing widely in Southern parts of Kenya (Mganga *et al.*, 2010). It has customarily been used medicinally in different communities in East Africa for the treatment of a variety of illnesses. In Kenya, a decoction of fresh leaves is drunk to relieve general pains in the body while the latex from the stem is used for wounds and cuts (Lemmens, 2013). To our knowledge, there is insufficient information on the antibacterial, antifungal and antioxidant activity of *Ipomoea hildebrandtii*. Hence, the objective of this paper was to evaluate the phytochemical analysis, antioxidant and antimicrobial properties of hexane, ethyl acetate and methanol leaf extracts of *Ipomoea hildebrandtii* Vatke shrub plant.

MATERIALS AND METHODS

Collection of plant material: The leaves of *Ipomoea hildebrandtii* were collected in March 2021 around the Ilbissil region of Kajiado County, Southern Kenya. Authentification of the plant was carried out by Mr. John Kamau Muchuku, a plant taxonomist from the Botany Department of Jomo Kenyatta University of Agriculture and Technology (JKUAT). The voucher specimen WJN–JKUATBH/001/A-2021 was deposited at the JKUAT Botany Herbarium.

Solvent extraction: The air-dried and powdered leaves of *I. hildebrandtii* (700 g) was succesively extracted using hexane, ethyl acetate and methanol at room temperature for 72 hours to obtain corresponding solvent extracts after evaporation under vacuum (Githua, 2013). Resulting extracts were stored in a refrigerator until when needed.

Phytochemical Analysis: Phytochemical study was performed to identify phytochemical components in the leaf extracts of *I. hildebrandtii* following the methods described for qualitative phytochemical screening (Roghini and Vijayalakshmi, 2018).

Chemical characterization of the extracts:
IR spectral analysis: About 2 mg of the methanol, hexane and ethyl acetate fractions was mixed with spectroscopic grade Potassium Bromide (KBr) salt and compressed into thin pellets. The functional groups in the leaf pellets of the extracts were detected using One Perkin-Elmer spectrometer between 400 to 4000 cm^{-1} at a resolution set at $4cm^{-1}$. Infrared absorptions obtained were recorded.

Gas chromatography/mass spectrometry (GC/MS) analysis: 500 mg of powdered *I. hildebrandtii* leaves was weighed in 50 mL Erlenmeyer flask sample and extracted with 10 mL each of hexane, ethyl acetate and methanol. The mixtures were allowed to stand for 24 hours with constant agitation and filtered with (Sartorius NY 0.45 µm, 47 mm) membrane filters prior to GC-MS analysis (Otieno, 2016). The Agilent 5975 GC-MS system was used to get the entire chemical profile. A BPX5 non-polar fused silica capillary column (30 m × 0.25 mm) was used under the following condition: temperature range 60 °C (2 mins) to 250 °C at 3 °C per min and the final temperature was retained for 20 mins, split injection mode; mass range of mass to charge ratio (m/z) 30 - 450 and helium as a carrier gas flow rate of 1.00 mL/min; temperature of ion source 200 °C and interface temperature 300 °C. The data were obtained using NIST-11, the National Institute of Standards and Technology's mass spectrum library. The unknown spectrum was compared to the WILEY 8 library database's standard spectrum to identify compounds present (Rhetso *et al*., 2020).

Antioxidant assay: Antioxidant activity was determined using a modified version of the DPPH radical-scavenging (Oyedemi *et al*., 2010). Two milliliters of different dilutions of the extracts (7.8125 – 500) µg/mL were mixed with 1 mL of freshly prepared DPPH solution (1 mM). The absorbance was measured at 517 nm after fifteen minutes of incubation in the dark. The ability to scavenge the DPPH free radical was determined with respect to reference

standard which contained all reagents without the test sample using Equation (1)

$$\% \text{ RSA} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100\% \quad (1)$$

Where RSA = percentage radical scavenging activity; Abs control is the absorbance value of blank solution and Abs sample is the sample's absorbance value.

To measure the quantity of plant extract necessary to scavenge DPPH by 50%, the percentage of DPPH radical scavenging was plotted against the plant extract concentration (µg/mL) (IC_{50}). Ascorbic acid was used as reference compound.

Antimicrobial Assay: Gram-negative bacteria (*Pseudomonus aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25992, *and Salmonella typhi* ATCC 14028), gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, and *Streptococcus mutans* ATCC 25175), and fungi (*Candida albicans* ATC 10231) were used as test microorganisms in this study.

Preparation of plant extracts for antimicrobial activity: An amount of 1 g of each extract were weighed into sterile universal flasks. To each flask, 1 mL of 99.9% pure dimethylsulfoxide (DMSO) (Sigma-Aldrich) was added to obtain a 1: 1 concentration and the mixture was agitated with a vortex mixer. All extracts were subjected to the same procedure (Otieno, 2016).

Preparation of the culture media: The susceptibility tests were carried out on Mueller-Hinton Agar (MHA) by dissolving 38 g of agar in 1 litre of distilled water. The medium was sterilized for 15 mins at 121 °C in an autoclave and was cooled to 40 °C. The agar was aseptically poured onto pre-labelled sterile petri plates (90 mm) on a levelled horizontal plane to a consistent depth of 4 mm, equating to about 25 mL of medium per dish. The dishes were covered and were left to stand at room temperature and stored at 4 °C (Otieno, 2016).

Preparation of inoculum: The colony suspension technique was used to make the test microorganism inoculum (EUCAST, 2003). The different bacteria strains and isolates were grown on nutrient agar overnight at 37 °C. The same bacteria colonies from the cultures were then suspended in sterile saline solution. Following the McFarland turbidity of 0.1 at 600 nm, the suspension was further adjusted to reach 5×10^5 colony forming units per/mL (Olajuyigbe, 2012).

Disc-diffusion technique: With some modifications, the bioassay was carried out using the Disc-diffusion technique (Khatiwora *et al*., 2012). Adequate amount of nutrient agar was prepared and dispensed into sterile plates and allowed to compact after cooling under aseptic conditions. Each extract was serially diluted to 1000, 100, 10, 1 and 0.1 mg/mL concentrations. A volume of 0.1 mL of each test organisms were inoculated with sterile spreader on the surface of solid medium in plates. The inoculated agar plate was incubated with the test organism for 1 hr before placing the extract impregnated paper discs on the plates. Negative control (DMSO) and positive control (Norfloxacin, Ofloxacin, Ceftriaxone, Sulphamethoxazole, Amoxylclav, Nitrofurantoin, Nalidixic acid, Gentamicin) discs were arranged on each plate. The plates containing bacterial isolates were incubated at 37°C for 24 hrs while those with fungal isolates at 25° C for 48 hrs. After incubation, all the plates were observed for zones of inhibition (ZOI) and the diameters of the ZOI were measured using a Vernier calliper and results recorded.

Statistical analysis: All the experiments were repeated three times. Origin Pro 21 was used to analyse a one-way analysis of variance (ANOVA) of the data. The results were expressed as mean ± SD and determined to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

The phytochemical screening of the methanol, hexane and ethyl acetate extracts of *I. hildebrandtii* revealed the presence of alkaloids, tannins, terpenoids, steroids, glycosides, flavonoids, phenols, quinones and saponins (Table 1). These phytochemicals have been reported to possess varying biological properties including antimicrobial, antidiabetic, antioxidative, anti-inflammatory, anti-tumoral, antibacterial properties. These reports correlate with the existing studies of other *Ipomoea* spp. (Aliyu *et al.*, 2010; Bhaigybati *et al.*, 2020).

Table 1: Preliminary phytochemical tests on the leaf extracts of *I. hildebrandtii*

Test	*Ipomoea hildebrandtii* Extracts		
	Hexane	Ethyl acetate	Methanol
Alkaloids	+	+	+
Tannins	-	+	+
Terpenoids	+	-	+
Steroids	+	-	+
Glycosides	+	-	-
Flavonoids	+	+	+
Phenols	-	+	+
Quinones	-	+	+
Saponins	-	-	+

+: Present, -: Not present

The IR spectra reveals the presence of different functional groups of the compounds present in *I. hildebrandtii* (Table 2; Figure 1, 2 and 3). The ethyl acetate and methanol extracts have a characteristic broad absorption band at around 3383 and 3370 cm^{-1} respectively which represents O-H of acids, alcohols and phenols. The hexane extract however showed a weak band at 3451 cm^{-1} which represents N-H stretch of an amine (Mandal and Bhattacharya, 2015). All the extracts show absorption around 2930 cm^{-1} assigned to the asymmetric C-H stretching indicates the presence of some alkane compounds. The band around 2860 cm^{-1} for ethyl acetate and methanol extracts is due to the symmetric C-H stretching of the alkane compounds present (Bellamy, 2013). The band at 1722 cm^{-1} for all samples represent C=O stretch of aldehydes, ketones, esters, amides & carboxylic acids (Coates, 2000). The medium peak observed at 1447 cm^{-1} indicates the presence of C-H bend alkanes and a relatively stronger peak around 1050 cm^{-1} and 1030 cm^{-1} signifies C-O of ethers, esters & glycosidic linkage (Maréchal and Chanzy, 2000). All the peak assignments are summarised in Table 2.

Table 2: FTIR Interpretation of compounds in leaf extracts of *I. hildebrandtii*

FTIR Peak positions (cm^{-1})				
Hexane	**Ethyl acetate**	**Methanol**	**Tentative assignment**	**Possible functional group**
3840			Si-O-H stretch	Kaolinite and Fe oxides
3742			R-NH-R stretch	Amines, Amides
3451			N-H stretch	Amines
	3383	3370	O–H stretch	Alcohols, aldehyde & Carboxylic acids
2930	2922	2926	C–H stretch asymmetric	Alkanes
	2862	2866	C–H stretch symmetric	Alkanes
2787			C–H stretch	Aldehydes
1736	1719	1722	C=O stretch	Aldehydes, Ketones, Esters, Amides & Carboxylic acid
		1640	C=C stretch	Alkenes and Aromatic compounds
1447	1447	1439	C–H bend	Alkanes
1366			C–H bend symmetrical	Alkane
		1389	O–H bend	Phenol or tertiary alcohol
1215			C–N stretch	Amine, Amide
	1252		C–O stretch	Alkyl ether
	1173		C–O stretch	Acyl group
1034	1030	1051	C–O–C	Ethers, Esters & Glycosidic linkage
714			C–H bend	Aliphatic halo compounds
525			C-I stretch	Halo compounds

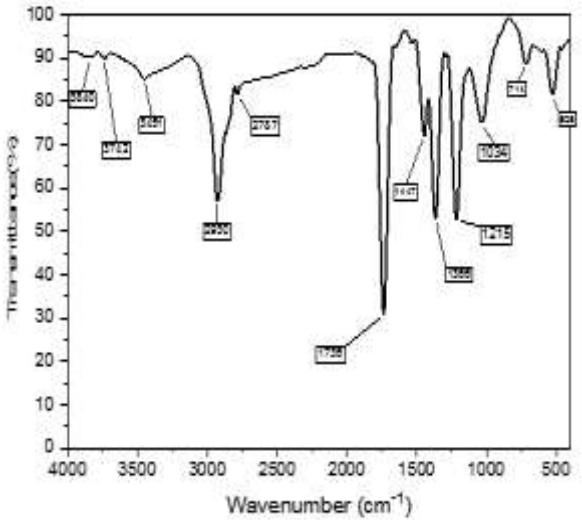

Fig 1: FTIR spectrum of hexane extract of *I. hildebrandtii*

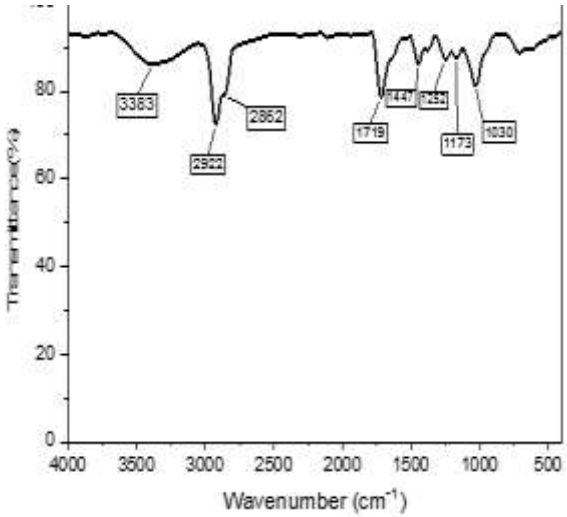

Fig 2: FTIR spectrum of ethyl acetate extract of *I. hildebrandtii* leaf

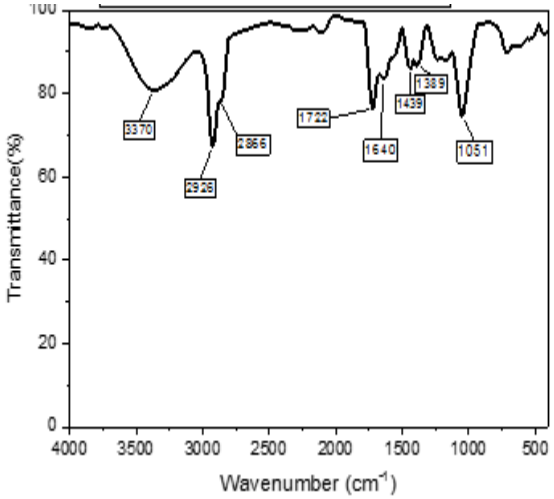

Fig 3: FTIR spectrum of methanol extract of *I. hildebrandtii* leaf

The GCMS results indicated that *I. hildebrandtii* hexane, ethyl acetate, and methanol leaf extracts contained several bioactive constituents (Tables 3, 4, and 5).

Table 3: Compounds identified in hexane extract of *I. hildebrandtii* by GC-MS

Peak number	RT (min)	Area %	Compound
	5.167	5.28	Pentanoic acid 2-propenyl ester (allyl valerate)
	5.604	0.51	4,4-dimethyl-(E)-2-Pentene
	17.838	0.30	Hexadecanoic acid, methyl ester (Methyl palmitate)
	19.637	2.27	Phytol
	22.462	1.29	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-Phenol
	22.834	0.55	2,6,10,15-tetramethylheptadecane
	25.301	2.46	(E)-1-(4-Hydroxy-3-methoxyphenyl)tetradec-3-en-5-one
	25.886	4.23	Squalene
	26.872	73.14	Tetratetracontane
	28.418	1.37	1-iodo-Dotriacontane
	31.712	0.95	Vitamin E

The most prominent compound in hexane extract was Tetratetracontane (73.14%) which is a long chain alkane reported to possess antibacterial, cytoprotective and antioxidative abilities (Parthasarathy *et al.*, 2018; Rhetso *et al.*, 2020). The compound; 9-octadecanamide, (Z)- was the most prominent in ethyl acetate (30.99%) and methanol extracts (43.09%). It is an amide derived from the fatty acid Oleic acid with strong antioxidant and antimicrobial properties (Kim *et al.*, 2020). The next most abundant compound was phytol (3.52%) and (12.64%) in ethyl acetate and methanol respectively. Phytol is an acyclic diterpene compound which is found abundantly in nature. It is said to exhibit antibacterial properties (Ghaneian *et al.*, 2015) anti-inflammatory (Silva *et al.*, 2014) antioxidative, antiarthritic, anti-inflammatory (Carvalho *et al.*, 2020) and immunostimulatory properties (Senguttuvan *et al.*, 2014). The potential antimicrobial and antioxidant properties of the extracts could be linked to some of the compounds identified.

Table 4: Compounds identified in ethyl acetate extract of *I. hildebrandtii* by GC-MS

Peak number	RT (min)	Area %	Compound
	6.771	0.85	1,2,3-Propanetriol, 1-acetate
	7.956	0.06	1-Dodecene
	8.919	1.55	1,2,3-Propanetriol, 1-acetate
	10.894	0.22	3-Hexadecene, (Z)-
	11.021	0.35	(S)-(-)-1,2,4-Butanetriol, 2-acetate
	12.393	0.35	1,2-Propanediol, diacetate
	12.687	0.56	2,4-Di-tert-butylphenol
	13.556	0.81	1-Octadecene
	15.193	0.08	Z-2-Octadecen-1-ol
	16.423	1.15	Neophytadiene
	16.554	0.10	2-Pentadecanone, 6,10,14-trimethyl-
	16.912	0.44	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
	17.455	0.28	Hexadecanoic acid, methyl ester
	17.526	0.02	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene
	18.099	0.14	Heneicosanol
	18.141	0.75	Hexadecanoic acid, ethyl ester
	19.140	0.08	1-Hexadecanol, 2-methyl-
	19.223	0.09	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
	19.358	3.52	Phytol
	19.845	0.33	trans,trans-9,12-Octadecadienoic acid, propyl ester
	19.927	0.35	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid
	20.057	0.06	n-Tetracosanol-1
	20.096	0.20	Nonadecanoic acid, ethyl ester
	20.211	1.86	Hexadecanamide
	20.265	0.35	Phytol, acetate
	20.862	0.07	9-Octadecen-1-ol, (Z)-)
	21.184	0.34	Agroclavine
	21.740	0.04	Dichloroacetic acid, tridec-2-ynyl ester
	21.974	30.99	9-Octadecenamide, (Z)-
	22.185	0.81	Octadecanamide
	23.007	0.36	Eicosane
	28.011	0.78	Squalene

Figure 4 shows the free radical scavenging ability of the three extracts of *I. hildebrandtii* and standard ascorbic acid. At a concentration of 500 µg/mL, the scavenging activity of methanol, ethyl acetate, and hexane extracts was 84.90 ± 0.17, 84.80 ± 0.17 and 78.18 ± 1.04% respectively whereas at the same concentration, ascorbic acid was 96.71 ± 0.06% (Figure 4). The IC_{50} of standard ascorbic acid was 21.24 ± 0.12 µg/mL. The free radical scavenging activity of the extracts and the ascorbic was in the following order Ascorbic acid > ethyl acetate >methanol > hexane extracts. Several illnesses have been related to excessive quantities of reactive oxygen

species in the cells of damaged organs (Zlatić *et al.*, 2019) and hence consuming antioxidant-rich plant products have been known to minimize the risk associated with these diseases (Lee *et al.*, 2004). The high scavenging activity of methanol and ethyl acetate extracts could be attributed to the high antioxidant potency of 9-Octadecenamide, (Z) a major compound in both extracts (Kim *et al.*, 2020). These findings support the existence of significant levels of phenolic compounds in Kenyan *Ipomoea* extracts, showing that this plant is a rich source of antioxidants that may offer consumers with health benefits.

Table 5: Compounds identified in methanol extract of *I. hildebrandtii* by GC-MS

Plant extract	RT (min)	Area %	Compound
	6.783	1.79	1,2,3-Propanetriol, 1-acetate
	9.568	3.37	1,2,4-Butanetriol
	11.038	2.31	(S)-(-)-1,2,4-Butanetriol, 2-acetate
	12.126	4.01	Benzaldehyde, 2-hydroxy-4-methyl-
	12.928	0.70	D-Allose
	13.190	0.74	D-Arabinitol
	14.399	0.76	Megastigmatrienone
	14.585	3.90	Hexanoic acid, 2-ethylhexyl ester
	16.413	0.61	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol
	17.456	3.10	Hexadecanoic acid, methyl ester
	18.142	2.33	Hexadecanoic acid, ethyl ester
	19.305	1.29	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)
	19.361	12.64	Phytol
	19.483	0.65	Methyl stearate
	20.219	3.04	Hexadecanamide
	20.267	1.84	Phytol, acetate
	21.179	6.99	N,N,1,3-Tetramethylbenzo[g]indol-9-amine
	21.977	43.09	9-Octadecenamide, (Z)-
	22.187	0.30	Octadecanamide
	22.552	3.98	2,5-Dimethyl-4-(4-acetylaminophenyl)pyridine
	23.576	1.66	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

I. hildebrandtii leaves extracts demonstrated considerable antimicrobial activity against test organisms as shown in Table 5. The methanolic extract was most active on *S. aureus, S. typhi, C. albicans, S. mutans, P. aeruginosa and B. subtilis* by showing ZOI of 15.00 ± 1.00, 18.33 ± 0.58, 11.33 ± 1.53, 12.00 ± 1.00, 13.00 ± 1.00 and 12.33 ± 0.58 mm respectively at 1000 mg/mL while at same concentration the hexane extract had the best antimicrobial activity against *E. coli* with a ZOI of 28.33 ± 0.58 mm. A previous study reported that methanol leaf extracts of *I. tuba* displayed higher degree of inhibition against pathogenic strains than the acetone and hexane extracts (Eswaraiah *et al.*, 2020). In this study, hexane and methanol extracts of *I. hildebrandtii* leaves showed higher inhibitory potency on gram-negative *E. coli* and *S. typhi*. Gram-negative bacteria in general are more resistant to most antibiotics (Koulenti *et al.*, 2019). *E. coli* is a bacterium commonly found in the gut and causes gastrointestinal and urinary tract infections while *S. typhi* infect the intestinal tract and in the bloodstream.

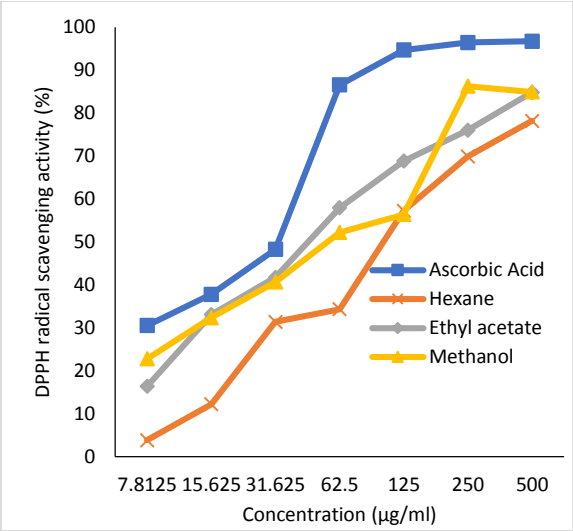

Fig 4: Percentage inhibition of the extracts of *I. hildebrandtii*

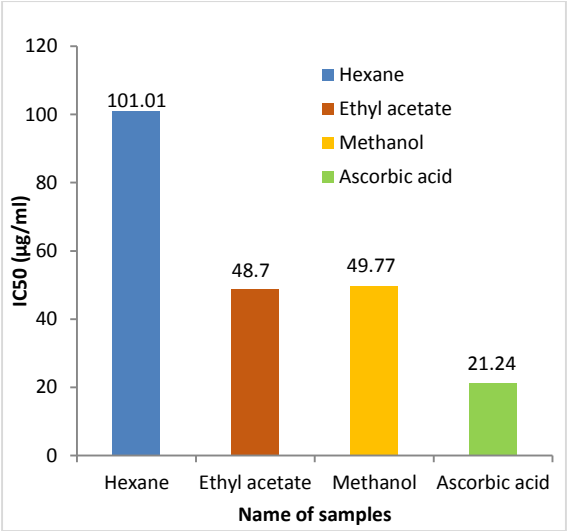

Fig 5: DPPH radical scavenging effect of leaf extracts of *I. hildebrandtii*

The susceptibility of *E. coli* and *S. typhi* to the extracts indicates the potential biological activity of the tested plant extracts against such infections. *S. aureus* has been implicated in infections related to the skin and soft tissue, thus, the active potential of the extracts on the gram-positive bacteria justifies the folkloric usage of the plant in the treatment of wounds, cuts and sores. The inhibitory effect observed on *P. aeruginosa*, an aerobic gram-negative bacterium was minimal by all the extracts; this organism is known to show resistance to many plant extracts (Aliyu *et al.*, 2010).

Differences in antimicrobial activity (Table 5) across extracts can be explained in part by qualitative and quantitative changes in secondary metabolites contained in the extracts, as detected by phytochemical and GC-MS analyses (Aliyu *et al.*, 2010). The extracts from the plant when compared to those of synthetic drugs used as positive control showed that generally all the extracts had significant antimicrobial activity against all microorganisms but some drugs did not have any effect against some of the microorganisms used. Amoxylclav only had antimicrobial activity towards *E. coli* and *S aureus*. This is also among other reasons why medicinal plants are preferred over commercial drugs because of their antimicrobial action against a wide range of pathogens.

Table 6: Antimicrobial activity (zone of inhibition in mm) of leaf extracts of *I. hildebrandtii*

Conc.	Inhibition zone (mm)						
(mg/ml)	*E. coli*	*S. aureus*	*S. typhi*	*C. albicans*	*S. mutans*	*P. aeruginosa*	*B. subtilis*
Hexane extract							
1000	28.33±0.58	8.33±0.58	9.00±1.00	9.33±1.53	8.00±1.00	8.33±0.58	8.67±0.58
100	20.00±1.00	8.00±1.00	7.67±0.58	8.00±1.00	7.67±0.58	0.00±0.00	7.67±1.15
10	18.00±1.00	7.67±0.58	7.67±0.58	7.67±0.58	7.67±0.58	0.00±0.00	7.33±0.58
1	15.00±1.00	7.33±0.58	7.67±0.58	7.67±0.58	7.33±0.58	0.00±0.00	7.33±0.58
0.1	11.00±1.00	7.00±0.00	7.33±0.58	7.33±0.58	7.00±0.00	0.00±0.00	0.00±0.00
Ethyl acetate extract							
1000	8.33±0.58	8.67±0.58	9.33±0.58	9.00±1.00	7.67±0.58	7.67±0.58	10.00±1.00
100	7.67±0.58	0.00±0.00	9.00±1.00	7.33±0.58	7.67±1.15	7.67±0.58	7.33±0.58
10	7.67±0.58	0.00±0.00	8.33±0.58	7.33±0.58	7.33±0.58	7.33±0.58	0.00±0.00
1	7.33±0.58	0.00±0.00	8.33±0.58	7.33±0.58	7.33±0.58	7.00±0.00	0.00±0.00
0.1	7.33±0.58	0.00±0.00	8.33±0.58	7.00±0.00	7.00±0.00	7.00±0.00	0.00±0.00
Methanol extract							
1000	17.67±4.62	15.00±1.00	18.33±0.58	11.33±1.53	12.00±1.00	13.00±1.00	12.33±0.58
100	10.00±1.00	11.00±1.00	10.00±1.00	7.33±0.58	9.33±0.58	11.67±1.53	11.00±1.00
10	8.67±1.15	8.33±0.58	7.67±1.15	7.00±0.00	7.67±1.15	7.00±0.00	8.33±0.58
1	8.00±1.00	7.67±1.15	7.33±0.58	0.00±0.00	7.00±0.00	0.00±0.00	7.33±0.58
0.1	7.00±0.00	7.33±0.58	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.00±0.00
Positive Controls (Antibiotics)							
Norfloxacin	12.00±0.00	13.33±0.58	14.33±0.58	19.67±0.58	0.00±0.00	19.00±0.00	0.00±0.00
OF	18.00±1.00	15.57±0.58	23.00±0.00	15.33±0.58	28.33±0.58	8.00±0.00	0.00±0.00
CTR	23.00±1.00	14.67±0.58	26.33±0.58	28.33±0.58	16.00±0.00	24.00±1.00	15.33±0.58
SX	10.33±0.58	11.67±0.58	15.67±0.58	0.00±0.00	17.33±0.58	10.33±0.58	12.33±0.58
AMC	12.33±0.58	0.00±0.00	14.00±0.00	0.00±0.00	11.00±0.00	13.33±0.58	0.00±0.00
NIT	10.67±0.58	10.00±0.00	0.00±0.00	13.33±0.58	0.00±0.00	11.67±0.58	0.00±0.00
NA	17.00±0.00	23.00±1.00	12.67±0.58	14.67±0.58	14.33±0.58	18.00±1.00	16.00±0.00
GEN	19.67±0.58	0.00±0.00	15.33±0.58	0.00±0.00	18.67±0.58	20.00±0.00	0.00±0.00
Negative control							
DMSO	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00

Conclusion: The methanol, hexane and ethyl acetate extracts of *I. hildebrandtii* contains several bioactive constituents that may be responsible for their biological activities. This study demonstrates that the leaves of *I. hildebrandtii* has outstanding antimicrobial and antioxidant characteristics, suggesting that it might be further explored for new therapeutic molecules.

Acknowledgement: The authors extend their appreciation to Pan African University Institute of Life and Earth Sciences (including Health and Agriculture) (PAULESI) and African Union for the financial support and scholarship awarded to WJN

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the first author or corresponding author.

REFERENCES

Aliyu, MS; Hanwa, UA; Tijjani, MB; Aliyu, AB; Ya'u, B (2010). Phytochemical and Antibacterial Properties of Leaf Extract of *Stereospermum kunthianum* (Bignoniaceae). *Nig. J. Basic. Appl. Sci.* 17(2). DOI: https://doi.org/10.4314/njbas.v17i2.49912

Bellamy, LJ (2013). *The infra-red spectra of complex molecules*. Springer Science & Business Media.

Bhaigybati, T; Gurumayum, J; Ranjit Singh, L; Grihanjali Devi, P; Sanasam, S; Bag, G (2020). Phytochemical profiling, antioxidant activity, antimicrobial activity and GC-MS analysis of *Ipomoea aquatica* Forsk collected from EMA market, Manipur. *Journal of Pharmacognosy Phytochemistry* 9(1): 2335-2342.

Carvalho, AMS; Heimfarth, L; Pereira, EWM; Oliveira, FS; Menezes, IRA; Coutinho, HDM; Picot, L; Antoniolli, AR; Quintans, JSS; Quintans-Junior, LJ (2020). Phytol, a Chlorophyll Component, Produces Antihyperalgesic, Anti-inflammatory, and Antiarthritic Effects: Possible NFkappaB Pathway Involvement and Reduced Levels of the Proinflammatory Cytokines TNF-alpha and IL-6. *J. Nat. Prod.* 83(4): 1107-1117. DOI: https://doi.org/10.1021/acs.jnatprod.9b01116

Coates, J (2000). Interpretation of infrared spectra, a practical approach. *Encyclopedia of analytical chemistry* 12: 10815-10837.

da Silva, AP; Nascimento da Silva, LC; Martins da Fonseca, CS; de Araujo, JM; Correia, MT; Cavalcanti Mda, S; Lima, VL (2016). Antimicrobial Activity and Phytochemical Analysis of Organic Extracts from Cleome spinosa Jaqc. *Front. Microbiol.* 7: 963. DOI: https://doi.org/10.3389/fmicb.2016.00963

Eswaraiah, G; Peele, KA; Krupanidhi, S; Kumar, RB; Venkateswarulu, TC (2020). Studies on phytochemical, antioxidant, antimicrobial analysis and separation of bioactive leads of leaf extract from the selected mangroves. *Journal of King Saud University - Science* 32(1): 842-847. DOI: https://doi.org/10.1016/j.jksus.2019.03.002

EUCAST (2003). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clin. Microbiol. Infect.* 9(8): ix-xv.

Ghaneian, MT; Ehrampoush, MH; Jebali, A; Hekmatimoghaddam, S; Mahmoudi, M (2015). Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. *Environmental Health Engineering Management Journal* 2(1): 13-16.

Githua, RN (2013). *Phytochemical and biological studies of the compounds from the root bark of Vernonia auriculifera; Hiern (Asteraceae)*. MSc. Jomo Kenyatta University of Agriculture and Technology, Nairobi Kenya.

Khatiwora, E; Adsul, VB; Kulkarni, M; Deshpande, N; Kashalkar, R (2012). Antibacterial activity of Dibutyl Phthalate: A secondary metabolite isolated from *Ipomoea carnea* stem. *J. Pharm. Res.* 5(1): 150-152.

Kim, BR; Kim, HM; Jin, CH; Kang, SY; Kim, JB; Jeon, YG; Park, KY; Lee, IS; Han, AR (2020). Composition and Antioxidant Activities of Volatile Organic Compounds in Radiation-Bred Coreopsis Cultivars. *Plants (Basel)* 9(6): 717. DOI: https://doi.org/10.3390/plants9060717

Koulenti, D; Song, A; Ellingboe, A; Abdul-Aziz, MH; Harris, P; Gavey, E; Lipman, J (2019). Infections by multidrug-resistant Gram-negative Bacteria: What's new in our arsenal and what's in the pipeline? *Int. J. Antimicrob. Agents* 53(3): 211-224. DOI: https://doi.org/10.1016/j.ijantimicag.2018.10.011

Lautie, E; Russo, O; Ducrot, P; Boutin, JA (2020). Unraveling Plant Natural Chemical Diversity for Drug Discovery Purposes. *Front. Pharmacol.* 11: 397. DOI: https://doi.org/10.3389/fphar.2020.00397

Lee, J; Koo, N; Min, DB (2004). Reactive Oxygen Species, Aging, and Antioxidative Nutraceuticals. *Compr Rev Food Sci Food Saf* 3(1): 21-33. DOI: https://doi.org/10.1111/j.1541-4337.2004.tb00058.x

Lemmens, RHMJ (2013). '*Ipomoea pes-caprae* (L.) R.Br. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(2): Medicinal plants/Plantes médicinales 2. PROTA'. Accessed 14 March 2022. Netherlands: Wageningen.

Mandal, S; Bhattacharya, S (2015). UV-VIS and FTIR spectroscopic analysis of phytochemicals and functional group in *Ocimum sanctum* and a few medicinal plants. *Romanian J. Biophysics* 25(4): 247-257.

Maréchal, Y; Chanzy, H (2000). The hydrogen bond network in Iβ cellulose as observed by infrared spectrometry. *J. Mol. Struct.* 523(1-3): 183-196. DOI: https://doi.org/10.1016/S0022-2860(99)00389-0

Meira, M; Silva, EPd; David, JM; David, JP (2012). Review of the genus Ipomoea: traditional uses, chemistry and biological activities. *Revista Brasileira de Farmacognosia* 22(3): 682-713. DOI: https://doi.org/10.1590/S0102-695X2012005000025

Mganga, KZ; Musimba, NK; Nyariki, DM; Nyangito, MM; Mwang'ombe, AW; Ekaya, WN; Muiru, WM (2010). The challenges posed by ipomoea Kituensis and the grass-weed interaction in a

reseeded semi-arid environment in Kenya. *International Journal of Current Research* 11: 001-005.

Olajuyigbe, OO (2012). In vitro pharmacological activity of the crude acetone extract of Erythrina caffra Thunb: antibacterial and antifungal assessment. *Journal of Medicinal Plants Research* 6(9): 1713-1720.

Otieno, AJ (2016). *Antimicrobial activity and phytochemical profiles of Warburgia ugandensis Sprague (canellaceae) extracts from different populations across the Kenyan Rift Valley*. M.Sc. thesis, Kenyatta University, Kenya.

Oyedemi, SO; Bradley, G; Afolayan, A (2010). In-vitro and-vivo antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. *Afr J Pharm Pharmacol* 4(2): 70-78.

Parthasarathy, A; Jayalakshmi, M; Pushpabharathi, N; Varadharaj, V (2018). Identification of Bioactive Components in *Enhalus Acoroides* Seagrass Extract by Gas Chromatography–Mass Spectrometry. *Asian Journal of Pharmaceutical and Clinical Research* 11(10): 131-137. DOI: https://doi.org/10.22159/ajpcr.2018.v11i10.25577

Rhetso, T; Shubharani, R; Roopa, MS; Sivaram, V (2020). Chemical constituents, antioxidant, and antimicrobial activity of *Allium chinense* G. Don. *Future Journal of Pharmaceutical Sciences* 6(1): 1-9.

Roghini, R; Vijayalakshmi, K (2018). Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. *International Journal of Pharmaceutical Science and Research* 9(11): 4859-64.

Senguttuvan, J; Paulsamy, S; Karthika, K (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. *Asian Pac J Trop Biomed* 4(Suppl 1): S359-67. DOI: https://doi.org/10.12980/APJTB.4.2014C1030

Silva, MS; Brandao, DO; Chaves, TP; Formiga Filho, AL; Costa, EM; Santos, VL; Medeiros, AC (2012). Study bioprospecting of medicinal plant extracts of the semiarid northeast: contribution to the control of oral microorganisms. *Evid. Based Complement. Alternat. Med.* 2012: 681207. DOI: https://doi.org/10.1155/2012/681207

Silva, RO; Sousa, FB; Damasceno, SR; Carvalho, NS; Silva, VG; Oliveira, FR; Sousa, DP; Aragao, KS; Barbosa, AL; Freitas, RM; Medeiros, JV (2014). Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. *Fundam. Clin. Pharmacol.* 28(4): 455-64. DOI: https://doi.org/10.1111/fcp.12049

Witt, A; Luke, Q (2017). *Guide to the naturalized and invasive plants of Eastern Africa*. CABI.

Zlatić, N; Jakovljević, D; Stanković, M (2019). Temporal, plant part, and interpopulation variability of secondary metabolites and antioxidant activity of Inula helenium L. *Plants* 8(6): 179. DOI: https://doi.org/10.3390/plants8060179