GC-MS ANALYSIS OF BIOMEDICAL CONSTITUENTS IN THE *EUPATORIUM ODORATUM L.* LEAVES (JAMANI) EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITY AGAINST MULTIPLE BACTERIAL PATHOGENS

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Abstract

The purpose of this study was to determine the biomedical constituents and antimicrobial properties of the crude extracts isolated from the leaves of *E. odoratum* collected in 2017 at Yadanabon University campus, Mandalay, Myanmar. The crude extracts, isolated by using maceration method with ethanol and hexane as solvents, were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The result showed the presence of *n*-Tetradecanol (52.98%, 37.42%) in both ethanol and hexane extracts but methyl hexadecanoate (40.47%) was recorded only in hexane extract as the main constituent. 2E-dodecanol (55.25%) was found to be the dominant constituents in ethanol extract of the leaves. The antimicrobial activity was evaluated by the paper disc diffusion method. Antimicrobial tests showed that ethanol extract of the leaves was active against all the clinically isolated gram-positive and gram-negative bacteria tested. The hexane extract was active against *E. coli* and *V. cholerae* but inactive against *K. pneumoniae* and all tested gram positive bacteria. The ethanol extracts which demonstrated good antibacteria activities and also showed better minimum inhibitory concentration (MIC) values on *V. cholera and S. aureus* (31.25 µg/ml). The results suggest once again that the antimicrobial activity of the extracts of the leaves is a resultant of the antibacterial properties of the major and minor components in their chemical composition. The skin irritant property of prepared ointments were examined by albino rabbit and there were not shown skin irritant property.

Keywords: *E. odoratum,* hexane, 2E-dodecanol, *E. coli,* antibacteria

Introduction

Globally, there is an ascending trend in life threatening diseases and emergence of drug resistant- bacterial pathogens which has become a serious health concern (WHO, 2001). Therefore, there is an urgent need to search for newer and effective novel compounds from various biota. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in the world. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Santos, Oliveira & Tomassini, 1995). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Pierangeli & Windell, 2009).

Eupatorium odoratum (formerly *Chromolaena odorata*) belongs to the family Asteraceae and it consists of about 60 species spread over tropical, sub-tropical and warmer parts of temperate regions of the world. The fresh leaves of *E. odoratum* or the decoction has been used by practitioners of traditional medicine for the treatment of human burns, soft tissue wounds, ulcerated wounds, burn wounds, postnatal wounds and also for the treatment of leech bites,

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indigestion and skin infection (Panyaphu et al. 2011). *E. odoratum* is used as a traditional medicine in Myanmar, where its Burmese common name is "Jamani or Bi-Zat". While it has been widely considered a weed by agriculturalists (Vaisakh & Pandey, 2012), the aqueous extract and the decoction from the leaves of this plant have been used throughout Vietnam for the treatment of soft tissue wounds, burn wounds, and skin infections. A number of clinical studies done by Vietnamese as well as foreign medical workers has demonstrated the efficacy of this extract on the wound-healing process. In Thailand, the leaves are also used as cataplasm to stem external hemorrhage (Tonzibo *et al*, 2007).

In the last few years gas-chromatography mass-spectrometry has become firmly established as a key technological platform for metabolite profiling in plant. Gas chromatography mass -spectrometry (GC-MS) based metabolome analysis has profound applications in discovering the mode of action of drugs or herbicides and helps unravel the effect of altered gene expression on metabolism and organism performance in biotechnological applications (Kanthal, 2014). Thus, the aim of this work was to characterize and chemically quantify the crude extracts of the *E. odoratum* leaves as well as to evaluate antimicrobial activities. Moreover, this work illustrates that traditional remedies that are used by folk practitioners to improve healing can be examined in a scientific manner using in vitro wound-healing models. It could be that the synergistic properties of components of the natural extract contribute to the positive effects demonstrated on various wound-healing mechanisms.

Materials and Methods

Materials

Eupatorium odoratum (Taw-Bizat) leaves were collected from around of the Yadanabon University, Amarapura Township. The plant was identified by the botanist, Dr. Soe Myint Aye, Professor of the Department of Botany, University of Mandalay, Myanmar. Ethanol, and hexane were used to extract the absolutes (crude extract) from the odorata leaves. These chemicals were purchased from Able Chemical Store, Mandalay.

Figure 1 *Eupatorium odoratum L.*

Methods

Maceration

The *E. odoratum* leaves were cleaned with water, dried under shade for about 7 days and powdered by an electrical grinder (MX-GM 1011, 1000 ml). The extract of the leaves was also obtained by using the maceration of 150 g of this leaves with hexane, and ethanol (500 ml each). The extraction process was done over the period of seven days at room temperature. After filtration and evaporation of all solvent under reduced pressure yielded *E. odoratum* crude extracts of hexane (yellow extract; 0.54 g), and ethanol (dark-brown extract; 2.62 g), respectively. The extracts were then stored at 4°C in the dark until further analysis.

Figure 2 (a) Dry *E. odoratum* leaves (b) Maceration

Analysis of Antibacterial Activity

The various extracts were tested for their antibacterial activities using the disc diffusion technique. *S. epidermidis* DMST 15505, *S. aureus* DMST 8804, *B. subtilis* TISTR 008, *K. pneumoniae* DMST 4739, *V. cholerae* DMST 2873 and *E.coli* DMST 4212 were testing of the antibacterial activity of *E. odoratum* crude extracts. The standard discs containing bacitracin and chloramphenicol were used as positive control while all used solvents including ethanol, and *n*-hexane were used as negative control.

Paper disc diffusion method: All extracts of *E. odoratum* leaves were tested for their antibacterial activity by disc diffusion method. To prepare the testing bacteria, a single colony of each bacterial culture was transferred to 3 mL nutrient broth (NB) pH 6.9 (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and incubated for overnight at 37 °C and each bacterial culture was then spread on the surface of the nutrient agar medium (NA) obtaining from 8.0 g/L of NB and 15.0 g/L of agar (Union Science Co. Ltd, Chiang Mai, Thailand) using sterile cotton swab. Subsequently, filter paper discs (6 mm in diameter (Whatman No.1, Maidstone, UK)) were placed on surface of each inoculated plate. The crude extracts were prepared at two fold concentrations (0.135, 0.625, 1.25, 2.5, 5, and 10, 20 µg/mL). A small amount, 20 µL, of each was then added into a disc plate using a sterile micropipette. These plates were then incubated overnight at 37 °C, and then the diameter of the clear zone around each disc plate was measured in mm after incubation and was expressed as the mean value +/- the standard deviation (± SD). This experiment was performed 3 times on each extract.

Preparation of Ointment

Materials

The three ingredients (bee wax, coconut oil and crude extract of *E. odoratum*) were used in the preparation of ointment. Bee wax was purchased from Min Thar shop, Zay Cho market, Mandalay. Coconut oil was purchased from Tadau market, Mandalay Region.

Preparation Procedure

Coconut oil (4 ml) and bee wax (1.2 g) were placed into a cleaned, dried and weighed test tube and heated at double boiler with stirring at 70 °C for 15 mins. When the wax has completely melted, 0.1 ml of crude extract (ethanol & hexane) of *E. odoratum* were added into the mixture and allowed the entire mixture to remain on the hot plate until liquefied. The resulted liquefied mixture was poured into the sterilized bottle and cooled at room temperature for 20 min.

Figure 3 Prepared Ointment

Evaluation of Properties of Ointment

Skin Irritation Test by Albino Rabbit (Draize Test)

Three sexually mature female albino rabbits, weighing 2.5 kg, originated from the breeding colony of laboratory animals were used. Test animals were kept within a limited-access rodent facility with environmental conditions set to a temperature of 25±2 °C, a humidity of 60-90% relative humidity (RH). Animals were provided with commercial rabbit-diet and drinking water. The 3 cm^2 area of hair from back quarter of rabbit was shaved by using blade and cleaned by the clean water. This shaved area of the skin of each rabbit was divided into three marked area. The first marked area was control position (no treatment) and another two marked areas were swabbed with 0.2 ml of sample prepared ointment (hexane, & ethanol) on shaved area (Figure 4). The animals were return to the cages. The skin area of rabbit was checked after 2 h, 24 h, 48 h and 72 h. If the area was showed redness (erythema), the animal was suffering the skin irritation. It was checked those skin areas daily for 3 days. This experimental test was conducted at Laboratory Animal Services Division, Department of Medical Research, Yangon.

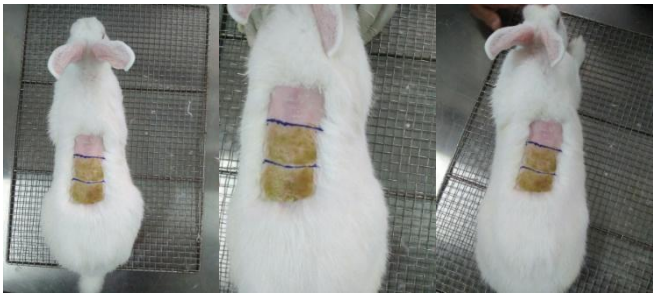


Figure 4 Skin Irritation Test

Results and Discussion

The extracts obtained from maceration were produced greenish colored with a strong pleasant odor and different yields. Higher yields were detected with ethanol maceration versus *n*-hexane maceration. A total of 64 volatile components were identified from two extracts by GC-MS analysis. The identified constituents, their percentages, and retention indices are listed in Table 1. Figure 5 and 6 have been shown for the chromatogram of extracts of *E. odoratum.* Significant qualitative and quantitative variations of most identified constituents were detected among these extracts. Thirty-seven components were detected in the *n*-hexane extract, representing 94.24% of the total. The major components were n-tetradecanol (52.98%) and methyl hexadecanoate (40.46 %), followed by canellal (0.15 %), and caryophyllene oxide (0.25 %).Thirty-five compounds were detected in the ethanol extract, representing 93.59% of the identified compounds, including 2E-dodecanol (55.24%), n-tetradecanol (37.42%), iso-longifolol acetate (8.09%), α-ylagene (4.73%), pregeijerene B (2.14%), and cis-carveol (1.70%) as the major constituents.

Most volatile components identified were similar to previous reports although the different quantities of compounds were detected. In the present investigation, monoterpenes and sesquiterpenes and their derivatives predominated in the hexane and ethanol extracts of *E. odoratum* leaves. Among identified compound, caryophyllene oxide, *n*-tetradecanol, methyl hexadecanoate, andn-heneicosane were found in this two extracts. In general, the application of extracts such as the biocide action are depend on the composition of these oils and extracts (Chamorro, Zambón, Morales, Sequeira, & Velasco, 2012).

Table 1 Chemical Composition of Various Extracts Obtained from *E. odoratum* Leaves

No.	Compound	RI	Extracts	
			Hexane	Ethanol
1	Isovaleric	835	-	0.057
2	2-Ethoxy ethyl acetate	904	-	0.012
3	Sabinene	975	0.02	-
4	<1,2,4>-Trimethyl benzene	1025	0.04	-
5	Methyl-(3E)-hexenoate	934	0.01	-
6	Mesitylene	995	0.02	-
7	Ethyl acetal-(Leaf Alcohol)	1088	-	0.002
8	Geijerene	1143	0.03	0.046
9	cis-Carveol	1229	-	0.185
10	3Z-Hexenyl 2-methyl butanoate	1232	t	-
11	Pregeijerene B	1276	t	0.127
12	Fenchol-<2-ethyl-endo>	1288	-	t
13	n-Tridecane	1300	t	-
14	2-Adamantanone	1311	t	-
15	a-Ylagene	1375	-	0.133
16	4aa,7a,7ab-Nepetalactone	1387	-	t
17	Damascone	1387	-	t
18	β-Cubebene	1388	0.042	-
19	β-Bourbonene	1388	t	-
20	3-Dodecanone	1390	-	t
21	n-Tetradecane	1400	0.006	-

No.	Compound	RI	Extracts	
			Hexane	Ethanol
22	α-Funebrene	1402	-	t
23	Z-Caryophyllene	1408	t	-
24	β-Ylangene	1420	0.014	-
25	Linalool butanoate	1421	t	-
26	1-Phenyl hexan-3-one	1425	-	0.016
27	Dictamnol	1429	-	t
28	γ-Elemene	1436	-	t
29	2E-Dodecanol	1466	-	55.24
30	cis-Muurola-4(14),5-diene	1466	-	t
31	neo-methyl lactate	1469	t	-
32	Dauca-5,8-diene	1472	t	-
33	β-Thujaplicin	1477	0.05	-
34	Butylated hydroxytoluene	1515	0.01	0.01
35	δ-Amorphene	1518	t	-
36	δ-Cadinene	1523	-	0.042
37	Laciniata furanone G	1529	t	-
38	Germacrene B	1561	-	0.023
39	Spathulenol	1578	-	0.003
40	Caryophyllene oxide	1583	0.256	t
41	1-Hexadecene	1589	-	0.003
42	β-Biotol	1613	-	t
43	Massoia dodecalactone	1686	t	0.0471
44	*n*-Tetradecanol	1672	52.98	37.417
45	Geranyl tiglate	1696	t	-
46	n-Heptadecane	1700	-	-
47	iso-Longifolol		-	0.094
48	iso-Longifolol acetate	1820	t	0.116
9	Acorone	1820	0.065	t
50	Avocadynofuran	1826	-	0.007
51	cis-Thujopsenic acid	1864	-	0.001
52	Methyl hexadecanoate	1921	40.469	-
53	Isohibaene	1937	0.03	-
54	Ethyl hexadecanoate	1993	-	t
55	Canellal	2046	0.157	-
56	Methyl linoleate	2085	t	-
57	Methyl linoleate	2095	-	t
58	Linoleic acid	2133	t	-
59	n-Heneicosane	2100	0.01	0.01
60	Methyl octadecanoate	2125	t	-
61	Linoleic acid	2133	-	t
62	Nezukol	2133	0.03	-
63	Incensole oxide	2237	t	-
64	<7-α-hydroxy->Manool	2237	t	-
Number of Compounds			38	35
% of Compounds			94.239	93.5911

RI: Retention index relative to C_8–C_{25}-alkanes on TG-5 column, MS: NIST and Wiley library, and the literature, t: trace (<0.1%).

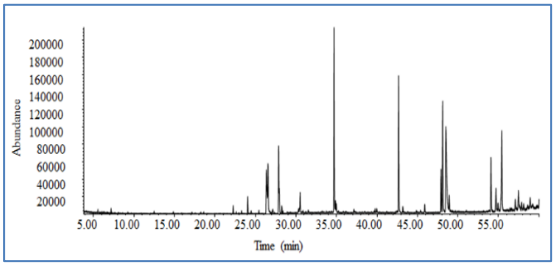


Figure 5 GC-MS Chromatogram of Hexane Extract of *E. odoratum* Leaves

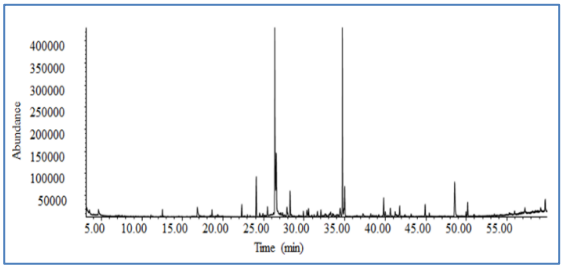


Figure 6 GC-MS Chromatogram of Ethanol Extract of *E. odoratum* Leaves

The observation of the antimicrobial activities has been tabulated in Table 2 and it was found to be varying between 31.25-1000 µg/mL, with respect to most of the test bacteria. The various extracts provided various efficiencies of antibacterial activity depending on the tested bacterial strains. The ethanolic extract had the greatest antibacterial activity against all tested Gram-positive and negative bacteria as compared with the hexane extract. An increased inhibition zone of the ethanol extract was observed with *B. subtilis*, followed by *S. epidermidis*, *K. pneumoniae*, and *E. coli* measured at 10.3, 10.1, 10 and 9.3 mm, respectively. Similar inhibition zone on *V. cholera* strains was detected in all extracts of the leaves. The hexane extract inhibited only two Gram-negative bacteria, *E. coli* (10.3 mm) and *V. cholera* (7.6 mm). As a result, *E. coli and V. cholera* were more sensitive to various extracts (see Table 2 & Figure7).

Table 2 Antibacterial Activity of Different Extracts of *E. odoratum* Leaves (diameter, mm ± SD)

Bacteria	Chloramphenicol	Bacitracin	Extract	
			Hexane	Ethanol
Gram-positive bacteria				
S. epidermidis	19.85 ± 0.75	27.8 ± 0.3	-	10.1±0.1
S. aureus	18.8 ± 0.1	19.45 ± 0.75	-	7.5±0.3
B. subtilis	9.75 ± 0.25	10.65 ± 0.35	-	10.3±0.35
Gram-negative bacteria				
E. coli	9.4 ± 0.4	16.4 ± 0.6	10.3±0.2	9.3±0.4
K. pneumoniae	12.7 ± 0.3	18 ± 0.5	-	10±0.5
V. cholera	11.05 ± 0.45	18.65 ± 0.65	7.6±0.13	8±0.2

–: not detected

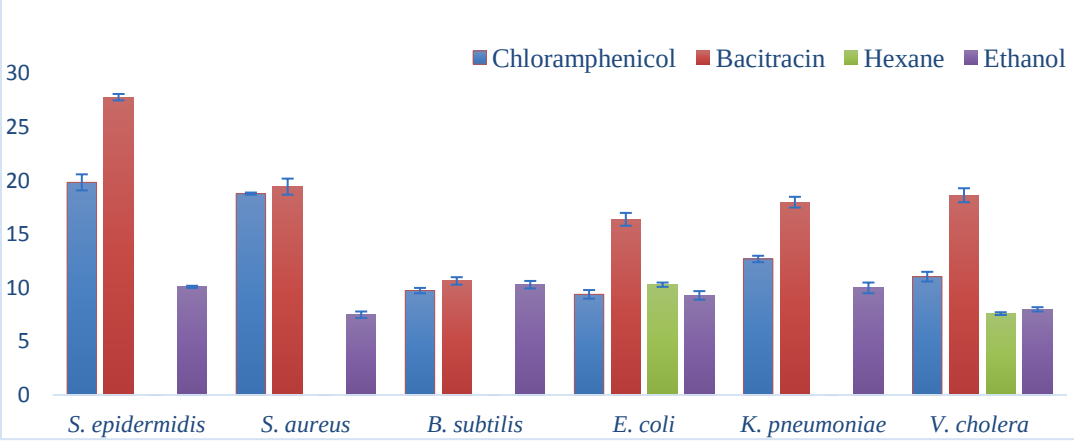


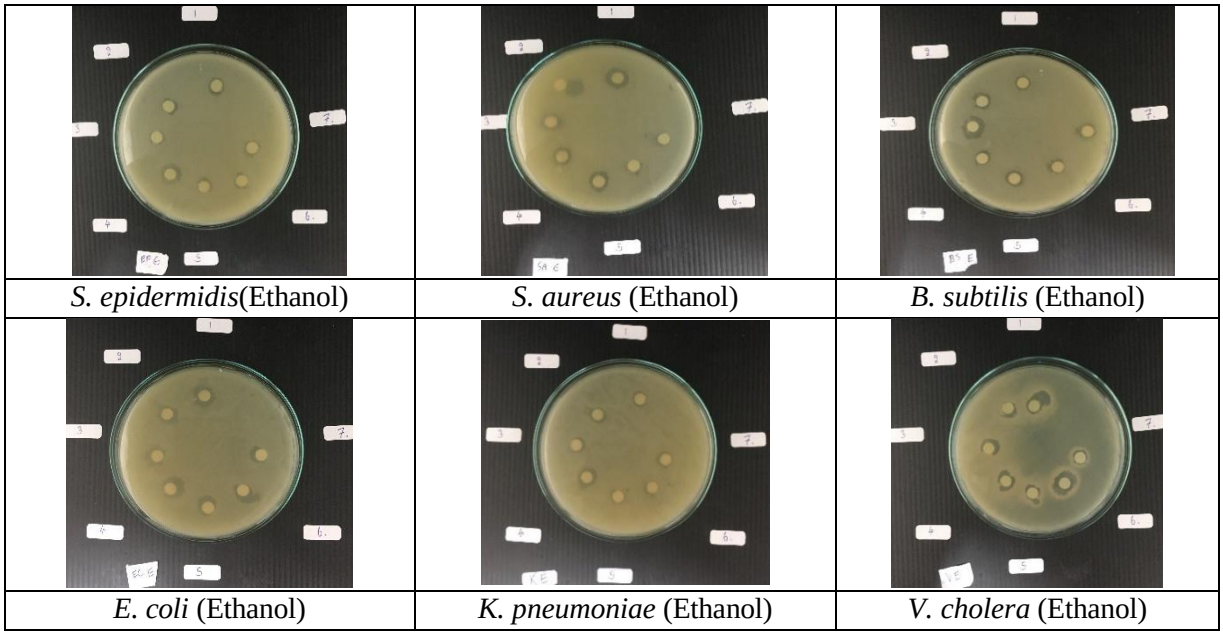
Figure 7 Zone of Inhibition produced by Crude Extracts of *E. odoratum* Leaves and Antibiotics

The antibacterial activities of all extracts were evaluated by the diameter of inhibition and MIC values compared with those obtained from positive control, bacitracin and chloramphenicol. MIC values of all samples are shown in Table 3, while the antibacterial activities of all samples on Gram-negative and Gram-positive bacteria are summarized in Figure 8 & 9. The ethanol extracts of *E. odoratum* leaves which demonstrated good antibacteria activities and also showed better minimum inhibitory concentration (MIC) values on *V. cholera and S. aureus* (31.25 µg/mL). The antimicrobial activities of crude extracts of *E. odoratum* for bacterial strains like *E. coli* DMST 4212 and *B. subtilis* TISTR 008 were inhibited at MIC value of 62.5 µg/mL. In addition, *S. epidermidis* DMST 15505, and *K. pneumoniae* DMST 4739 for the ethanolic extract were inhibited at MIC value of 125 µg/mL, while the hexane extract presented higher MIC values ranging from 62.5 to 500 µg/mL (see Table 3).). According to this information, it was clearly found that *E. odoratum* has antimicrobial activity against the pyogenic pathogens. Among the extracts selected for the study, the ethanolic extract of *E. odoratum* shows highest antimicrobial activity against the target microbial flora, when compared with hexane extract.

It was reported that the plant extract have significant antimicrobial activity against pyogenic microorganism due to the presence of various phytochemical constituents such as alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins. Moreover, the presence of bioactive compounds and the supporting studies accompanied with them depicts that the *E. odoratum* is highly active against pyogenic pathogens. This shows that the phytochemical constituents can be used to treat pyogenic infection. The potency of the extract is also based on the method of extraction and concentration of plant extract. In addition, diversity of major and minor constituents in the extracts due to the synergistic effects could be affected on the consideration to account for their biological activity (Giweli *et al*., 2013).

Table 3 Minimum Inhibitory Concentration of Crude Extracts of *E. odoratum* Leaves

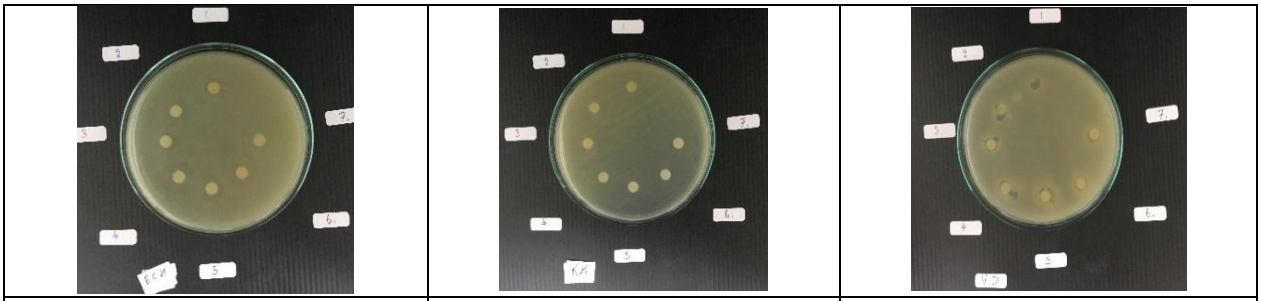
Sr. No.	Bacteria	MIC of Hexane Extracts	MIC of Ethanol Extracts
Gram-positive bacteria			
1.	*S. epidermidis* DMST 15505	-	125 µg/mL
2.	*S. aureus* DMST 8840	-	31.25 µg/mL
3.	*B. subtilis* TISTR 008	-	62.5 µg/mL
Gram-negative bacteria			
4.	*E. coli* DMST 4212	62.5 mg/ml	62.5 µg/mL
5.	*V. cholerae* DMST 2873	500 mg/ml	31.25 µg/mL
6.	*Ps. aeruginosa* DMST 4739	125mg/ml	125 µg/mL



S. epidermidis(Ethanol) | *S. aureus* (Ethanol) | *B. subtilis* (Ethanol)

E. coli (Ethanol) | *K. pneumoniae* (Ethanol) | *V. cholera* (Ethanol)

Figure 8 Inhibition Zones of the Tested Bacteria by Ethanol Extract of *E. odoratum* Leaves



E. coli (Hexane) | *K. pneumoniae* (Hexane) | *V. cholera* (Hexane)

Figure 9 Inhibition Zones of the Tested Bacteria by Hexane Extracts of *E. odoratum* Leaves

Skin irritant property of ointment was shown in Table (4). Rabbit with ointment was not shown the sign of skin redness (erythema) after 2 h, 24 h, 48 h and 78 h. From the above results, ointments formulated with the two crude extracts were not shown skin irritation sign. The results are shown in Table (4) and Figure (10). Plant products have been shown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing due to the presence of active terpenes, alkaloids and flavonoids (Ibrahim, 2018). The study reveals that both ethanolic and hexane extracts of

E. odoratum possesses good skin irritation properties which may be attributed to the individual or combined action of phytoconstituents like alkaloids, and terpenoids present in the extracts.

Table 4 Skin Irritant Property of Prepared Ointments

Animal	Sample	Interval of skin exposure			
		2h	24h	48h	72h
Albino rabbit	Ointment (Ethanol)	0.0	0.0	0.0	0.0
	Ointment (Hexane)	0.0	0.0	0.0	0.0
	Control	0.0	0.0	0.0	0.0

Note: no erythema = 0.0, slightly erythema = 0.2-0.4, erythema = 0.6

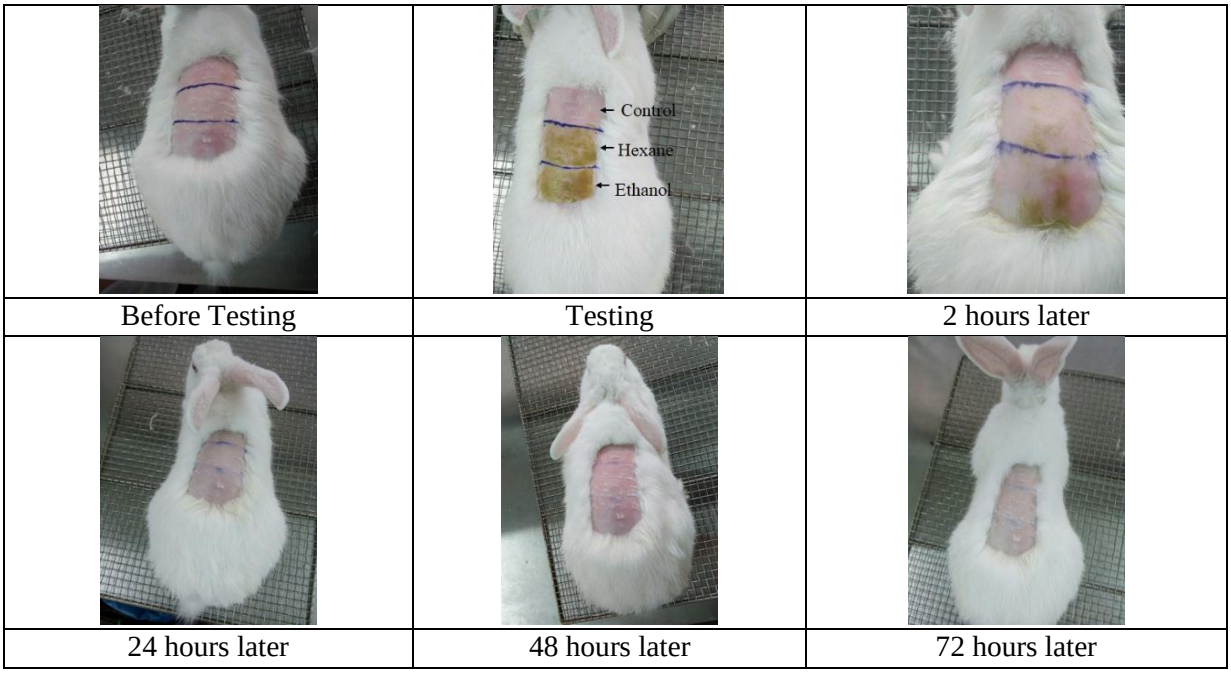


Figure 10 Skin Irritation score observation

Conclusion

The present work has been designed to evaluate the chemical constituents and antimicrobial potential of *E. odoratum* with a view to contributing to the search for beneficial uses of this invasive plant which is a menace to farmers. It is quite evident from this work that *E. odoratum* is an important medicinal plant. It contains a number of phytoconstituents, which are the key factors in the medicinal value of this plant. According to all of positive findings, it is concluded that the traditional plants may represent new sources of antimicrobial with stable, biologically active compounds that can establish a scientific base for the use of plants in modern medicine. Further investigations are necessary to determine the bioactive constituents present in the extracts to prove its potential in clinical studies.

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