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ARTICLE

Antimicrobial Effect of Ethyl Acetate Extract and Eluted Fraction of Ethyl Acetate Extract of *Chromolaena Odorata* with Standard Antibiotics on Different Bacteria.

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Abstract

The present study was aimed for the comparative analysis of antimicrobial effect of ethyl acetate extract and eluted fraction of ethyl acetate extract of *Chromolaena odorata* with standard antibiotics on different bacteria including Pseudomonas, Enterococcus, Staphylococcus and Streptococcus. Dried powdered plant material was used in this study, extracted with ethyl acetate using soxhlet apparatus. Column chromatography was used for the separation and purification of active compounds in ethyl acetate extract of *Chromolaena odorata*. The antimicrobial effect of the crude extract and eluted fraction was evaluated using well diffusion method. The antimicrobial assay confirmed that the eluted fraction showed greater antimicrobial activity than crude extract. Comparative analysis of antimicrobial effect of crude extract and eluted fraction with standard antibiotics clearly exhibited the antimicrobial competency.

Keywords: *Chromolaena odorata*, antimicrobial, extraction, Elution, antibiotic, separation

Introduction

Chromolaena odorata is a species of flowering shrub in the sunflower family, Asteraceae. It is native to North America, from Florida and Texas to Mexico and the Caribbean, and has been introduced to tropical Asia, West Africa, and parts of Australia. It is used as a traditional medicine in Indonesia. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds.

The several parts of this herb have been used to treat burns, and skin infections. Furthermore, it has also been shown to possess anticancer (Adedapo et al., 2016, Kouame et al., 2013), antidiabetic (Onkaramurthy et al., 2013, Ijioma et al.,2014, Uhegbu et al., 2016), anti-hepatotoxic (Asomugha et al., 2014), anti-inflammatory (Owoyele et al.,2005, Hanh et al.,2011,Pandith et al.,2013), antimicrobial (Mullika et al., 2005), and antioxidant properties (Phan et al., 2001, Akinmoladun et al., 2007, Rao et al., 2010). Its phytochemical components are alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids.

Kamath N et al., (2015) reported that the ethanolic, ethyl acetate and petroleum ether extract of *Chromolaena odorata* leaves showed antimicrobial activity against all microorganisms whereas the aqueous extract revealed variable degree of antibacterial activity. Lovet T. Kigigha and Douye Victor Zige (2013) studied the antibacterial effect of *Chromolaena odorata* extract against the enteric pathogenic bacteria forms showed very low. Olukoya, (1986) revealed that the plants contain substances that are antimicrobial. Foluke Odutayo et al., (2017) reported that in methanolic and ethyl-ether extract of *Chromolaena odorata* how low antibacterial effect against K.pseudomonas, P.aeruginosa and E.facecalis when compared to other bacteria taken. Nwachukwu et al., (2016) stated that the crude extract of *Chromolaena odorata* was effective on the isolated bacteria on wounds. Hridhya K V et al., (2017) reported that the plant extract have significant antimicrobial activity against pyogenic microorganism due to the presence of various phytochemical constituents. Pierangeli G Vital et al., (2009) *Chromolaena odorata* extracts revealed antibacterial activities, inhibiting the growth of *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium*. Elijah I. Ohimain et al., leaf extracts of *Chromolaena odorata* were found to exhibit a significant antibacterial activity against S. Typhi as compared to *E. coli*, revealing that the extract of *Chromolaena odorata* is more effective on *S. Typhi* than *E. coli*. Afolayan Michael et al., (2015) results indicated that the leaf extract was the most active against all tested microorganisms with the minimum inhibitory concentration (MIC) ranging from 7.81 – 31.25 µg/ml. N Nanadini et al., (2014) results revealed that the leaf extracts of *Chromolaena odorata* contains bioactive compounds having antimicrobial activity and so useful in human medicine.

Now a day, antibiotics are overused or used incorrectly was the major reason for the emergence of multi drug resistance strains. Some bacteria develop genes for drug resistance in plasmids, they are able to spread drug resistance to other strains. The overuse of antibiotics leads to major side effects such as nephron toxicity, kidney failure etc. For centuries plants have been used throughout the world as

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drugs and remedies for various diseases (UNESCO, 1996).

Hence, in the present study, an attempt was made for extraction, elution of phytochemicals from the ethyl acetate extract of selected medicinal plant *Chromolaena odorata* on various bacteria and comparative analysis among them and thereby providing a ray of hope to find alternative solutions for antibiotics to prevent the emergence of resistant strains of bacteria.

Materials and Methods

Chemicals

The chemicals such as Hexane, Chloroform, Ethyl acetate, DMSO, and other chemicals (analytical or equivalent grade of high purity) used for the experiment were purchased from SRL, Bombay.

Plant material

Chromolaena odorata were locally collected from Thiruvananthapuram district, Kerala, and botanically authenticated. The whole plants collected were washed thoroughly 2 – 3 times with running water and dried in 40 – 60 ^{o}C in hot air oven. The dried materials were finely powdered and stored in air tight bottle and used for soxhlet extraction.

Isolation of ethyl acetate fraction

A quantity of 500 gm of hot air dried powdered plant material was extracted in 2.5 L of ethyl acetate using soxhlet. The soxhletion with ethyl acetate was done for one week to obtain extract. The final extract of *Chromolaena odorata (C. odorata)* collected was concentrated under rotary evaporator and preserved at 4°C in air tight bottles for conducting the further experiment (study material I).

Isolation of active ingredients

A part of the concentrated extract obtained from ethyl acetate, which is to be separated was first applied to the column at the top. When all the concentrated extract had been adsorbed on the top of the column, more hexane was added and the column was allowed to run. Some compounds of the mixture are adsorbed strongly, while others less strongly. The more strongly a substance is adsorbed, the more slowly it moves down the column. The elution was carried out using the mixture of hexane and chloroform (9:1 i.e. 90 ml: 10 ml Hexane: Chloroform). Each 50.0 ml fraction was separately collected for a period of 30 days. Identical fractions were pooled together (Bobbit 1963).

The eluted pooled fractions (9:1 of Hexane:Chloroform) were concentrated under rotary evaporator. The concentrated material was crystallized and re-crystallized using chloroform and preserved in the desiccator at 4°C in air tight bottles as study material II for conducting the further experiment.

Experimental organisms

Pure slant cultures of different bacterial strains such as Pseudomonas, Staphylococcus, Streptococcus and Enterococcus were collected from the Research laboratory of South Travancore Hindu College, Nagercoil, Kanyakumari District. The slant cultures were brought to the laboratory within a short time to avoid any possible contamination. It was stored at 4 ^{o}C in refrigerator for future preparation of sub culture in nutrient agar.

Experimental design

All the Pseudomonas inoculated plates were divided into three groups. (Each group contains two (2) Pseudomonas inoculated plates respectively).Group I: has 4 sterilized well received 10 μl, 20 μl, 30 μl and 40 μl of study material I. Group II: has 3 sterilized well received 10 μl of study material I, 10 μl of study material II and 10 μl DMSO. Group III: only standard antibiotics discs.

All the Staphylococcus inoculated plates were divided into two groups. (Each group contains two (2) Staphylococcusinoculated plates respectively). Group I: has 4 sterilized well received 10 μl, 20 μl, 30 μl and 40 μl of study material I respectively. Group II: has 3 sterilized well received 10 μl of study material I 10 μl of study material II and 10 μl DMSO. All the Streptococcus inoculated plates were divided into two groups. (Each group contains two (2) Streptococcus inoculated plates respectively).Group I: has 4 sterilized well received 10 μl, 20 μl, 30 μl and 40 μl of study material I respectively. Group II: has 3 sterilized well received 10 μl of study material I, 10 μl of study material II and 10 μl DMSO. All the Enterococcus inoculated plates were divided into two groups. (Each group contains two (2) Enterococcus inoculated plates respectively).Group I: has 4 sterilized well received 10 μl, 20 μl, 30 μl and 40 μl of study material I respectively. Group II: has 3 sterilized well received 10 μl of study material I, 10 μl of study material II and 10 μl DMSO.

Antibacterial Susceptibility Test

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disc-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (10–100 μL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. 10 μL, 20 μL, 30 μL and 40 μL of ethyl acetate extract and 10μL of eluted fraction of ethyl acetate extract of Chromolaena odorata and 10 μL of DMSO were added to each well (8 mm diameter holes cut in the agar gel, 20 mm apart from one another) separately. The systems were incubated for 24 h at 36°C ± 1°C, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm.

Statistical Analysis

The ANOVA-Two Factor was conducted with inhibition zone diameters of treated groups (Group I – 10 μl , 20 μl, 30 μl, 40 μl of ethyl acetate extract with 10 μl of control

(DMSO alone)),(Group II - 10 µl of ethyl acetate extract, 10 µl of eluted fractions with 10 µl of control (DMSO alone)). The ANOVA-One Factor was conducted with inhibition zone diameters of treated group III-antibiotics disc in the pseudomonas inoculated plates with control).The results are expressed as mean + SE, and Paired Samples test (SPSS - 19 Computer package) was used to assess statistical significance.

Results

Antimicrobial activity of four different concentration of ethyl acetate extract of Chromolaena odorata (C. odorata) on Pseudomonas, Staphylococcus, Streptococcus and Enterococcus. Results are given in Table no: 1

Administration of four different concentrations of ethyl acetate extract of *Chromolaena odorata (C. odorata)* on the separate wells made on the Pseudomonas bacterial culture individually showed a well-marked inhibition zone in dose dependent manner. The increased inhibitory zone was observed in the well received 40 µg (40 µl) of ethyl acetate extract dose (Fig 1) The wells treated with different concentrations of 10µg, 20µg, 30µg and 40 µg of ethyl acetate extract of *Chromolaena odorata* individually on Streptococcus and Staphylococcus inoculated plates also showed concentration dependent inhibition zone (Fig 2, 3) But different concentration of study material I did not show any inhibitory activity on Enterococcus inoculated plates (Fig 4).

Comparative effect of antimicrobial activity of ethyl acetate extract and eluted fraction (hexane: Chloroform (9:1)) of ethyl acetate extract of Chromolaena odorata (C.odorata) on Pseudomonas, Enterococcus, Streptococcus and Staphylococcus. Results are given in Table no: 2

The wells treated with the concentrations of 10µg ethyl acetate extract and 10µg eluted fraction (hexane : Chloroform (9:1)) of ethyl acetate extract of *Chromolaena odorata* individually on Pseudomonas inoculated plates showed well marked inhibition zone in both (Fig 5) From the experiment we found increased inhibition zone was observed in well received the eluted fraction. Administration of ethyl acetate extract and eluted fraction on the separate well made on Streptococcus and Staphylococcus inoculated plates also showed inhibition zone (Fig 6, 7). The better inhibitory activity was noted in the well administered with eluted fraction. Treatment of extract did not show any inhibitory activity on Enterococcus inoculated plates whereas the eluted fraction showed a well-marked inhibition zone in Enterococcus inoculated plates (Fig 8).

Antimicrobial activity of standard antibiotics on Pseudomonas

Table 3 clearly indicated the inhibitory zone diameters of various standard antibiotics available in markets. In Pseudomonas inoculated plates the maximum inhibitory activity expressed by the standard antibiotics such as Levofloxa-

Table 1. Dosage effect of antimicrobial activity of four different concentration of ethyl acetate extract of *Chromolaena odorata* (*C. odorata*) on Pseudomonas Enterococcus, Streptococcus and Staphylococcus.

Inhibition Zone (IZD) in mm	Sl.No.	Name of the bacteria	Different concentration of ethyl acetate extract as Study material I				Control (DMSO alone)
			10µl (10µg/ml)	20µl (20µg/ml)	30µl (30µg/ml)	40µl (40µg/ml)	10µl (10µg/ml)
	1.	Pseudomonas	16 ± 0.30	16 ± 0.30	16 ± 0.30	22 ± 0.50	8 ± 0.01
	2.	Enterococcus	8 ± 0.01	8 ± 0.01	8 ± 0.01	8 ± 0.01	8 ± 0.01
	3.	Streptococcus	16 ± 0.30	18 ± 0.40	18 ± 0.40	19 ± 0.40	8 ± 0.01
	4.	Staphylococcus	19 ± 0.40	19 ± 0.40	19 ± 0.40	21 ± 0.50	8 ± 0.01

Table 1A. ANOVA Two Factor – showing significance of IZDs (inhibition Zone diameters) obtained between the effect of antimicrobial activity of four different concentration of ethyl acetate extract of *Chromolaena odorata* on Pseudomonas, Enterococcus, Streptococcus and Staphylococcus.

Source of variation	Sum of square	Degree of freedom	Mean square	F – value calculated	P- value	F- critical value
Between groups	519.5	3	173.1667	951.0472	9.85E-22	3.098391
Within groups	414.6	4	103.65	569.2553	2.73E-20	2.866081

Table 2. Evaluation of comparative effect of antimicrobial activity of the ethyl acetate extract of *Chromolaena odorata (C. odorata)* and eluted fraction (hexane: Chloroform (9: 1)) of ethyl acetate extract of *Chromolaena odorata (C. odorata)* on different bacteria such as Pseudomonas, Staphylococcus, Streptococcus and Enterococcus.

Inhibition Zone (IZD) in mm	Sl. No.	Name of the bacteria	Study Material I Ethyl acetate extract	Study Material II Eluted fraction (90:10 hexane : Chloroform)	Control (DMSO alone)
			10µl (10µg/ml)	10µl (10µg/ml)	10µl (10µg/ml)
	1.	Pseudomonas	16 ± 0.30	18 ±0.40	8 ± 0.01
	2.	Enterococcus	8 ± 0.01	22 ± 0.50	8 ± 0.01
	3.	Streptococcus	16 ± 0.30	20 ± 0.40	8 ± 0.01
	4.	Staphylococcus	19 ±0.40	22 ± 0.50	8 ± 0.01

Table 2A. ANOVA Two Factor – showing significance of IZDs (inhibition Zone diameters) obtained between the comparative effect of antimicrobial activity of ethyl acetate extract and eluted fraction (hexane : Chloroform (9:1)) of ethyl acetate extract of *Chromolaena odorata (C. odorata)* on Pseudomonas, Enterococcus, Streptococcus and Staphylococcus.

Source of variation	Sum of square	Degree of freedom	Mean square	F – value calculated	P- value	F- critical value
Between groups	41.83333	3	13.94444	72.09536	6.05E-08	3.490295
Within groups	626.3333	2	313.1667	1619.13	2.53E-15	3.885294

cin (QB), Ciprofloxacin (RC), Ampicillin / Sulbactum (AS) and Roxythromycin (AT). The other antibiotics like Cephalexin (PR), Linezolid (LZ), Cloxacillin (CX), Gentamycin (GM), Co-trimoxazole (BA), Tetracycline (TE), Cefotaxime (CF) and Lincomycin (LM)showed only very little inhibition zone diameters on Pseudomonas inoculated plates. The result is graphically represented in graph no: 1.

Comparative study on the antimicrobial effects of 10µl of crude extract, 10µl of eluted fraction and standard antibiotics in Pseudomonas.

The Pseudomonas inoculated plates received 10µg ethyl acetate extract and 10µg eluted fraction (hexane: Chloroform (9:1)) of ethyl acetate extract of Chromolaena odorata and standard antibiotics showed inhibition zone. Comparative analysis of antimicrobial effect of study material I, study material II with standard antibiotics clearly exhibited the antimicrobial competency. The statistical analysis clearly revealed that the eluted compound has significant antibacterial activity against the tested microbe. The analysis were graphically represented in graph no: 2.

Discussion

Our study revealed that the wells treated with different concentrations of 10µg, 20µg, 30µg and 40 µg of ethyl acetate extract (crude) of *Chromolaena odorata* on Pseudomonas, Staphylococcus and Streptococcus inoculated plates showed well marked antibacterial activity in dose dependent manner. Our results are in agreement with Olukoya and Kigigha and Zige (2013). The increased inhibitory zone was developed in wells received 40 µg of ethyl acetate extract of Chromolaena odorata. But different concentration of ethyl acetate extract (study material I) did not show any inhibitory activity on Enterococcus inoculated plates.

The results obtained from the work revealed that the maximum inhibition zone diameters produced by 10µg of eluted fractions of ethyl acetate extract of *Chromolaena odorata* than 10µg of ethyl acetate crude extract of Chromolaena odorata. The eluted fraction showed greater antimicrobial activity than crude extract. This may be due to the active compound purity in eluted fraction than the crude extract. Maximum inhibition zone exhibited by the eluted fraction is due to compound mobility and may be permeability through the bacterial cell membranes. So it is necessary to isolate, identify and characterize the active principal present in the crude extract.

Comparative analysis of antimicrobial effect of study material I, study material II with standard antibiotics clearly exhibited the antimicrobial competency. So the antimicrobial property of medicinal plant is perhaps due to the presence of various secondary metabolites such as alkaloids,

Table 3. Antimicrobial activity of standard antibiotics on Pseudomonas.

Antibiotics	Pseudomonas IZD (mm)	Control (DMSO alone)
Ampicillin / Sulbactum (AS)	15 ± 0.20	6 ± 0.01
Co-trimoxazole (BA)	8 ± 0.01	6 ± 0.01
Cephalexin (PR)	9 ± 0.01	6 ± 0.01
Tetracycline (TE)	8 ± 0.01	6 ± 0.01
Cefotaxime (CF)	8 ± 0.01	6 ± 0.01
Ciprofloxacin (RC)	12 ± 0.10	6 ± 0.01
Levofloxacin (QB)	11 ± 0.10	6 ± 0.01
Linezolid (LZ)	9 ± 0.01	6 ± 0.01
Cloxacillin (CX)	9 ± 0.01	6 ± 0.01
Lincomycin (LM)	8 ± 0.01	6 ± 0.01
Roxythromycin (AT)	10 ± 0.10	6 ± 0.01
Gentamycin (GM)	9 ± 0.01	6 ± 0.01

Table 3A. ANOVA-Single Factor -– showing significance of IZDs (inhibition Zone diameters) obtained between the comparative effect of antimicrobial activity of ethyl acetate extract, eluted fractions of the extract of *Chromolaena odorata* and standard antibiotics on pseudomonas.

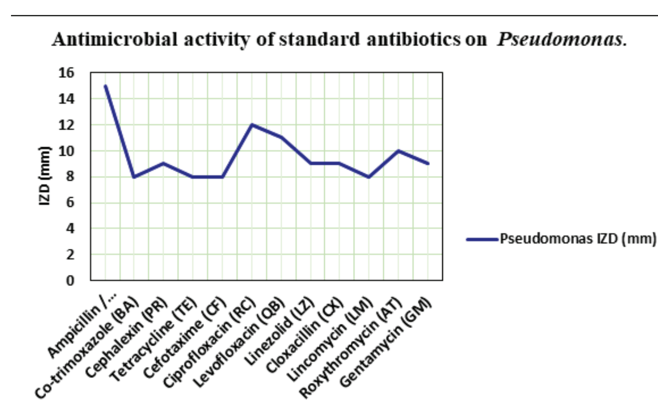
Source of variation	Sum of square	Degree of freedom	Mean square	F – value calculated	P- value	F- critical value
Between groups	327.2	14	23.37143	499.5318	2.15E-17	2.424364

flavonoids, glucosides, phenol, saponins and sterols etc.

The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal. Antibiotics like Penicillin and Erythromycin, which used to be one-time miracle cures are now less effective because bacteria have become more resistant. Inappropriate antibiotic treatment and overuse of antibiotics have been a contributing factor to the emergence of resistant bacteria.

In recent times, due to several intricacies of modern antibiotics, there has been significant shift towards alternative treatment and herbal remedies Patwardhan B, Vaidya ADB and Chorghade M (2004). Antibiotic screening of plants and natural products used in alternative systems of medicines like Ayurvedic and Unani is a major thrust of R&D, in the Indian pharmaceutical sector today Afaq SH et al, (2004) and Baijal R, Patel N and Kolhapure SA (2004).

Graph 1. Antimicrobial activity of standard antibiotics on Pseudomonas.



Dosage effect of four different concentration of ethyl acetate extract of *Chromolaena odorata (C. odorata)*

Fig. 1. on Pseudomonas

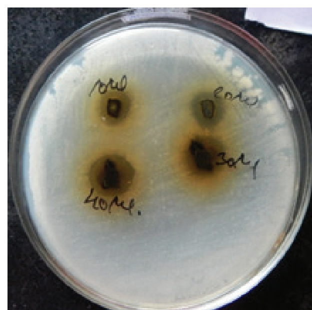


Fig. 2. on Streptococcus

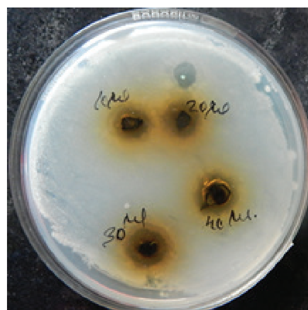
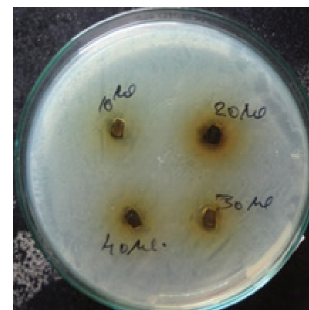


Fig. 3. on Staphylococcus

Fig. 4. on Enterococcus



Comparative effect of extract and eluted fraction of ethyl acetate extract of *Chromolaena odorata (C. odorata)*

Fig. 5. on Pseudomonas

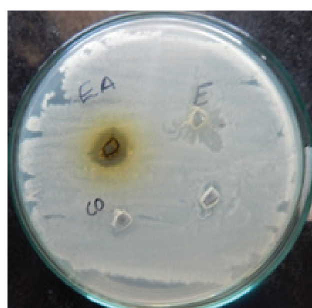


Fig. 6. on Streptococcus

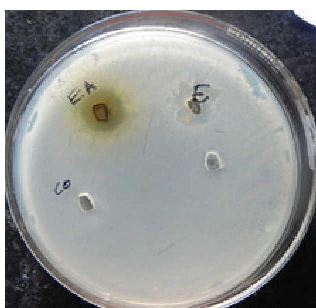
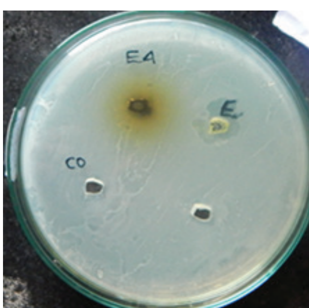
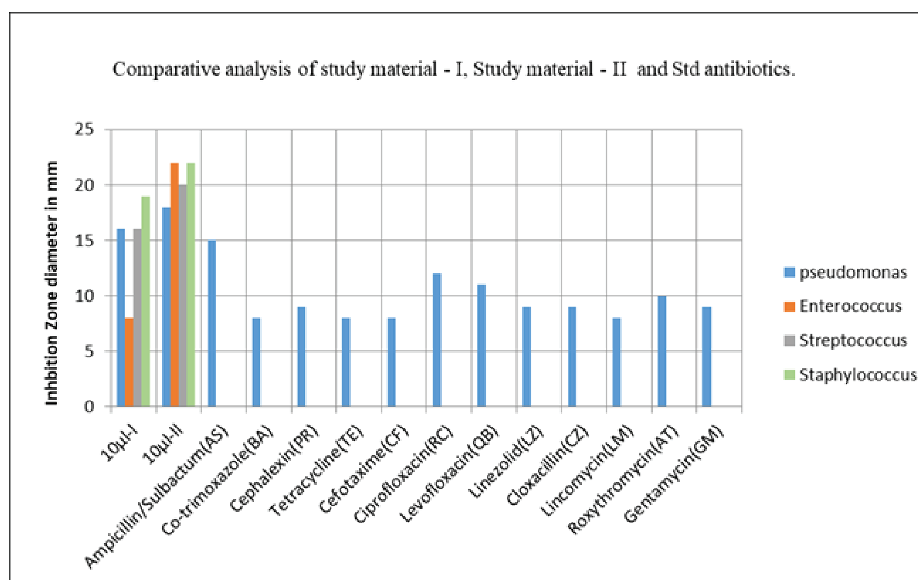


Fig. 7. on Staphylococcus

Fig. 8. on Enterococcus



Graph 1. Comparative analysis of study material - I, Study material - II and Std antibiotics.



The results obtained from the biological experiment revealed that the selected exotic medicinal plant extract and eluted fraction of ethyl acetate extract have significant antimicrobial activity against the tested microbes. This comes to the conclusion that medicinal plant *Chromolaena odorata* which are traditionally used in Ayurveda or in other herbal medical practices have scientific basics and can be modified to produce specific medicines against each bacterium.

References

1. Adedapo A A, Oyagbemi A A, Fagbohun OA, Omobowale T O, Yakubu MA (2016) Evaluation of the anticancer properties of the methanol leaf extract of *Chromolaena odorata* on HT29 lung cancer cell line. The FASEB Journal; 30: Supplement 1193.6.
2. Afaq S H, Shehbaz A, Masood A K, Owais M , Nadeem A. Antibiotic screening of certain Unani medicinal plants. Indian Drugs, 2004; 41:236-239.
3. Afolayan Michael , Fatokun Olakunle , Olajide Olutayo, Akolade Jubril ,

Fagbohun Adebisi and Orishadipe Abayomi.2015.Journal of Pharmaceutical and Biological Sciences ISSN: 2320-1924; CODEN: JPBSEV.

4. Akinmoladun A C, Ibukun E O, Dan-Ologe I A (2007) Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata* . Sci Res Essays 2007; 2:191-4.

5. Asomugha R N, Okafor P N , Ijeh I I , Orisakwe O E , Asomugha A L(2014) Hepatic effects of aqueous extract of *Chromolaena odorata* in male Wistar albino rats. vol.1 :127-136

6. Baijal R , Patel N, Kolthapure S A. Evaluation of efficacy and safety of Liv 52 DS tablets in acute viral hepatitis: A prospective, double-blind,randomized, placebo-controlled, phase III clinical trial. Med Update, 2004; 12: 41-53.

7. Bobbit J N 1963. Thin Layer Chromatography. Reinhold publishing corporation, Newyork, Chapman and Hall Ltd London.

8. Douye V. Zige , Elijah I. Ohimain and Medubari B. Nodu.2013.Antibacterial Activity of Ethanol, Crude and Water Extract of *Chromolaena odorata* Leaves on S Typhi and E Coli.Greener Journal of Microbiology and Antimicrobials. ISSN: 2354-2284 Vol. 1 (2), pp. 016-019.

9. Evans C E, Banso A, Samuel O A.Efficacy of some nupe medicinal plants against Salmonella typhi: an invitro study, J Ethnopharmacol, 2002; 80:21-24.

10. Facklam R .What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin Microbiol,2002Rev; 15(4):613–30. doi:10.1128/CMR.15.4.613-630,2002. PMC 126867. PMID 12364372.

11. Foluke Odutayo, Cajethan Ezeamagu, Taofikat Kabiawu, Daniel Aina and Grace Mensah – Agyei.2017.Phytochemical Screening and Antimicrobial Activity of *Chromolaena odorata* Leaf Extract against Selected Microorganisms.Journal of Advances in Medical and Pharmaceutical Sciences 13(4): 1-9;Article no.JAMPS.33523.

12. Hanh T T, Hang D T, Minh C V, Dat N T 2011 Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*. Asian Pac J Trop Med; 4: 760-3.

13. Hridhya K V, Kulandhaivel M.2017.Antimicrobial Activity of *Chromolaena odorata* Against Selected Pyogenic Pathogens.International Journal of Pharmacognosy and Phytochemical Research; 9(7); 1001-1007 ISSN: 0975-4873.

14. Ijioma S N, Okafor A I, Ndukuba P I, Nwankwo A A, Akomas S C 2014Hypoglycemic, hematologic and lipid profile effects of *Chromolaena odorata* ethanol leaf extract in alloxan induced diabetic rats. Ann Biol Sci; 2: 27-32.

15. Kouamé P B, Jacques C, Bedi G, Silvestre V, Loquet D, Barillé-Nion S, Richard J Robins , Illa Tea (2013) Phytochemicals isolated from leaves of *Chromolaena odorata*: Impact on viability and clonogenicity of cancer cell lines. Phytother Res; 27:835-40.

16. Lebreton F, Manson AL, Saavedra JT, Straub TJ, Earl AM, Gilmore MS. Tracing the Enterococci from Paleozoic origins to the hospital Cell,2017; 169(5):849-861.e13. doi: 10.1016/j.cell.2017.04.027.

17. Lovet T. Kigigha and Douye Victor Zige Activity of *Chromolaena odorata* on enteric and superficial etiologic bacterial agents. American Journal of Research Communication, 2013; Vol 1(11).

18. Mullika Traidej Chomnawang, Suvimol Surassmo, Veena S Nukoolkarn, Wandee Gritsanapan (2005) Antimicrobial effects of thai medicinal plants against acne inducing bacteria, Journal Of Ethno Pharmacology.

19. N.Nanadini, P.Nagababu, V. Umamaheswara Rao,N.Venugopal.2014.International Journal of Phytomedicine 6;286-292.

20. Nagesh Kamath, Madhusudan P. Joshi, Maria H. S Godinho, Yogita Sardessai, Raghu Jetti Screening of various leaf extracts of *Chromolaena odorata* for biochemical constituents and antimicrobial sensitivity. Int J Basic Clin Pharmacol, 2015; 4(1):89-97.

21. Nwachukwu,Ifeanyi; Aliga, Chinanu; Upabi,Chibueze F. Ukogo, Ifeoma. 2016. IOSR Journal of Pharmacy and Biological Sciences (IOSR_JPBS),e-ISSN:2278-3008,p-ISSN:2319-7676. Volume 11,Issue 6 Ver.II,PP 49-52.

22. Olukoya D K, Odugbemi T O and Bamgbose S O A. Some Aspects of Traditional Therapy ofGonorrhoea in Lagos, Nigeria. Journal of Research in Ethno-Medicine, 1986; 1: 26-29.

23. Onkaramurthy M, Veerapur VP, Thippeswamy B S, Reddy T N, Rayappa H, Badami S (2013) Anti-diabetic and anti-cataract effects of *Chromolaena odorata* Linn. in streptozotocin-induced diabetic rats. J Ethnopharmacol; 145:363-72.

24. Owoyele V B, Adediji J O, Soladoye A O (2005) Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata* . Inflammopharmacology; 13:479-84.

25. Pandith H, Zhang X, Thongpraditchote S, Wongkrajang Y, Gritsanapan W, Baek S J (2013) Effect of Siam weed extract and its bioactive component scutellarein tetramethyl ether on anti-inflammatory activity through NF-κB pathway. J Ethnopharmacol; 147:434-41.

26. Patwardhan B, Vaidya A D B, Chorghade M. Ayurveda and natural products drug discovery. Current Science, 2004; 86:789-799.

27. Pelletier LL Jr.1996. Microbiology of the Circulatory System. In Baron S;Baron's Medical Microbiology (4th ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1.

28. Phan T T , See P, Lee S T, Chan S Y (2001) Anti-oxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage. Burns; 27:319-27.

29. Pierangeli G. Vital and Windell L. Rivera. 2009. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L.f.) King and Robinson and Uncaria Perrottetii(A. Rich) Merr.extracts.Journal of Medicinal Plants Research Vol.3(7),pp.511-518.

30. Rao K S, Chaudhury P K, Pradhan A (2010) Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. Food Chem Toxicol; 48:729-32.

31. Ryan KJ, Ray C G. 2004.Sherris Medical Microbiology. 4th edition. McGraw Hill. ISBN 0838585299.

32. Uhegbu F O, Imo C, Onwuegbuchulam C H (2016) Lipid lowering, hypoglycemic and antioxidant activities of *Chromolaena odorata* (L) and Ageratum conyzoides (L) ethanolic leaf extracts in albino rats. J Med Plants Stud; 4 :155-9.

33. UNESCO. Culture and Health Orientation, 1996.World Decade for Culture Development 1988-1997. Paris, France: Document CLT/DEC/PRO.

34. Williams J E. Review of antiviral and immunomodulating properties of plants of the Oeruvian rainforest with a particular emphasis on Una de Gato and Sangre de Grado. Altern Med Rev, 2001; 6(6):567-579.