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Preliminary Phytochemical Screening, Characterization of Bioactive Compounds and Anti-bacterial Properties of *Eupatorium odoratum* from Agbor, Nigeria.

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Abstract

Medical professionals have used synthetic medicine to treat bacterial infections. Scientists globally have been on the search for the utilization of natural plant products as alternatives. Therefore, this study investigated the preliminary phytochemical screening, and characterization of bioactive compounds and anti-bacterial properties of *Eupatorium odoratum*. Phytochemicals were detected using standard methods, certain bioactive compounds were detected using thin layer chromatographic (TLC) technique, ultra-violet (UV) visible and Fourier transform infra-red (FTIR) spectroscopic techniques. Antimicrobial screening was carried out using the punched agar diffusion method, the minimum inhibitory concentration and minimum bactericidal concentration of test organisms were determined by using serial doubling dilution method. Saponins, tannins, glycosides, alkaloids, steroids, terpenoids, proteins, oil were detected in the crude extract and some bioactive compounds which denoted the presence of various phytochemicals were found in the three solvent extracts used. All extracts showed good antibacterial activities with zone inhibition of 15-25 mm against all micro-organism specimens; however, the chloroform extract was more potent in causing inhibition with *Staphylococcus aureus* (25 mm), having the highest zone of inhibition. *Eupatorium odoratum* can therefore be utilized as an alternative medicinal agent against a variety of bacterial infections.

Keywords: *Eupatorium odoratum, Antibacterial Agent, Phytochemicals and Bioactive Compounds.*

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Introduction

Pharmaceuticals derived from plants have gained popularity due to their biocompatibility and fewer adverse effects compared to synthetic drugs. This has spurred interest in identifying plant species with potential medicinal properties [1-2]. Extensive research has been conducted on the therapeutic uses of plant extracts [3-4], particularly focusing on their antibacterial properties [5-7]. *Eupatorium odoratum* is a plant of the family *Asteraceae* and a well-known traditional medicinal herb. It is also known as common floss flower, baby tea, Santa Maria, Christmas bush, devil weed, French weed, siam

weed, and bitter bus [8-11]. In the open, it can reach heights of 3–7 meters. *E. odoratum* is a perennial shrub with a long, winding growth habit. Its damaging nature makes it a hazard since it damages estates and other ecosystems [12]. Previous study on *E. odoratum* have shown medicinal effects on the body; as a result, it has been widely and regularly used as traditional herbal medicine to treat wounds, burns, stomachache etc [13]. It has been demonstrated that *E. odoratum* leaf extracts have numerous medically significant qualities, including cytoprotective, analgesic, antibacterial, antioxidant, and anti-inflammatory effects [1, 14, 15]. Furthermore, according to Vijayaraghavan *et al* [16], it is also used as a vermifuge and to treat rheumatism, catarrh, diabetes, diarrhea, fever, pertussis, and nasal congestion Alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids are among the chemical compound components of E. odoratum [17]. Given the ongoing need for new antimicrobial agents to combat emerging infectious diseases [18], this study investigated the preliminary phytochemical screening, characterization of bioactive compounds, and antibacterial properties of *E. odoratum.*

Materials and Methods

Collection, identification and extraction of plant

The leaves of the plant were collected fresh from University of Delta, Agbor and identified in the Department of Botany, Delta State University Herbarium as *Eupatorium odorantum* (*Chromolaena odorata*) with voucher number DELSUH: 270 by Mr Michael O.E. The plant leaves were destalked, washed and left to air drying at ambient temperature. 20 g of the dried leaves were pulverized using an electric grinder (QASA QBL-15L40 model) and homogenized in 160 ml of 90% methanol and 40 ml of water for about 5 minutes. Whatman filter paper was used to filter the homogenized sample. Rotary evaporator (Supervac, India) was used to evaporate the filtrate. The crude extract was used for phytochemical analysis. The methanol-water crude extract was further fractionated with a separating funnel using chloroform, chloroform-methanol and aqueous solvent respectively [19, 20].

Phytochemical screening and identification of bioactive compounds of *E. odoratum* leaf extracts.

Qualitative phytochemical screening was carried out according to the method of Borokini and Omotayo [21], Njoku and Obi [22]. The chloroform, chloroform-methanol and aqueous extracts of leaf samples were analyzed for bioactive compounds using thin layer chromatography (TLC) (Silica gel 60 GF254, Merck) and were further identified by Fourier Transform Infrared Spectrometer (FTIR) (Perkin-Elmer 1605 FT-IR spectrophotometer) as well as Ultraviolet (UV) Spectrometer (Unicam UV-Visible Spectrophotometer vision 32 software V1.21)) according to the method of Kwekowe et al [23] and Aye and Noe Oo [24] with slight modification.

Antimicrobial properties of *E. odoratum* leaf extracts

The test organisms (*Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris*) were acquired from Pharmaceutics and Pharmaceutical Microbiology Laboratory, Sir Ahmadu Bello University, Zaria, Kaduna State. The punched agar diffusion method according to Yazdi *et* al., [25] was used. The sensitive plates were prepared as recommended by the manufacturer. Each plate had three 6 mm wells formed with sterile 6 mm diameter punches and labeled accordingly. The punched plates were inoculated with the various organisms (10 CFU/ml) and controls using a sterile swab stick and were left for a short while to dry. The inoculum was carefully and evenly spread across the whole surface of the agar plate using a sterilized glass rod. To protect the agar surface, the spreading motion was soft and soothing. 50 µL of the different plant extracts ($CHCl_3$, $CHCl_3$-MeOH and H_2O) were added to the labeled well. The zones of inhibition were determined after the agar plate was cultured for 24 hours at 37 °C to enable isolated colonies to proliferate and become noticeable colonies on the agar plate.

The serial doubling dilution approach was utilized to determine the minimum inhibitory concentration (MIC). For every extract, six McCartney bottles holding 2 ml of glucose indicator broth were utilized. In order to correspond with the bacterial species and extract layers, the bottles were labelled 1-6. Dilutions of each extract layer were made (1:2, 1:4, 1:8, 1:16, 1:32, 1:64) by adding 2 ml of each extract into 2 ml broth. Controls were also included. Media and organism were put into Tube 7, broth and extract were in Tube 8, and broth and solvent were in Tube 9. Then, one drop of the inoculums was added to each dilution using a sterile pipette. After

properly mixing them, they were incubated for 48 hrs at 37°C and the bottles were checked for growth. To obtain the MIC of the extract, all tubes showing no growth were subcultured on fresh nutrient broth with a wire loop and incubated at 37°C for 24 hrs. The last dilution tube showing growth was the MIC of the extract and were indicated using (+) sign.
Also, the minimum bactericidal concentration (MBC) was the last dilution tube showing no growth and were indicated using (-) sign.

Results

Figure 1 shows the percentage yield of different solvents used for the extraction of *E. odoratum* leaf. The chloroform extract had the highest percentage yield of 4% and was followed by the Chloroform-methanol extract (1.55%) and the lowest yield was seen in the aqueous extract (1%).

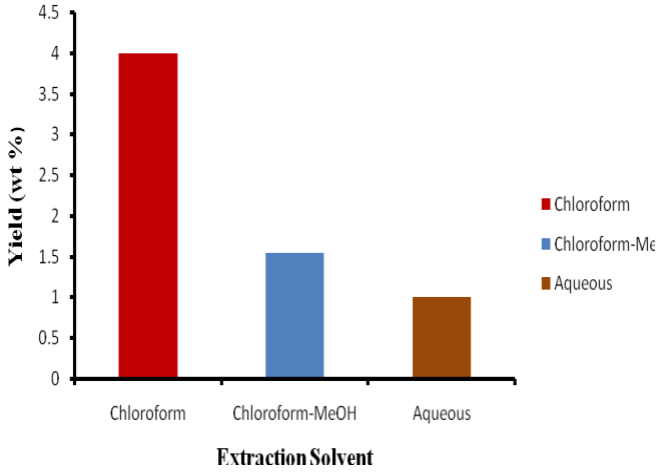

Figure 1: Percentage yield of E. odoratum leaf extracts in different extraction solvent

Preliminary Phytochemical Screening of E. odoratum leaf extracts

Table 1 shows the preliminary phytochemical composition of crude leaf extract of *E. odoratum*. Test for saponins, tannins, glycosides, alkaloids, steroids and terpenoids, proteins, oils, were positive while flavonoids, reducing sugar and carbohydrate tests were negative.

Table 1: Preliminary phytochemical screening of crude (Methanol-water) leaf extract of *E. odoratum*.

Phytochemicals	Result
Saponin	++
Tannin	+++
Alkaloid	+
Proteins	++
Oils	++
Steroids and Terpenoids	++
Flavonoids	-
Glycosides	++
Reducing sugar	-
Carbohydrates	-

- = Absent
+ = Present in low concentration
++ = Present in moderate concentration
+++ = Present in high concentration

TLC, FTIR, and UV spectra analysis of *E. odoratum* leaf.

TLC, FTIR, and UV spectra analysis of group of bioactive compounds in chloroform, chloroform-methanol and aqueous extracts are presented in Tables 2, 3 and 4 respectively. The FTIR absorption spectrum of the chloroform extract (Table 2) between 3400 – 3200 cm^{-1} showed the presence of amines (N-H) stretch and O-H stretch for alcohols and phenols, while the absorption between 2973 – 192 cm^{-1} was C-H stretch for alkyl and aryl groups. At 1659 cm^{-1}, there appeared C=O stretch for ketones, amides and amines. At 1455 cm^{-1}, C-H stretch indicate a terminal $-CH_3$ and absorption at 1384 – 1274 cm^{-1} was a C-O stretch for alcohols, esters, phenols and amides. Between 1089 – 1050 cm^{-1} was C-H deformation bond for five- or six-members ring. C-H deformation bonds were found between 946 and 804 cm^{-1} for both aryl and alkyl groups. At 518 – 430 cm^{-1} was a C-H out of plane deformation. The UV spectrum gave λmax at 480 nm and 650 nm. The TLC gave retardation factor (Rf) value of 0.20.

Table 2: TLC, FTIR, and UV spectra of chloroform leaf extract of *E. odoratum*

Wave numbers(cm^{-1})	**Description**
3400 3200	O – H Stretch for alcohol and phenols N- H stretch for amines, C-H stretch for aromatic
2973 2851 2724	C-H stretch for alkyl and aryl groups.

1659	C=0 stretch for ketones and amides and imides
1659	C=C stretch for aromatic group
1455 1384	Indicative of terminal – CH_3. C-O stretch for alcohols, ester, phenols and amides
1274	C-O deformation
1089 1050	C-H deformation indicative of five- or six-member ring.
946 841	C-H deformation bonds for alkyl and aryl groups
518 430	C-H deformation (out of plane).
UV λmax 480nm 650nm	18 Showing highly conjugated aromatic compounds.
Rf Value	0.20

The FTIR for the chloroform-methanol extract (Table 3) had a broad absorption band at between 3600-3300 cm^{-1}, showing the N-H and O-H stretches for primary amines and alcohol/phenols respectively. The band between 2934 – 2708 cm^{-1} was C-H stretch for methyl and aryl groups, while between 2343 – 1922 cm^{-1} was C=N stretch for nitriles. Absorption at 1639 – 1610 cm^{-1} was for C=O stretches for aldehydes, ketones and carboxylic acid as well as N-H stretch for amines and amides. Between 1557 – 1430 cm^{-1} exists the C=C stretch for alkenes and arenes. Aldehydes, ketones, and carboxylic acids had C-O stretches between 1383 and 1332 cm^{-1}, whereas C-O deformation bonds are absorbed between 1275 and 1203 cm^{-1}. The C-H deformation bond for alkyl and aryl groups ranged from 952 to 800 cm^{-1}. C-H deformation bonds for methyl groups were absorbed between 669 and 418 cm^{-1}. The UV absorption peaks at λmax of 460 nm, 650 nm and 680 nm indicated highly conjugated and substituted aromatic compounds. The TLC gave Rf value of 0.16.

Table 3: TLC, FTIR, and UV spectra of chloroform-methanol leaf extract of *E. odoratum*

Wave numbers (cm^{-1})	Description
3600 3300	N-H stretch for 1° amines (broad band) O-H stretch for phenols & alcohols
2934 2708	C-H stretch for methyl group and aryl groups attached to an aromatic ring.
2343 2135 1922	C-N stretch for nitrates.
1639 1610	C-O stretch for aldehydes, ketones & carboxylic acid N-H stretch for amines and amides.
1557 1430	C-C stretch for alkenes and arenes.
1383 1332	C-O stretch for aldehydes, ketones and carboxylic acid.
1275 1203	C-O deformation for amides, esters and alcohol.
952 848	C-H deformation bond for alkyl and aryl groups.
UV λmax 460nm 650nm	Indicating highly conjugated and substituted aromatic compounds.
Rf Value	0.16

The FTIR results of the aqueous extracts (Table 4) showed absorption peaks at 2633 – 2539 cm^{-1}, indicative of C-H stretches for alkyl and aromatic systems and N-H stretch for amines and amides. At 2412 cm^{-1} exists C=C stretch for alkyl group or N=C=N for aromatic C-N bond. At 1842 – 1650 cm^{-1} was the C=O stretch for amides, imides and Ketones and Stretch C=C for arenes and alkenes. At 1330 cm^{-1} was the C-O stretch for amides, alcohols and esters. Between 947 – 804 cm^{-1} was the absorption band region for bonds of C-H deformation for aromatic and alkyl groups. Between 649 – 432 cm^{-1} was the distortion of C-H for alkyl group. The UV spectrum at 480 nm and 650 nm showed also the presence of a highly conjugated

aromatic system and the Rf value from TLC was 0.38.

Table 4: TLC, FTIR, and UV spectra of aqueous leaf extract of *E. odoratum*

Wave numbers	Description
2633 2539	C-H stretch for alky and aromatic groups N-H stretch for amines and amides
2412 1924	C-C stretch for aromatics and alkyl groups or -N=C=N- stretch for aromatics C-N bond.
1842 1650	C=O stretch for amides and imides and ketones.
1639 1610	C-O stretch for aldehydes, ketones & carboxylic acid N-H stretch for amines and amides.
1540 1330	Stretch C=C for arenes and alkenes.
1274	C-O deformation for amides, esters, ketones and alcohols.
947 881	Bonds of C-H deformation for aromatic and alkyl groups.
649	Distortion of C-H for alkyl group.
UV λmax 480nm 650nm	Indicating highly conjugated aromatic ring.
Rf Value	0.38

Antibacterial screening

Antibacterial screening of various leaf extracts of *E. odoratum* are shown in Table 5. The chloroform extract had inhibition zone range of 19 mm to 25 mm on test organisms. *Staphylococcus aureus* had the highest zone of inhibition of 25 mm while *Escherichia coli* had the lowest zone of inhibition of 19 mm in the chloroform extract. The chloroform-methanol extract had zone of inhibition ranging from 16 mm to 21 mm. *Staphylococcus aureus* had the highest inhibition zone of 21 mm while *Escherichia coli* and *Proteus vulgaris* had the lowest inhibition zone of 16 mm in the chloroform-methanol extract. The aqueous extract had inhibition zone ranging from 15 mm to 22 mm. *Staphylococcus aureus* had the highest inhibition zone of 22 mm while *Proteus vulgaris* had the lowest inhibition zone of 15 mm in the aqueous extract. The chloroform extract showed more potent antibacterial capacity as it had the highest zone of inhibition in all organisms when compared to the chloroform-methanol and aqueous extracts, although the highest inhibition zone was against *Staphylococcus aureus*.

Table 5: Antibacterial screening of leaf extracts of *E. odoratum*

Extracts		Average diameter of zone of inhibition on test organisms (mm)			
		Salmonella enterica	*Escherichia coli*	*Staphylococcus aureus*	*Proteus vulgaris*
Chloroform ($CHC1_3$)		21	19	25	20
Chloroform-methanol (CHC13-MeOH)		20	16	21	16
Polar		20	16	21	15
Controls	50% $CHC1_3$	No inhibition	No inhibition	No inhibition	No inhibition
	50%$CHCl_3$-MeOH	No inhibition	No inhibition	No inhibition	No inhibition

Extracts	Samples Codes	Dilution	Presence or absence of test organisms			
			Salmonella enterica	*Escherichia coli*	*Staphylococcus aureus*	*Proteus vulgaris*
$CHC1_3$		Stock	-	-	-	-
Layer	A	1:2	-	-	-	-
		1:4	-	-	-	-
		1:8	-	+	-	+
		1:16	+	++	+	++
		1:32	++	++	++	++
		1:64	++	++	++	++
	Control	7	++	++	++	++
	Tubes	8	-	-	-	-
		9	-	-	-	-
MIC			1:16	1:8	1:16	1:8
MBC			1:8	1:4	1:8	1:4
$CHCI_3$-		Stock	-	-	-	-
MeOH	A	1:2	-	-	-	-
extract		1:4	-	-	-	-
		1:8	+	-	-	+
		1:16	++	+	-	++
		1:32	++	++	+	++
		1:64	++	++	++	++
	Control	7	++	++	++	++
	Tubes	8	-	-	-	-
		9	-	-	-	-
MIC			1:8	1:16	1:32	1:8
MBC			1:4	1:8	1:16	1:4
Polar		Stock	-	-	-	-
Exract	A	1:2	-	-	-	-
		1:4	-	-	-	-
		1:8	+	-	-	+
		1:16	++	+	-	++
		1:32	++	++	+	++
		1:64	++	++	++	++
	Control	7	++	++	++	++
	Tubes	8	-	-	-	-
		9	-	-	-	-
MIC			1:8	1:16	1:32	1:8
MBC			1:4	1:8	1:16	1:4

Table 6 depicts the results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the leaf extracts of *E. odoratum*. *Staphylococcus aureus* was more sensitive in all extract with MIC of 1:16, 1:32, 1:32 and MBC of 1:8, 1:8, 1:16 in the chloroform, Chloroform-methanol, and aqueous extracts respectively while *Proteus vulgaris* was more resistant with MIC of 1:8 and MBC of 1:4 in all extracts.

Table 6: Minimum inhibition concentration and minimum bactericidal concentration of various leaves extract of *E. odoratum* on bacterial species.
Tube 7 = Media + Organism, Tube 8 = Media + Extract only, Tube 9 = 50% Solvent.

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration.

+ = Slight growth i.e MIC, ++ = Visible growth in media, - = No growth.

Discussion

Researchers all over the world have given priority to the investigation of plant sources as alternative to synthetic medicine as the overuse of synthetic medicines have given rise to resistant microbes [26]. The polarity index determines the solvent to be used for extraction [27]. The extraction of *E. odoratum* from the three types of solvents gave different results because of their polarity index. Chloroform with polarity index of 4.1 gave the highest yield while the aqueous with polarity index of 9.0 gave the lowest yield. This shows that *E. odoratum* leaves are better extracted in solvent with low polarity. This is contrary to the work of Ahmed and Yasser [28], that reported maximum yield in methanol extract of *Spathodea nilotica* leaves followed by distilled water and the chloroform extract.

The results of this investigation demonstrated the existence of terpenoids, cardiac glycosides, alkaloids, steroids, saponins, proteins and oils in the

crude extract of *E. odoratum*, however, flavonoids, reducing sugar and carbohydrates were absent. This is similar to earlier report by *Mishra et al* [29], that documented the presence of alkaloids, triterpenoid, tannins, sterols, steroids, cardiac glycosides, flavons and flavonoids in various extracts (petroleum ether, chloroform and ethanol) of *E. odoratum*. Although, their study showed the presence of flavonoid in petroleum ether and chloroform extract, it was absent in the ethanol extract. Yusuf and Fahriani [30], also reported the presence of phytochemicals in the leaf extract of *Chromolaena odorata Linn*. The phytochemicals found in this plant provide early insights into a variety of classes of active secondary metabolites that are essential to physiological and therapeutic benefits, including anti-diabetic, antibacterial, antioxidant, and anticarcinogenic properties [31]. The antibacterial properties of some phytochemicals have been demonstrated by previous studies [32-35].

The presence of compounds (with varied wavelengths) belonging to the groups of alcohols, amines, esters, aldehydes, alkanes, alkenes, alkyls, nitrates, carboxylic acids, aromatic compounds, etc. was discovered during the determination of bioactive compounds in the extracts using TLC, FTIR, and UV spectra. These differences in the wavelength of the bioactive compounds of the different extracts correspond to the kind of solvent that was utilized during the extraction procedure. Numerous studies have demonstrated the existence of several phytochemicals, including phenolics, terpenoids, and alkaloids, which have been extensively documented to exhibit antioxidant and anti-inflammatory characteristics [36, 37]. Different solvent systems yield varying retardation factor (Rf) values for different phytochemicals. This variance in the phytochemicals' Rf values offers a crucial hint for determining their polarity and aids in choosing the right solvent solution for the separation of pure substances [38].

Human pathogenic bacteria, such as *Escherichia coli*, can cause neonatal meningitis; *Staphylococcus aureus* can cause foodborne illnesses like endocarditis; and *Salmonella enterica sp*. can cause localized infections like gastroenteritis [39-42]. *P. vulgaris* is the primary cause of urinary tract infections (UTIs), which are a major risk factor for infections in the community and hospital [43].

Staphylococcus aureus was more sensitive to the different extracts; all extracts had good antimicrobial efficacy against every microbe examined, with the chloroform extract causing higher inhibition against all tested species. Previous studies by Jai *et al* [44] and Devi *et al* [12] have reported that antibacterial and antifungal properties were demonstrated by the ethanol and methanol extracts of *E. odoratum* against a range of tested species. The presence of active ingredients in the extracts, which may function singly or in combination, may be the cause of the microorganisms' in vitro growth inhibition [45]. Table 2 and 3, indicated the existence of the N-H group and Table 4 in addition to the N-H group, stated the presence of -N=C=N-, which are characteristics of alkaloids as supported by Table 1 that is present in the leaf extract of *E. odoratum*. Alkaloids work as efflux pump inhibitors to produce antibacterial effects [46, 47]. This corresponds with the work of Maurya et al., [48], Sridevi et al., [49] that opined that Lysergol and Reserpine which are alkaloids inhibited the growth of *E. coli* and *Staphylococcus sp* respectively via efflux pump inhibitor. Biorational plant extracts rich in alkaloids, derived from a variety of families such as *Amaryllidaceae, Burseraceae, Capparaceae, Mimosaceae, Vitaceae* and *Tiliaceae* have demonstrated exceptional antibacterial efficacy against several pathogens, including as *P. aeruginosa*, *E. Coli*, *S. aureus*, *S. Typhimurium*, and *K. pneumonia*. [50, 51]. Additionally, it's commonly known that the antibacterial substances found in *E. odoratum*, such as flavonoids, tannins, and alkaloids, can help prevent pathogens from synthesizing their cell walls, thereby limiting their ability to develop [52-53]. Previous report by Akinmoladun *et al.,* [54], made clear that *E. odoratum* may have the ability to cure a variety of illnesses because of its bioactive components.

Variations in the phytochemical composition and antibacterial activity may result from different extraction techniques, solvent efficiency, plant sample to solvent ratios, and other factors [55, 56].

Conclusion

This research revealed that the leaves of *E. odoratum* contains phytochemicals and some bioactive compounds such as alkaloids, saponins, tannins, cardiac glycosides, steroids, terpenoids, oils etc. which is the reason for its potency against various bacteria used for the study. Based on the antibacterial study, we recommend the use of chloroform solvent extract as the best, as it triggered higher inhibition in all test organisms studied. Thus, *E. odoratum* could be used as medicine to combat

various health challenges caused by bacteria such as *S. aureus*, *E. coli*, *S. enterica*, *P. vulgaris* and other bacteria species.

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