ANTIMICROBIAL ACTIVITY OF EXTRACTS FROM *ENHYDRA FLUCTUANS* LOUR. (KA-NA-HPAW)

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Abstract

Three selected medicinal plants grown in Pathein were collected on June, 2019. These plants are *Clitoria ternatea* L. (Aung-me-nyo) belonging to Fabaceae, *Melastoma malabathricum* L. (Say-o-boke) belonging to Melastomataceae and *Enhydra fluctuans* Lour. (ka-na-hpaw) belonging to Asteraceae. The fresh samples of these plants except flowers were air-dried and powdered for extraction. The powder of each plant (5 g) was extracted with 50 mL of acetone, ethanol, methanol and water on water-bath at 80℃. These extracts were tested the antimicrobial activity on eight different test organisms using the paper disc diffusion assay method. According to their antimicrobial activity, the ethanol extract of *Enhydra fluctuans* Lour. (20.74 mm of inhibitory zone) was selected for further investigation.

Keywords: medicinal plants, extracts, antimicrobial activity

Introduction

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs. The flora is rich in medicinal plants which are usually exploited by herbal doctor otherwise called "native doctor". Some of the plant collections are used against a variety of diseases such as typhoid, fever, gastroenteritis, dysentery, malaria and others which are typical diseases of tropical countries (Akinyemi *et al*, 2006).

The bioactivity of plant extracts is attributed to phytochemical constituents present in the extracts. However, the constituents of a plant extracts are extremely dependent on the polarity of solvents, solvent to plant material ratio, particle size of plant material, temperature and extraction method (Kannamba, *et al*, 2017).

The aim and objectives of this research are to apply the extract as substituent crude compound in practical, to study the outstanding characters of some medicinal plants, to investigate the extraction from some medicinal plants with different solvents, to study the antimicrobial activity with different test organisms by paper disc diffusion assay method.

Materials and Methods

Collection of plants materials

Three selected medicinal plants grown in Pathein were collected on June, 2019. The fresh samples of except flowers were air-dried for 45 days and ground to fine powder using a blender. The outstanding characters of these three medicinal plants are checked by Hooker (1897).

Extraction of the three selected plants (M. M. Nyein, 1976)

The polar solvents like acetone, ethanol, methanol and water were selected as extraction solvents. Exactly 5 g of each three selected plants powder were extracted with 50 mL of acetone, ethanol, methanol and water separately on water-bath at 80℃ . The extracts were filtered and the filtrate was undertaken to be semi-solid.

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Table 1 Three Medicinal Plants for Extraction

No	Scientific Name	Myanmar Name	Family
1.	***Clitoria ternatea* L.**	**Aung-me-nyo**	**Fabaceae**
2.	***Melastoma malabathricum* L.**	**Say-o-boke**	**Melastomataceae**
3.	***Enhydra fluctuans* Lour.**	**Ka-na-hpaw**	**Asteraceae**

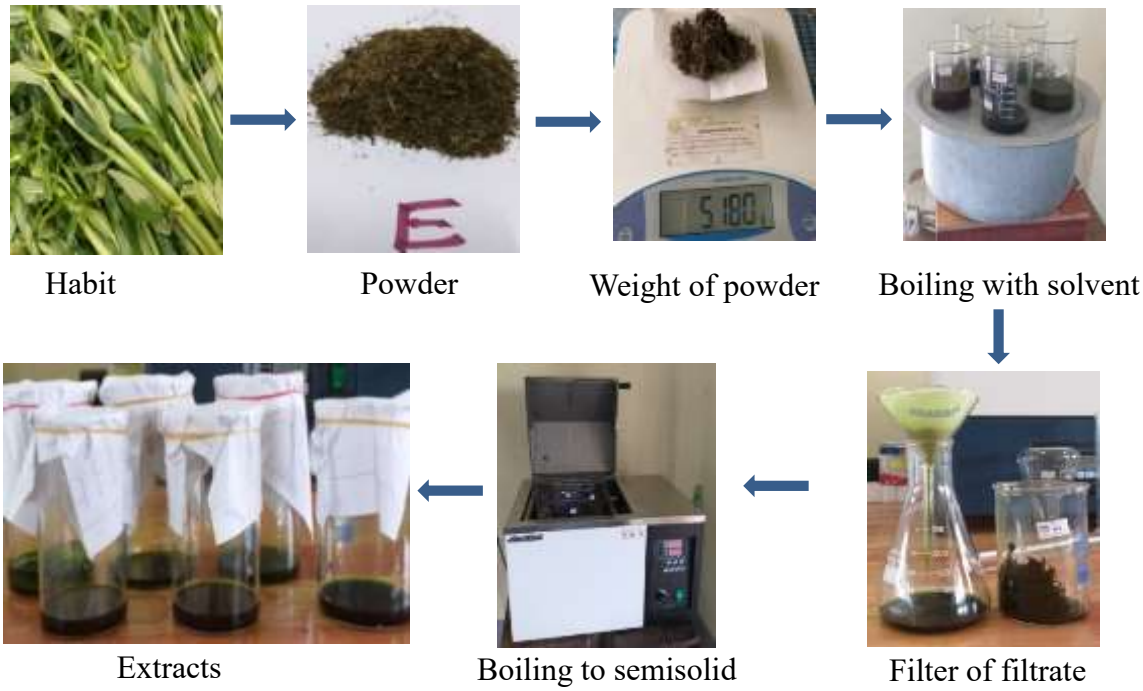

Figure 1 Extraction procedure of *Enhydra fluctuans* Lour. (M.M.Nyein, 1976)

Paper disc diffusion assay method (NITE, 2004)

Paper disc diffusion assay method was followed to determine the antimicrobial activity by the paper discs (6 mm in diameter) soaked with the respective extracts. The culture (Glucose 1 g, peptone 0.3 g, DW 100 mL) was used for respective bacteria and fungi as 8 different test organisms.

Testing the antimicrobial activities (Hassawi, and Kharma 2006)

Nutrient agar medium (Glucose 1 g, peptone 0.3 g, Agar 1.8 g, DW 100 mL) was prepared and autoclaved at 121℃ under pressure for 30 min. After cooling about 65℃, 100 mL of medium before solidification was added the test organism broth (1 mL) and poured into petri-dish. The plates were kept at room temperature for solidification and then the paper discs soaked with the respective extracts were put on agar plate for testing the antimicrobial activity. The zone of clearance around the paper disc was measured for indication of the antimicrobial activity of the extract.

Table 2 Test organisms for antimicrobial activity

No.	Test organisms	Causes of Diseases
1	***Agrobacterium tumefaciens* NITE 09678**	**Crown gall diseases**
2	***Bacillus subtilis* IFO 90571**	**DNA topoisomerase I**
3	***Bacillus pumilis* IFO 12092**	**Wound and burn infection, Fever**
4	***Candida albicans* NITE 09542**	**Candidiasis**
5	***Escherichia coli* AHU 5436**	**Diarrhoea**
6	***Malassezia furfur* AVU0255**	**Danddruff, Seborrhoeic dermatitis**
7	***Pseudomonas fluorescens* IFO 94307**	**Rice disease**
8	***Staphylococcus aureus* AHU 8465**	**Food poisoning, Methicillin Resistance**

Results

Outstanding Characters of *Enhydra fluctuans* Lour. (ka-na-hpaw) (Asteraceae)

Herbs; serrate leaves 1-3 inches variable in breadth with opposite sessile, linear oblong, base narrowed; stem elongated, hollow, thinly glabrous, rooting at the nodes; inflorescence heads terminal and axillary, heterogamous, obscurely radiate, ray florets numerous, seriate, fertile ligule minute broad 3-4 toothed and disk florets numerous, fertile, tubular, limb campanulate 5-fid, epigynous, flowers tipped with glandular hairs; corolla greenish white, two forms; anther basifixed; ovule solitary, style-arms obtuse, tip hispid; fruit dry, indehiscent.

Figure 2 Habit and inflorescence of *Enhydra fluctuans* Lour**.**

In the extraction procedure, acetone, ethanol, methanol and watery extracts from *Clitoria ternatea* L., *Melastoma malabathricum* L. and *Enhydra fluctuans* Lour. were utilized for the antimicrobial activity on 8 test organisms. All extracts of *Melastoma malabathricum* L. and *Clitoria ternatea* L. showed no antimicrobial activity. All extracts of *Enhydra fluctuans* Lour. showed antimicrobial activity on 7 test organisms except *Staphylococcus aureus.* Then, watery extract of *Enhydra fluctuans* Lour. gave the less antibacterial activity on *Bacillus pumilis.* Ethanol extract of *Enhydra fluctuans* Lour. exhibited the best antibacterial activity (20.74 mm of inhibitory zone) on *Bacillus subtilis.*

Table 3 Antimicrobial activity (inhibitory zone) of all extracts on different test organisms

Collected Plants	Extracts	*Agrobacterium tumefaciens*	*Bacillus subtilis*	*Bacillus pumilis*	*Candida albicans*	*Escherichia coli*	*Malassezia furfur*	*Pseudomonas fluorescens*	*Staphylococcus aureus*
Clitoria ternatea L.	Acetone	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-
	Water	-	-		-	-	-	-	-
Melastoma Malabathricum L.	Acetone	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-
Enhydra fluctuans Lour.	Acetone	14.33 mm	-	15.14 mm	12.41 mm	-	15.63 mm	14.58 mm	-
	Ethanol	16.24 mm	20.74 mm	10.74 mm	15.97 mm	14.21 mm	13.01 mm	11.47 mm	-
	Methanol	12.16 mm	+	10.74 mm	11.56 mm	20.71 mm	12.32 mm	10.00 mm	-
	Water	-	-	+	-	-	-	-	-

Paper disc is 6 mm in diameter, - is no activity, + is <10 mm

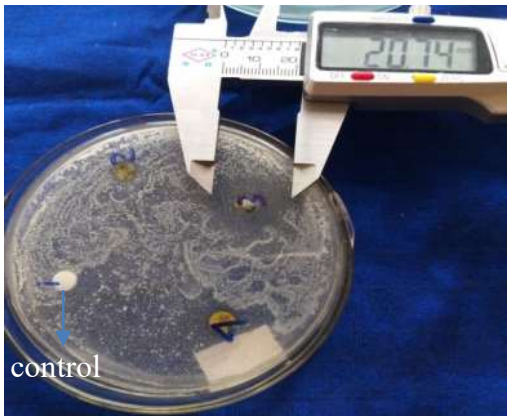

Figure 3 The best antibacterial activity of ethanol extract from *Enhydra fluctuans* Lour. on *Bacillus subtilis*

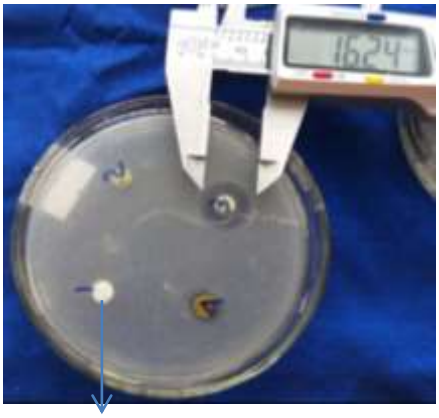

Figure 4 Antibacterial activity of ethanol extract on *Agrobacterium tumefaciens*

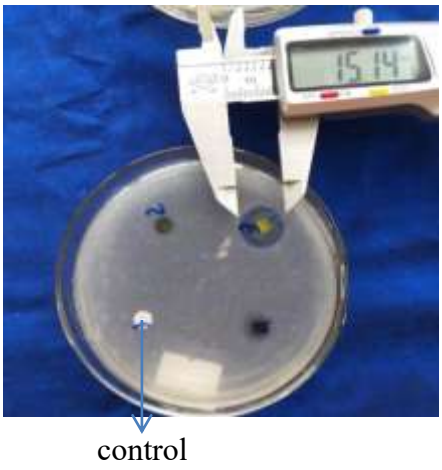

Figure 5 Antibacterial activity of acetone extract on *Bacillus pumilis*

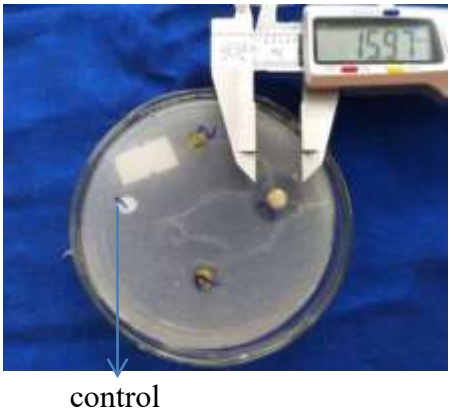

Figure 6 Antifungal activity of ethanol extract on *Candida albicans*

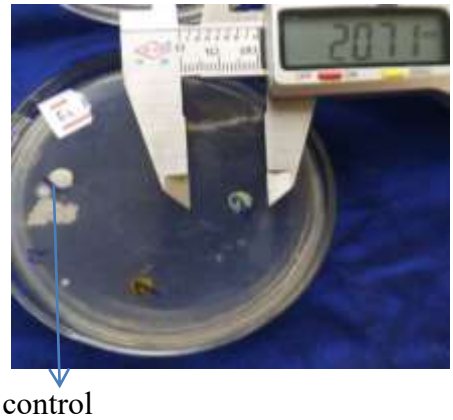

Figure 7 Antibacterial activity of methanol extract on *Escherichia coli*

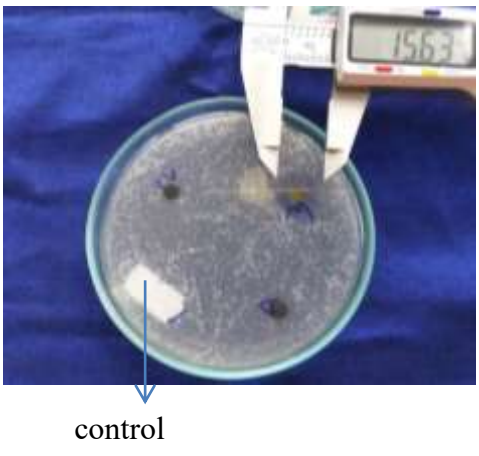

Figure 8 Antibacterial activity of acetone extract on *Malassezia furfur*

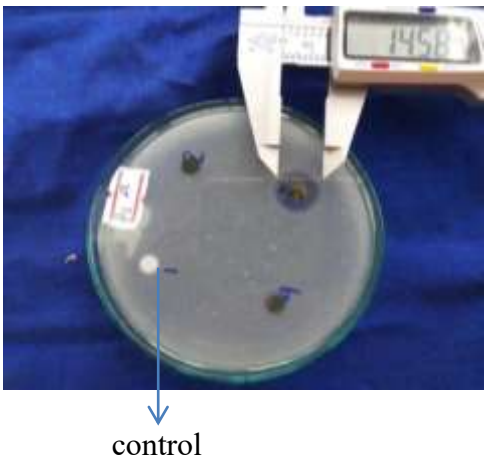

Figure 9 Antibacterial activity of acetone extract on *Pseudomonas fluorescens*

Discussion and Conclusion

This study is reported for the antimicrobial activity. Some of the medicinal plants are potentially effective antimicrobial agents. The resulting information will contribute to a better understanding of antimicrobial activity of the plant.

The plant species could have an antimicrobial agent that caused the antimicrobial activity. Also, they could have the different concentrations that cause high variations in their antimicrobial activity (Hassawi and kharma, 2006).

The possible phytochemical constituents, thrombolytic and membrane stabilizing activities of the crude ethanolic extract of *Enhydra fluctuans* (CE) were investigated along with the antimicrobial, antioxidant and cytotoxic potentials of its petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions (Kamal *et al*., 2019).

Enhydra fluctuans is one such plants which is available abundantly in India especially in the North-Eastern states. It has immense potential as a medicinal plants and also has many beneficial effects such as anticancer, antioxidant, antidiabetic, anti-inflammatory, antimicrobial, anti-diarrheal, hepatoprotective and even neuropharmacological effects, These activities can be attributed mainly to the presence of phytochemicals such as flavonoids, alkaloids, saponins, tannins, phenols and carbohydrates (Sarma *et al.*, 2014).

Screening of extracts from *Enhydra fluctuans* Lour. with acetone, ethanol, methanol and water were utilized. These semi-solid extracts were used for testing of antimicrobial activity by paper disc diffusion assay method on eight different test organisms.

Among three medicinal plants, almost extracts of *Enhydra fluctuans* Lour. showed the antimicrobial activity on seven test organisms. According to these activity, 20.74 mm of inhibitory zone of ethanol extract from *Enhydra fluctuans* Lour. showed the best antibacterial activity on *Bacillus subtilis.* Other two medicinal plant extracts showed no antimicrobial activity.

The second-best antibacterial activity of methanol extract is 20.71 mm of inhibitory zone on *Escherichia coli.* The acetone, ethanol and methanol extracts also showed the antibacterial activity on *Agrobacterium tumefaciens* (14.33 mm, 16.24 mm and 12.16 mm of inhibitory zones), *Bacillus pumilis* (15.14 mm, 10.74 mm and 10.74 mm of inhibitory zones), *Malassezia furfur* (15.63 mm, 13.01 mm and 12.32 mm of inhibitory zones) and *Pseudomonas fluorescens* (14.58 mm, 11.47 mm and 10.00 mm of inhibitory zones) and then the antifungal activity on *Candida albicans* (12.41 mm, 15.97 mm and 11.56 mm of inhibitory zones). The watery extract showed the less antibacterial activity on *Bacillus pumilis* but the acetone extract showed no antibacterial activity on *Bacillus subtilis* and *Escherichia coli.* Moreover, all extracts of *Enhydra fluctuans* Lour. showed no antibacterial activity on *Staphylococcus aureus.*

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