Comparative Studies on the Antioxidant and Antimicrobial Activity on different Extracts of *Eupatariumodoratum*(L.)(Bea-zert) and *Chromolaenaodorata*(L.)(Taw-chin-paung)Leaves

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ABSTRACT

Eupatorium odoratum (L.) and *Chromolaenaodorata* (L.) are traditional medicinal plants that are widely used for theirwound healing property.They have been reported to have antispasmodic, antiprotozoal, antibacterial and antihypertensive activities.Antioxidant activity were determined by DPPH assay method. IC_{50} value of EtOH extract (13.04 μgmL^{-1})and H_2O extract(7.6 μgmL^{-1}) were observed in the extracts of *Eupatariumodoratum*(L.).IC_{50} value of EtOH extract (8.18 μgmL^{-1})and H_2O extract(4.73 μgmL^{-1}) were observed in the extracts of*Chromolaenaodorata*(L.)**.***In vitro* antimicrobial activity of some crude extracts such as ethanol and water extracts of *Eupatorium odoratum*(L.) and *Chromolaenaodorata*(L.) were tested against the following microorganism like *Staphylococcus aureus*, *Bacillus pumilus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*by Agar-well diffusion method. The results showed that both samples extracted with water and ethanol had maximum inhibition zone against Germ positive, Germ negative and fungi. The main contribution of the present research is that antioxidant activity and antimicrobial activities in the samples can be known. Moreover, these can be suited for skin cover, wound drafty system and other biomedical application.

Keywords: Antioxidant , antimicrobial ,and *Eupatorium odoratum*(L.)

INTRODUCTION

Eupatorium odoratum(L.) and *Chromolaenaodorata*(L.)are locally known as "Bea-zet and Taw-chin-paung "respectively. This species is a perennial weed of plantation crops and cleared lands and it comes from the family of Asteraceae. The fresh leaves and extract of these plants are traditionally used as herbal treatment in some developing countries for burns, soft tissue wounds and skin infections.The plants are widely distributed in Myanmar, so it has a potential to be commercialize as a herbal medicine. There is a need to characterize the chemical compound in these plants.*Eupatorium odoratum*(L.) and *Chromolaenaodorata*(L.)are perennial herb distributed throughout India, tropical Asia, Mexico, Africa and other parts of the world.Medicinal plants remain the source of inspiration of novel drug compounds as they afford key chemical structure for the progress of new antimicrobial drugs as well as phytomedicine (Ahukakar, *2008)*.A total of twenty-nine compounds have been identified, accounting 97.6% of the total oil. The signature compounds from the leaves oil of this plant reported from different countries were almost the same with quantitative differences. The major constituents, pregeijerence, epi-cubebol, cubebol, cis-sabinene hydrate, germacrene-D-4-ol, germacrene D, geijerence, cyperene, α-muurolol, khusimone, β-copaen-4α-ol, camphor, limonene, vestitenone, bulnesol and trans-ocimene were reported from the leaves oil of *Eupatorium odoratum*(L.)and *Chromolaenaodorata*(L.).There are some benefits of *Eupatorium odoratum*(L.)and*Chromolaenaodorata*(L.). Suchas Cyst Medicine, Pain Reliever, Prevent Cervical Cancer, Maintain the Health of Reproduction Organ on Women,Prevent Diabetes ,Vertigo Medicine , Maintain the Heart's Health ,Decrease

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the Cholesterol level and Decrease the Blood Pressure.It has been reported to have antispasmodic, antiprotozoal, antibacterial and antihypertensive activities.

MATERIALS AND METHODS

Collection of Leaves

The leaves of *Eupatorium odoratum*(L.) and *Chromolaenaodorata*(L.)were chosen for the present research. The sample was collected from Panglong Township, Loilem District, and Southern Shan State, Myanmar. The collected sample was identified in Department of Botany, University of Panglong.

Preparation of *Eupatorium odoratum*(L.)and *Chromolaenaodorata*(L.)leaveextracts

The dried sample was ground into parley fine powder by using an electric grinder. The powdered sample was labeled and stored in air tight plastic bottle to prevent moisture and other contaminations. The powdered material (15)g was extracted with 100 mL of water and ethanol separately.

Screening of Antioxidant Activity of *Eupatorium odoratum(L.) and Chromolaenaodorata*(L.)leave samples

Antioxidant activity of 95% ethanol and watery extracts were carried out by DPPH (2,2)Diphenyl, 1-picryl-hydrazyl) radical scavenging assay using UV-visible spectrophotometer. The control solution was prepared by mixing of 60µM DPPH solution and 1.5 mL of 95% ethanol using vortex mixer. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solution and 1.5 mL of test sample solution. The solution was allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by UV-visible spectrophotometer.

Screening of Antimicrobial Activity

Test organisms

Screening of antimicrobial activity of various crude extracts such as 95% EtOH and watery extract of *Eupatorium odoratum(L.) and Chromolaenaodorata(L.)leave*samples were done by Agar Well Diffusion Method. In the present work, the test microorganisms were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.*

Antimicrobial Activity Test

The agar well wasspread on nutrient agar and A. niger& A. flavus spread on rose bengal agar using sterile cotton swabs. The wells (6 mm in diameter) were cut from the agar plates using a cork horer.30µL of the extracts (7mg/mL) were poured into the well using a sterile micro pipette. The plates were incubated at 37±2 ° C for 24 hs for bacterial activity and 48 hs for fungal activity .The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

RESULTS AND DISCUSSION

In order to determine the phytochemical constituent in the *Eupatorium odoratum*(L.) leaves and *Chromolaenaodorata*(L.)leaves, preliminary phytochemical test were carried out according to the standard procedure. The results obtained are

summarized in Table 1 and figure 1, 2,3 and 4.According to this table, carbohydrates, phenolic compounds,tannins, saponins, flavonoids, alkaloids, reducing sugar, α-amino acid and coumarins are present but starch is absent in *Eupatorium odoratum*(L.)and *Chromolaenaodorata*(L.).Therefore, *Eupatorium odoratum*(L.) and *Chromolaenaodorata*(L.)contains functional compounds such as phenolics and alkaloids. The present of phenolic compounds in medicinal plants are responsible for the antioxidant and anti-inflammatory activities of these species.Antioxidant activity were determined by DPPH assay method. IC_{50} value of EtOH extract (13.04 μgmL^{-1})and H_2O extract(7.6 μgmL^{-1}) were observed in the extracts of *Eupatariumodoratum*(L.). IC_{50} value of EtOH extract (8.18 μgmL^{-1})and H_2O extract(4.73 μgmL^{-1}) were observed in the extracts of*Chromolaenaodorata*(L.)**.**The antioxidant potential of sample can be determined by IC_{50} (50% inhibition concentration). The IC_{50} values for each sample were determined by linear regressive excel program. By using DPPH free radical scavenging assay, watery extract was found to the most potent antioxidant activity than 95% ethanol extract of both sample.

Table 1 Results of Phytochemical Test of *Eupatorium odoratum*(L.)and *Chromolaena odorata*(L.)

No.	**Constituents**	***Eupatorium odoratum*(L.) (Bea-zert)**	***Chromolaenaodorata*(L.)(Taw-chin-paung)**
1	Alkaloids	+	+
2	α-amino acids	+	+
3	Carbohydrates	+	+
4	Coumarins	+	+
5	Flavonoids	+	+
6	Glycosides	+	+
7	Phenolic Compounds	+	+
8	Reducing Sugars	+	+
9	Saponins	+	+
10	Starch	-	-
11	Tannins	+	+

(+) presence (-) absence

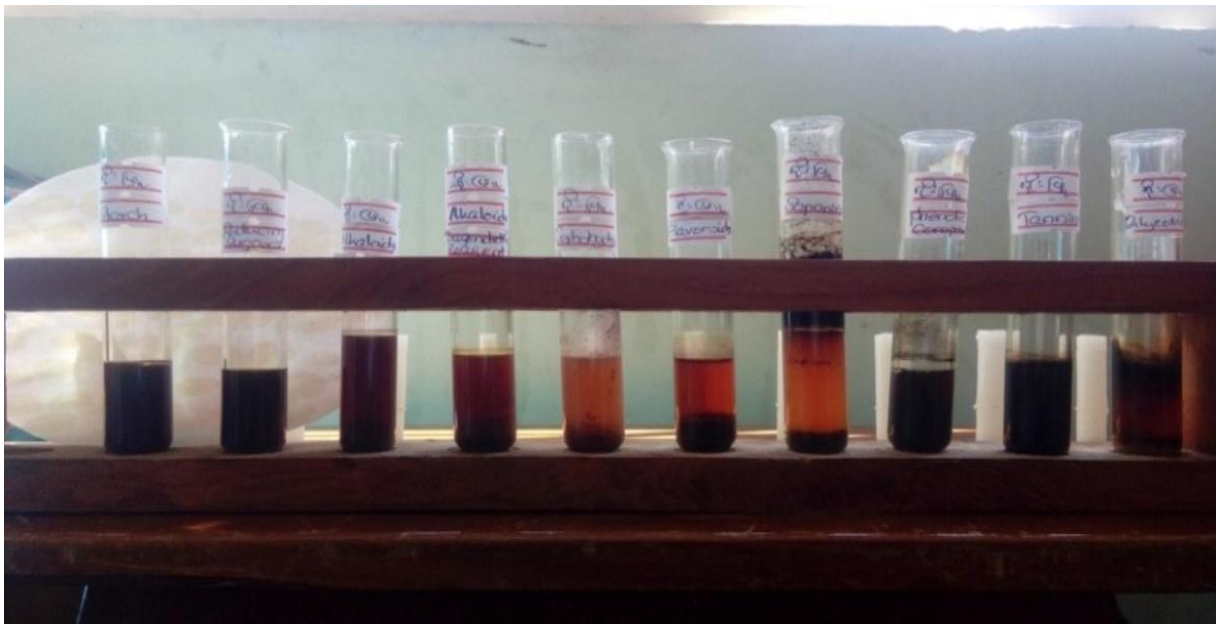

Figure 1 Preliminary phytochemical tests on *Eupatorium odoratum*(L.)(Bea-zert)

Figure 2 Coumarinstestof *Eupatorium odoratum*(L.) (Bea-zert)

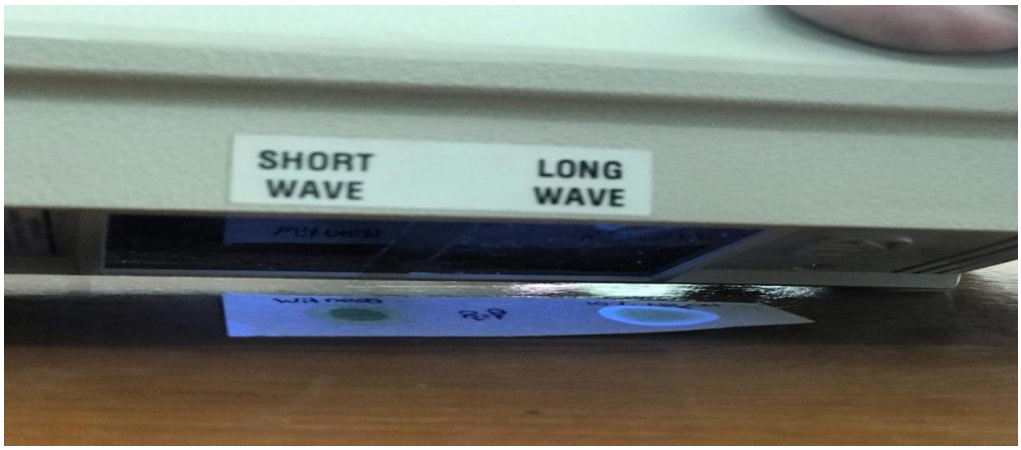

Figure 3 Coumarins test of *Chromolaenaodorata*(L.) (Taw-chin-paung)

Figure 4 Preliminary phytochemical test on *Chromolaenaodorata(*L.) (Taw-chin-paung)

The results of antioxidant activity are shown in Table (2) and Figure (5,6).The antimicrobial activity of various crude extracts such as ethanol, methanol and water extracts were investigated by agar well diffusion method.The test microorganisms were *Bacillus subtilisSatphylococcusaureus, Pseudomonusaeruginosa, Bacillus pumilus, Candida albicans and Esherichia coli.*In this study, methanolextract showed significant inhibition against all microorganism.The ethanol and water extracts responded medium activity on all tested organisms.The results indicated that *Eupatorium odoratum***(**L.)*andChromolaenaodorata*(L.) have shown the maximum antibacterial and antifungal activities against all tested microorganism as shown in Table 3.

Table 2 Radical Scavenging Activity (IC_{50}) of Water and EtOHLeafy Crude Extracts of *Eupatorium odoratum*(L.),*Chromolaenaodorata*(L.)And Ascorbic Acid

TestedSample	**% RSA (mean ± SD) In different concentration (µg/mL)**						**IC_{50} (µg/mL)**
	1.25	2.5	5	10	20	40	
***Eupatorium odoratum*(L.)(Water)**	32.83 ± 1.46	37.33 ± 1.46	41.33 ± 1.46	58 ± 1.46	80.67 ± 1.46	93.67 ±1.46	7.6 93.67
***Chromolaenaodorata*(L.) (Water)**	27.83 ± 3.12	32.5 ± 2.08	38.67 ± 1.04	56.5 ± 1.04	78.83 ± 3.12	94.33 ± 2.08	8.18

***Eupatorium odoratum*(L.)(EtOH)**	18.17 ± 1.40	24.83 ± 2.10	43.56 ± 2.80	45.33 ± 2.10	60.67 ± 0.00	85.17 ±0.59	13.04
***Chromolaenaodorata*(L.) (EtOH)**	33.17 ± 1.46	40.17 ± 1.04	51.17 ± 1.04	69.5 ± 1.04	93.83 ±0.59	96 ±0.55	4.73
Ascorbicacid	45.17 ±0.59	53.5 ±0.55	70.5 ±0.59	92.83 ±0.78	96.17 ±0.48	99 ± 0.00	1.97

CONCLUSION

Preliminary phytochemical qualitative analysis of *Eupatorium odoratum*(L)(Bea-zert) and *Chromolaenaodorata*(L.) (Taw-chin-paung) leaves were revealed that the presence of alkaloids, α-amino acids, flavonoids, glycosides, phenolic compounds, saponins, reducing sugars, and tannins but starch was absent. Phytochemicals of nutraceuticals importance are bioactive constituents that sustain or promote health and occur at the intersection of food and pharmaceutical industries.Medicinal plants contain compounds exhibiting antioxidant properties as phenolic compounds, which possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals.These secondary metabolites are reported to have many biological and therapeutic propertiesAntioxidant activity of watery extracts of *Eupatariumodoratum*(L.)and *Chromolaenaodorata*(L.) were obtainedthe maximum amount of H_2O extract(7.6 μgmL^{-1}) of *Eupatariumodoratum*(L.)and H_2O extract(4.73 μgmL^{-1})of*Chromolaenaodorata*(L.)**.**So, this species is expected to have medicinal uses. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases. In vitro antimicrobial activity of some crude extracts (water, ethanol and methanol extracts) of *Eupatorium odoratum(*L.)(Bea-zert) and *Chromolaenaodorata*(*L.)* (Taw-chin-paung) were screened by agar-well diffusion method against six organisms . Such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*and*Escherichia coli*. The leaves of both the plants were observed that the methanol extract and ethanol extract showed significant inhibition on all tested organisms. The results showed that both samples extracted with methanol and ethanol had maximum inhibition zone against Germ positive, Germ negative and fungi. The main contribution of the present research is that main phytoconstituent in the samples can be known. Moreover, it can be suited for skin cover, wound drafty system and other biomedical application.

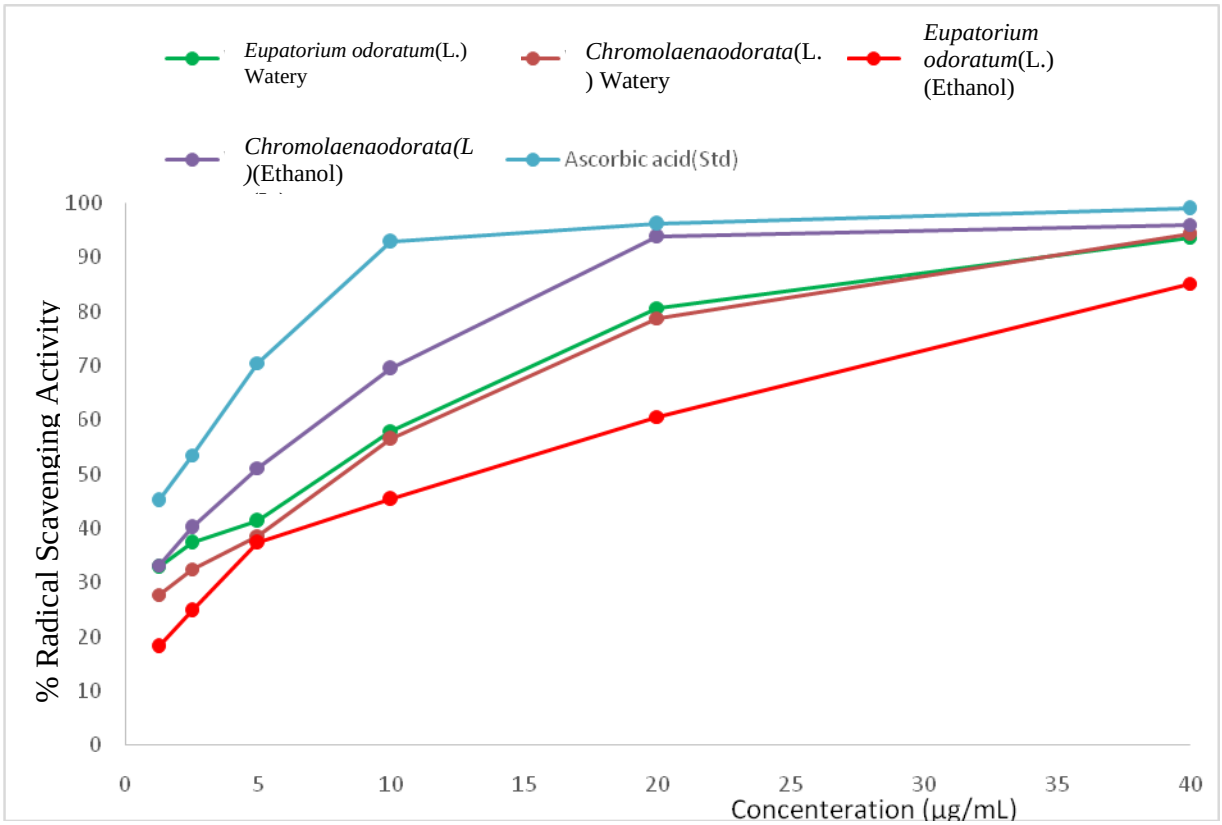

Figure 5 Radical scavenging activity of different concentrations water and EtOHleafy Crude Extracts of *Eupatorium odoratum*(L.), *Chromolaenaodorata*(L.) and Ascorbic Acid

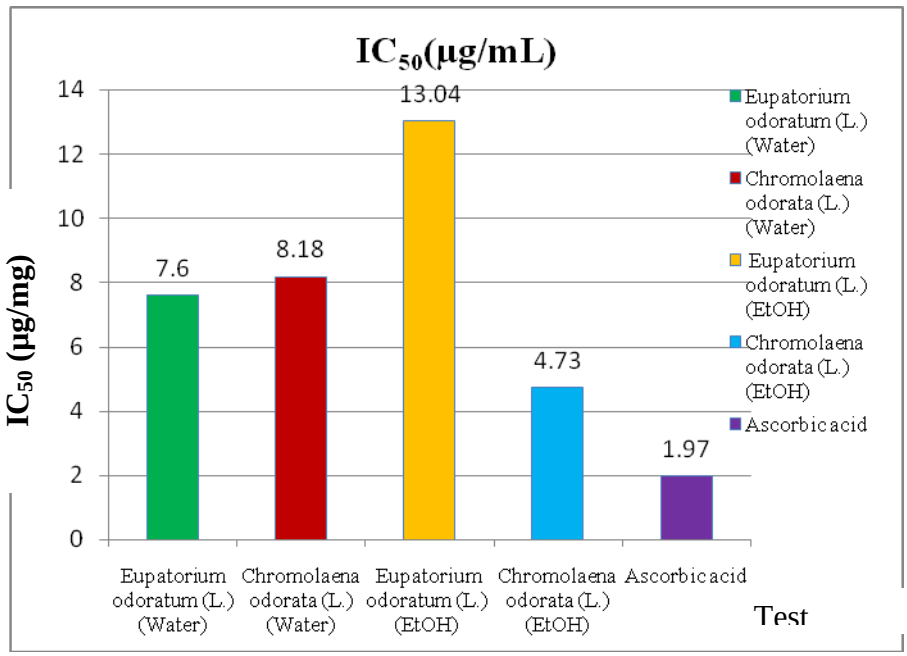

Figure 6 A bar graph of IC_{50} (µg/mL) of water and EtOH leafy Crude extracts of *Eupatorium odoratum*(L.), *Chromolaenaodorata*(L.)and Ascorbic Acid

Table 3Diameter of Inhibition Zone of Crude Extracts on Different Bacterial Strains

Compound	Zone of inhibition (mm)					
	Gram- positive bacteria			Gram-negative bacteria		Fungi
	B. Sub	*S.aureus*	*Pseudomonas*	*B.Pumilus*	*E.coli*	*Candida*
Eupatoruimodoratum L. **(Water Extract)**	12mm (++)	15mm (++)	15mm (++)	17mm (++)	16mm (++)	15mm (++)
Eupatoruimodoratum L. (EtOH)	19mm (++)	18mm (++)	20mm (+++)	18mm (++)	18mm (++)	18mm (++)
Eupatoruim odoratum L. (MeOH)	19mm (++)	20mm (+++)	20mm (+++)	20mm (+++)	20mm (+++)	20mm (+++)
Chromoleanaodorata L. (Water Extract)	17mm (++)	15mm (++)	20mm (+++)	-	18mm (++)	-
Chromoleanaodorata L. (EtOH)	17mm (++)	18mm (++)	26mm (+++)	14mm (++)	15mm (++)	15mm (++)
Chromoleana odorata L. (MeOH)	17mm (++)	18mm (++)	27mm (+++)	17mm (++)	15mm (++)	13mm (++)

Agar well – 10mm , 10mm ~ 14mm (+) , 15mm ~ 19mm (++) , 20mm above (+++)

Organisms

Bacillus sublilis (N.C.T.C-8236), *Pseudomonas aeruginosa* (6749) ,

Staphylococcus aureus (N.C.P.C-6371),*Bacillus pumilus (N.C.I.B- 8982),*

E-coli (N.C.I.B -8134), Candida albican

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