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Chemical Composition, Antioxidant and Antimicrobial Activities of *Chenopodium Ambrosioides* Leaf Essential Oil

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Abstract: Chenopodium ambrosioides is a herbal plant belonging to the family of Amaranthaceae Traditionally, the leaves of Chenopodium ambroisiodes have been used in the treatment of A a variety of illnesses, such as malaria, nausea, back pain, toothaches, skin infections etc Fresh leaves of Chemopodium ambrosioides (300 g) were successively packed into the hydrodistillation apparatus (Clevenger) for 3 h using the Clevenger apparatus. The essential oil characterization from the plant above was achieved through gas chromatography mass spectrometry (GC-MS). The volatile oil obtained were screened for their antibacterial activity using the resazurin-based 96-well plate micro-dilution method. The essential oil's antioxidant potential was evaluated through 2, 2 diphenyl-1-picrylhydrazyl (DPPH) and 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The GC-MS analysis of the volatile oil revealed thirteen components summing up to 99.96%. The chief constituent in the volatile oil of Chenopodium ambrosiodioces is m-cymene (56.20%). The phyto-architectural composition of this plant is mainly made up of monoterpene hydrocarbons (93.61%); the 6.39% remaining 6components are distributed along oxygenated sesquiterpene, saturated fatty acid, and diterpene alcohol. Antimicrobial studies revealed that the volatile oil of Chemopodium ambrosioides was able to limit the development of Serratia marscescens, Klebsiella penumoniae, and Pseudomonas aeruginosa, which the usual antibiotic streptomycin was unable to do. The visible growth, after the incubation period, observed when aliquos from all MIC wells were streaked on sterile nutrient agar plate devoid of antimicrobial agents showed that the essential oil do not exhibit bactericidal effect at the highest concentration of 50% v/v tested but their ability to exhibit bacteriostatic activity against the test bacteria is worth documenting. C.ambroisiodes has the ability of reducing the DPPH radicals by 50% with an IC50 0.070±0.04, while the standard employed for the plant sample, vitamin C, had an IC50 of 0.026 mg/mL. For the ABTS assay, the volatile oils obtained from the C.ambrosioides revealed free radical scavenging potentials; the plants exhibited a 50% reduction of 2.07±0.07. Since the studied plant possess volatile oil that contain bioactive compounds, it is a good source of antioxidant and antibacterial agents.

Keywords: Chenopodium ambrosioides, antimicrobial, antioxidant, bioactive compounds,essential oil

Introduction

Since the earliest days of humanity, medicinal plants have formed the basis of healthcare throughout the world (Ojha *et al.*, 2020). Health-related problems of the global populace are cured by phytomedicines, as stated by the World Health Organization (Balyan *et al.*, 2021). Medicinal plants are gaining popularity by virtue of their therapeutic potential. Also, several therapeutic remedies are acquired through plants, and the significance of medicinal plants has expanded in recent years (Aye *et al*., 2019). Many drugs, such as anti-tumor, antimicrobial, anti-hepatotoxic compounds among others are made with the aid of therapeutic plants. (Howes *et al*., 2020). About 70% of all drugs currently available are linked to medicinal plants, making nature the foundation of drug synthesis. (Dar *et al.,* 2017). Several studies reported that about 80% of effective medications, including immunosuppressive, anticancer, antibacterial, and cardiovascular medicines, are obtained from plants (Kasali *et al.,* 2021*).* Reports have shown that high population of the world, rely on traditional medicine with herbal plants predominantly employed for this purpose (Sharma *et. al* 2023). Traditional medicine usage is not limited to developing countries, this is evident in the significant increase in herbal practice in developed countries (Sharma *et. al,* 2023). In recent times, despite the emphasis placed on research on synthetic drugs, interest in medicinal plants has reawakened. Traditional systems of medicine are widely practiced due to many reasons: an increase in the population of the world, scarcity of drugs, high cost of orthodox treatments, side effects of some allopathic drugs, and drug resistance by many infectious microbes. (Zavala-Ocampo *et al.*, 2022).

Figure 1: Pictorial view of *Chenopodium ambrosioides*

The genus *Chenopodium* (Chenopodiaceae) is made up varieties of weedy herbs (more than 200 species) and this plant originates from Europe and it can also be found in countries like India, China, North and South America (Drioua *et al.*, 2024). *C. ambrosioides* is commonly known as India worm seed, sweet pig weed, or Mexican tea. This plant is a hairy, with a very strong pungent smell, annual or short-lived perennial herb and it also grows widely in West Africa countries especially in Nigeria, Senegal, Ghana, and Cameroon. It is mostly consumed in the form of leafy vegetables or herbs (Drioua *et al.*, 2024). *C. ambrosioides also* found its usage in the food, cosmetics, and pharmaceutical industries. Traditional medicine practioners also use this plant to treat several diseases including back pain, nausea, skin infections, fever, digestive problems, intestinal worms, asthma and so on (Kasali *et al*., 2021). The bioactive components found in *C. ambroisioides* possess many medicinal properties which enables the plant's use in folk medicine such as laxative, anti-insecticidal, antioxidant, antibacterial and antimalarial (Assaidi *et al*., 2019). Also, the leaves of *C. ambrosioides are* used for the treatment of abscesses, epilepsy, and nausea (Alain *et al.,* 2012). While, the whole plant has been investigated and found to possess ability to be used as a vermifuge (Kuete, 2014).

Essential oils are complex mixtures of volatile compounds obtained from plants, where they play aromatic, communicative, and defensive roles. They possess a broad spectrum of biological activities such as antimicrobial, antiviral, anti-inflammatory, antioxidant, and smooth muscle relaxant properties (Żukowska and Durczyńska, 2024). Essential oils are intricate mixes of volatile substances created by living things; they are colorless, liquid at room temperature, and made up of around 200 chemical compounds with carbon, hydrogen, and oxygen serving as the building blocks. Terpenoids and non-terpenoids are the two groups that they fall under. Both of the sources are composed of hydrocarbons or their oxygenated derivatives, and occasionally either sulfur or nitrogen is added. They could exist as hydrocarbons (such as pinene, limonene, and myrcene), alcohols (such as linalol and santalol), acids (such as benzoic acid and geranic acid), aldehydes (such as citral), cyclic aldehydes (such as cuminal), ketones (such as camphor), lactones (such as bergaptene), phenolic esters (such as geranyl acetate), oxides (1, 8 cineole), and ethers (such as anethole) (Mohammed and Leila, 2022).The aim of this research work is to investigate the chemical composition, antioxidant activity and antimicrobial activity of leaf essential oil of *Chenopodium ambrosioides.*

Materials and Methods

Plant Sample Collection and Extract Preparation

Fresh leaves of *Chenopodium ambrosioides were* obtained from Ilora town, Oyo State, Nigeria. The sample was identified and authenticated at Forestry Research

Institute of Nigeria, (FRIN), Jericho,Ibadan,Oyo State,Nigeria where a voucher number, FHI 113656 was assigned to the plant..300 g of the fresh plant sample of *Chenopodium ambrosioides* were separately packed into a hydro distillation Clevenger apparatus and the volatile oil was isolated for about 3 h in accordance with the guidelines of European Pharmacopoeia Commission, 2004.The essential oil obtained was kept in different vials, labelled and preserved in the refrigerator at 4° C to avoid chemical transformation of the various constituents until the period of Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.

GC-MS Analysis of Bioactive Compounds

Using the method described by Adams *et.al*, 2007, a Hewlett-Packard HP 5973 mass spectrometer coupled with an HP-6890 gas chromatograph was the GC-MS instrument used.70°C was the initial column temperature adopted. This device has non-polar column and polar doubling capillary (25.0 m internal diameter, 0.25 m film thickness). Helium gas was used as the carrier gas, flowing at a rate of 0.99 mL/min. The column's linear velocity was set at 36.8 cm/s, the solvent cut duration was also set at 3 min, the E.I mode was readjusted to 70 eV, and the starting and end temperatures was programmed at 60 °C and 280 °C, respectively, with a heated rate of 3 °C/min that was constant isothermally for 6 min.

Detection of Components

Based on their respective retention times, the chemical components of the oil were determined with references to homologous series of n-alkanes as obtained in the comprehensive National Institute of Standards and Technology, (NIST) Library 2014. The compounds' mass spectra were compare to the information that was known (Adams, 2007).The total ion chromatograms (TICs) for the sample was also produced.

***In vitro* Antioxidant Action**

DPPH (2,2-diphenyl -1-picrylhydrazyl) assay)

The radical scavenging and antioxidant activities of the oil from the leaves of *C. ambrosioides* were evaluated against the free radical DPPH using the method described by Okoh *et.al,* 2011. This was carried out by incubating five different concentrations (33.3, 50.0, 66.6, 99.0 and 130.0 mg mL^{-1}) of the oil and commercial antioxidants namely β-carotene and vitamin C with a Dimethylsulfoxide solution of DPPH for 30 minutes at ambient temperature in the dark. The mixtures were then shaken separately using a vortex machine and their respective absorbances were taken at 517 nm. The volatile oil ability to scavenge DPPH free radical were calculated using the equation below.

$$\%\text{Inhibition} = \frac{A_{control} - A_{vo}}{} \times 100$$

Acontrol

Where $A_{control}$ is the absorbance of DPPH+DMSO; and A_{vo} is the absorbance of DPPH with volatile oil or the commercial antioxidant. The dose response curve was plotted and the IC50 value of the commercial antioxidant and volatile oil was calculated (Okoh *et al*., 2011).

ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) assay

The modified method of Nantitanon *et al*., 2007 was used to evaluate the ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) activity of the volatile oil extracts. The working solution was obtained by the oxidation of ABTS stock solution (7mM) with 2.4 mM of potassium persulfate in equivalent amounts and the mixture was permitted to react for 12 hours at 25°C. A portion (1 mL) of the resultant solution was further diluted using 60 mL of methanol to obtain an absorbance of 0.706 ± 0.001 at 734 nm after 7 min using a UV-spectrophotometer. The procedure was repeated by mixing five different concentrations (33.3, 50.0, 66.6, 99.0 and 130.0 mg mL^{-1}) of the volatile oil were mixed with methanolic solution of ABTS for 7 minutes at 25°C in dark. The absorbance was then measured spectro-photometrically at 734 nm and the % inhibition of ABTS radical by the volatile oils and commercial antioxidants was calculated by means of the equation portrayed for DPPH assay above.

Antimicrobial screening assay

The activity of the essential oil of *Chenopodium ambrosioides* was evaluated against four Gram-negative and four Gram-positive bacteria pathogens using the resazurin-based 96-well plate micro-dilution method, as previous study in the literature (Balouiri *et al*., 2016).

Results and Discussion

The result of the GC- MS analysis of *C. ambrosioides* leaf essential oil is presemted in Table 1 below:

Table 1: Chemical Constituent of *C. ambrosioides* essential oil

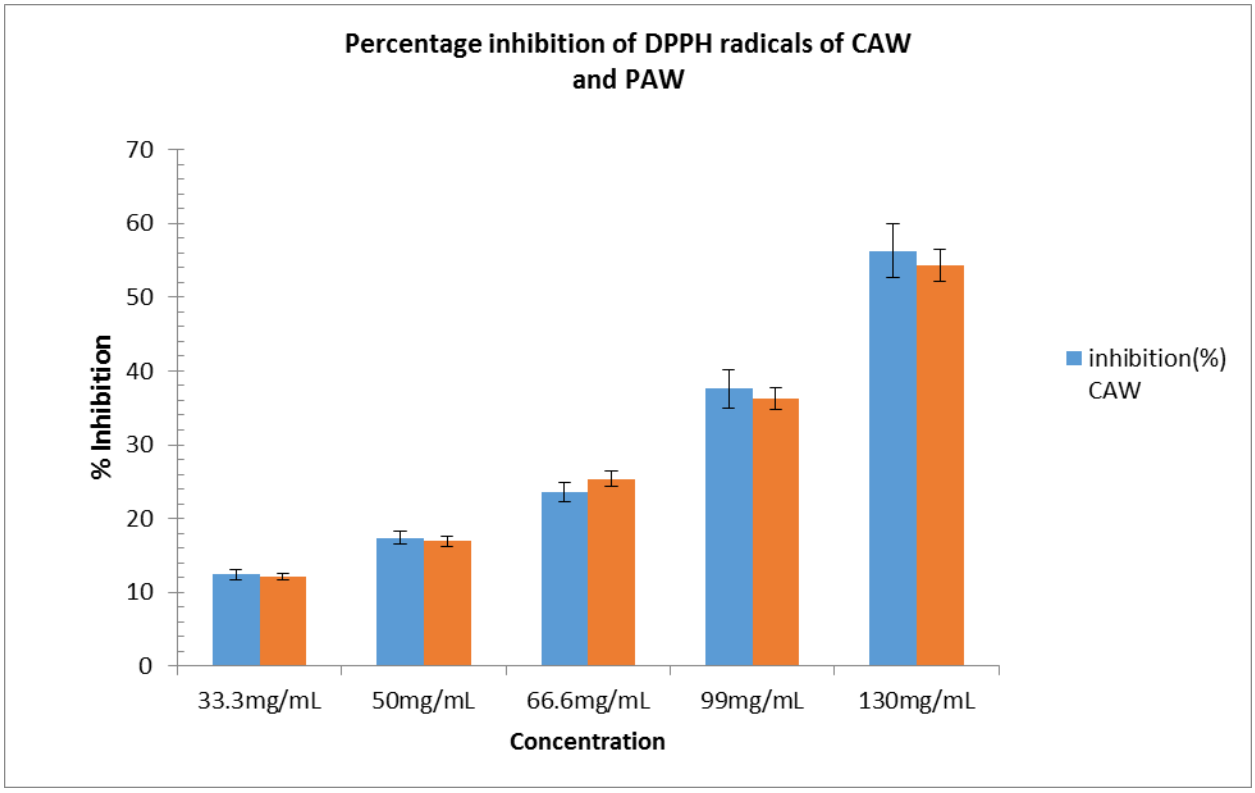
S/N	RT	Components	Molecular Formular	Molecular Weight	% Composition	Class Of Compound
1	8.466	m-Cymene	$C_{10}H_{14}$	134	56.20	Monoterpene hydrocarbon
2	8.586	p-Cymene	$C_{10}H_{14}$	134	11.34	Monoterpene hydrocarbon
3	8.660	D-Limonene	$C_{10}H_{16}$	136	0.61	Monoterpene yydrocarbon
4	9.164	γ-Terpinene	$C_{10}H_{16}$	136	0.86	Monoterpene

						hydrocarbon
5	11.075	Ethanone, 1-(1,3-dimethyl-3-cyclohexen-1yl	$C_{10}H_{16}O$	152	0.38	Oxygenated Hydrocarbon
6	12.099	2-Bornene	$C_{10}H_{16}$	136	24.22	Monoterpene hydrocarbon
7	12.260	Cis,trans-1,2,3-Trimethylcyclohexane	C_9H_{18}	126	0.44	Hydrocarbon
8	12.323	Cyclohexane, 3-ethyl-5-methyl-1-propyl	$C_{12}H_{24}$	168	1.18	Hydrocarbon
9	12.898	4-Hydroxy-3-methylacetophenone	$C_9H_{10}O_2$	150	0.30	Oxygenated Hydrocarbon
10	13.009	Isoascaridol	$C_{10}H_{16}O_2$	168	3.08	Oxygenated Hydrocarbon
11	17.478	Fragranyl isovaalerate	$C_{15}H_{26}O_2$	222	0.33	Oxygenated Sesquiterpene
12	17.583	Palmitic acid	$C_{16}H_{36}O_2$	256	0.52	Saturated Fatty Acid
13	20.557	Phytol	$C_{20}H_{40}O$	296	0.50	Alcohol
				Total	99.96%	

A yellow-coloured essential oil (EO) was obtained by hydro distillation procedure yielding 0.75 % W/W. According to the result above, thirteen components accounting for 99.96% of the entire oil were determined. The result from this research work is in line with previous studies of the plant in Nigeria with little variations (Kasali *et al*, 2016). Little variation in the composition of the oils was noticed from the essential oil from the leaves of *C. ambrosioides* from North Central Nigeria which contained nineteen bioactive compounds amounting to 98.37% of the total oil. The majpr compounds found in the North Central Nigeria grown *C. ambrosioides* are 2- arene (17.80%) 2-bornene (14. 79%) p-Cymene (12.93%) a-Terpinene (13.98%), o.-Terpinolene (7. 90%), and y-Terpinene (6. 94%). *C. ambrosioides* (Larayetan *et al*., 2015). This present study also contains 2-bornene (24.22%) p-Cymene (11.34%) and y-Terpinene (0.86%).

The leading compound in the volatile oil of *C. ambrosioides* is m-cymene (56.20% as shown in Table I, m-cymene is a geometrical isomer of o-cymene; it has a benzene ring ortho substituted with a methyl substituent and an isopropyl group. Followed in this predominant ranking of chemical components of *C. ambrosioides* is 2-bornene with chemical formula ($C_{10}H_{16}$). Another geometric isomer of o-cymene (p-cymene) is ranking third in the chemical components with a percentage composition of 11.34%.It is an alkyl-substituted aromatic compound naturally occurring in essential oils (EOs) of various aromatic plants. This is followed by isoascariodol (a peroxy monoterpenoid). The phyto-architectural composition of this plant is mainly made up of monoterpene hydrocarbons (93.61%); the 6.39% remaining components are distributed along with oxygenated sesquiterpene, saturated fatty acid, and diterpene alcohol. M-cymene is used in the synthesis of fungicides and pesticides, it is also used in perfumery and in the production of some precursors of standard antioxidants such as p-cresol. M-cymene has shown a lot of pharmacological properties including antimicrobial (Tian *et al*, 2012), antioxidant (de Oliveria *et al,* 2015), anti-inflammatory (Santana *et al*, 2015), antiparasitic (Adams *et al*, 2015) and antidiabetic (Abbasi *et al*, 2018). 2-bornene has also been reported to have several pharmacological potentials such as antioxidant and anti-inflammatory (Kumar *et al*, 2013), antimicrobial (Sharma *et al*, 2014), anticancer (Srivastava *et al.,* 2017), analgesic, anti-nociceptive (Sharma *et al*, 2016), antidiabetic (Joshi *et al,* 2019) and anti-ulcer (Kumar *et al,* 2020). The therapeutic properties include antioxidant and anti-inflammatory (Younis *et al*, 2022), antiparasitic, anticancer (Dhfi *et, ai*, 2016), antidiabetic, antiviral, antitumor and antimicrobial (Chouhan *et al*, 2017), antibacterial and antifungal activities (Younis2022).

***In vitro* Radical Scavenging of *Chenopodium ambrosioides* (*C.ambrosioides*)**



CA = *Chenopodium ambrosioides*, PA = *Petiveria alliacea*

Figure 1: Pecerntage inhibition of DPPH radicals of C.A and P.A

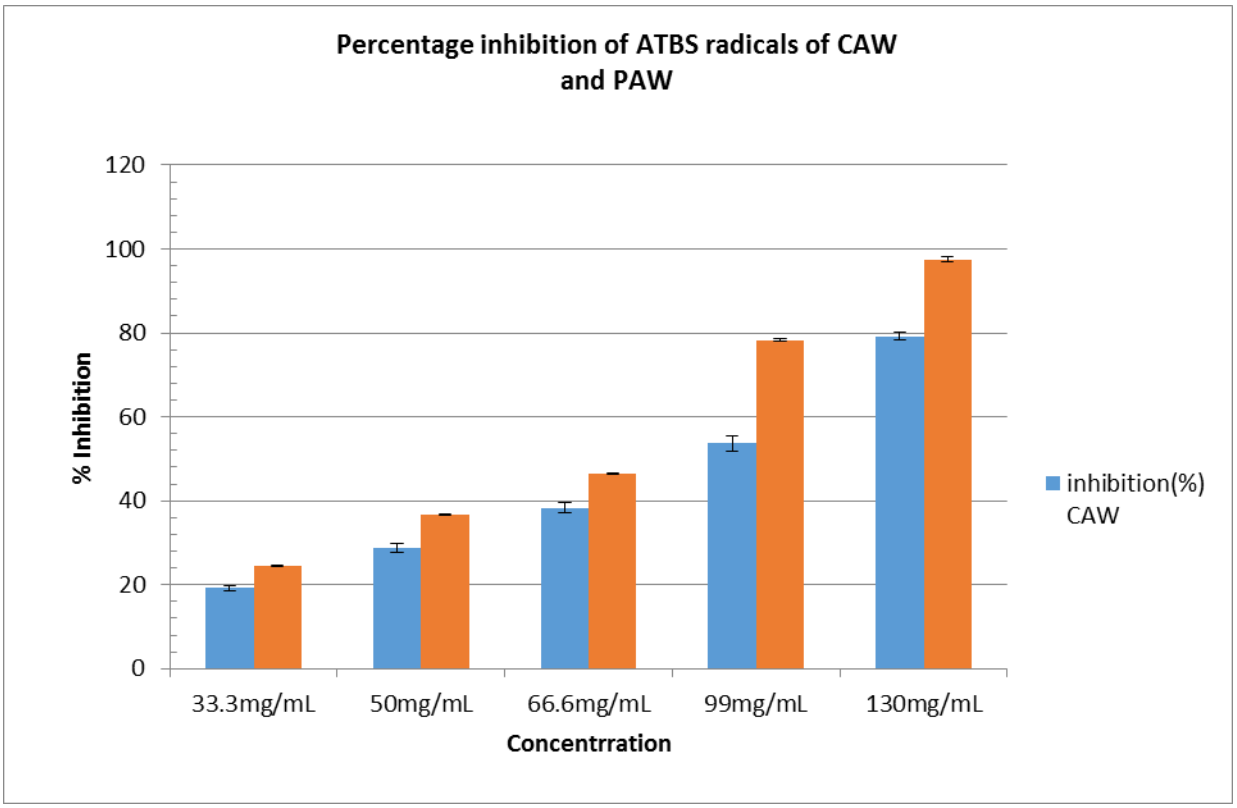


Figure 2: Pecerntage inhibition of ABTS radicals of C.A and P.A

The % DPPH inhibitions for *C. ambrosioides at* varying concentrations (33.3, 50.0, 66.6, 99.0 and 130.0 mg/mL) and it ranges from 12.43 ± 0.71 to 56.28 ± 3.67 %. The essential oil of *C. ambrosioides* was able to reduce the DPPH radicals by 50% and its IC50 is 0.070±0.04 mg/ml. The standard employed for the plant sample: vitamin C had an IC_{50} of 0.026 mg/mL. The synthetic drug employed as the standard had a better radical scavenging potency (IC_{50}) of 0.026 mg/mL than the volatile oil extract used in this work. For the ABTS assay the essential obtained from the *C. ambrosioides* revealed free radical scavenging potentials having a maximum activity of 53.70 ± 1.85 at 130 mg/mL. The essential oil obtained from this herbal plant exhibited a 50% reduction of 2.07± 0.07. This denotes that *C. ambrosioides* possess a lower scavenging radical action than the positive control (BHT) with IC50 of 0.19 mg/mL.

The radical scavenging activities of the volatile oil examined using DPPH and ABTS models showed that he visible violet colour observed when DPPH was dissolved in DMSO was owing to the unpaired electron on nitrogen. The DPPH radical was reduced in the process to DPPH-H when the *C. ambrosioides* essential oil was added to it thereby changing from violet to purple colour. This color change is due to the reduced form which no longer absorbs at this wavelength 517 nm. This shows that the essential oil of *C. ambrosioides* has an antioxidant capacity and it is able to catch free radicals and prevent their harmful effects.

Antibacterial potential of *C. ambrosioides*

Table 2.: Antimicrobial activity of the two essential oils against test bacteria

Bacteria	**row on plate**	**CA(%v/v)**		**Streptomycin (ug/mL)**	
		MIC	**MBC**	**MIC (ug/mL)**	**MBC(ug/L**
***Staphylococcus aureus* (NCIB 8588)**	**A**	**NVI**	**ND**	**7.5**	**7.5**
***Clostridium sporogenes* (NCIB 532)**	**B**	**NVI**	**ND**	**3.75**	**3.75**
***Bacillus cereus* (NCIB 532)**	**C**	**NVI**	**ND**	**3.75**	**7.3**
***Serratia marcescens* (NICB 1377)**	**D**	**50**	**NBE**	**ND**	**ND**
***Pseudomonas aeruginosa* (NCIB 950)**	**E**	**NVI**	**ND**	**ND**	**ND**
***Micrococcus* luteus(196)**	**F**	**50**	**NBE**	**3.75**	**15**
***Klebsiella pneumoniae* (NCIB 418)**	**G**	**50**	**NBE**	**ND**	**ND**

***Salmonella typhimurium* (ATCC14028)**	**H**	**50**	**NBE**	**7.5**	**15**

Keyμ: NVI = no visible antibacterial effect observed, NBE = no bactericidal effect observed, MIC = Minimum inhibitory concentration, MBC = minimum bactericidal concentration, ND=Not detected

***Note: The starting concentration in column 1 was 50% v/v* for the essential oil.**

Plate2 : Determination of MIC for CA against the eight test bacteria

Resazurin-based 96-well plate micro-dilution method as earlier articulated in the literature (Balouiri *et al*., 2016) was used to determine the activity of the essential oil against four Gram-negative and four Gram-positive bacteria pathogens. The result of the antimicrobial activity of the essential oils against test bacteria is given in Table 4 and in the plate above. For the plate, each row represents a test bacterium while each column represents a concentration of the essential oil. After the period of incubation, resazurin dye was added to all the wells in the micro-titter plate. Column 13 confirms (essential oil at 50% v/v and Mueller Hilton broth) that no contamination occurred while preparing the plate since the natural (blue/purple) colour of the dye was maintained after the incubation period. Column 12 (standardized bacteria solution and Mueller Hilton broth), a negative control shows a change of resazurin's natural colour (blue/purple) to the reduced form (pink-colourless) and this confirmed the viability of the test bacteria solution. The highest concentration of the essential oil incorporated into the plate (column 1 cells) was 50% v/v and the lowest achieved through double-fold serial dilution (column 11 cells) was 0.049% v/v.

The essential oil of *Chenopdium ambrosioides* (CA) exhibited an inhibitory effect against only four of the eight test bacteria as shown in Table 4 and Plate 1 at varying concentrations. The MIC of the Essential oil CA against *Serratia marcescens* and *Salmonella typhimurium* was 50% v/v while that against *Micrococcus luteus* and Klebsiella *pneumoniae* was 0.098% v/v and 12.5% v/v respectively. The visible growth, after the incubation period, observed when aliquots from all MIC wells were streaked on sterile nutrient agar plates devoid of antimicrobial agents showed that the essential oil do not exhibit bactericidal effects against any of the test bacteria.

Although the essential oil did not exhibit bactericidal activities against any of the test bacteria at the highest concentration of 50% v/v tested, their ability to exhibit bacteriostatic activity against the test bacteria (50% of the test bacteria inhibited by CA) is worth documenting. The bacteriostatic activity observed was against both Gram-negative and Gram-positive bacteria adjudging that the essential oil has broad-spectrum bacteriostatic activity. Essential oil with only bacteriostatic activity has been reported previously (Radaelli *et al*., 2016) and a standard drug such as tetracycline exhibits only bacteriostatic activity(Stein *et al*., 2018). . It is also worth noting that the essential oil inhibited the growth of *Serratia marcescens*, *Klebsiella penumoniae* and *Pseudomonas aeruginosa* (only CA) that the standard antibiotic (Streptomycin) could not inhibit in this study. This suggests that the essential oil contains bio molecules that could be effective against multi-drug resistant bacteria because the aforementioned bacteria are known to exhibit multi-drug characteristics (Kijineh e*t al*., 2024; Pang *et al*., 2019).

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