DETERMINATION OF ANTIMICROBIAL, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES FROM THE ROOT OF *Leea Macrophylla* Roxb.ex Hornem. (Kya hpetgyi)

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Abstract

In this research, *Leea macrophylla* Roxb. ex Hornem., a medicinal plant native to Myanmar, was selected for investigation of its phytochemical constituents, antibacterial activity, antioxidant property, anti-inflammatory effect. It gave rise to positive tests for alkaloids, flavonoids, glycosides, phenols, polyphenols, saponins, steroids, reducing sugars, terpenes and tannins, respectively. The antimicrobial activity of crude extracts from this sample was tested by using paper disc diffusion assay method on six selected microorganisms. The results indicated that the ethyl acetate extract exhibited the highest antimicrobial activity against all tested organisms compared to the other extracts. Moreover, the antioxidant activity of ethyl acetate extract and *n*-hexane extract of this sample was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay method using UV-Visible spectrophotometer. The ethyl acetate extract revealed stronger antioxidant activity with an IC_{50} value of 20.341 μg/mL compared to the IC_{50}value of 31.313 μg/mL for the *n*-hexane extract. In addition, the anti-inflammatory activity of the ethanol extract was assessed by Croton Oil-Induced Ear Edema method. The results showed that the anti-inflammatory activity of groups A, B and C showed (36 % for 0.75 mg/ear, 45 % for 1.15 mg/ear, and 48 % for 2.25 mg/ear) respectively.

Keywords: *Leea macrophlla* Roxb. ex Hornem, Antioxidant activity, Antimicrobial activity, Anti-inflammatory

Introduction

Medicinal plants represent a rich source of new molecules with pharmacological properties, which are lead compounds for the development of new drugs. Since the chemical constituents of medicinal plants, particularly the secondary metabolites (alkaloid, sterol, terpene, flavonoid, saponin, glycoside, tannin, etc.) have pronounced pharmacological action on animal systems and organs, they are capable of mitigating sufferings and curing ailments. Natural products and related drugs are used to treat 87 % of all categorized human diseases including bacterial infections, cancer and immunological disorders (Newman DJ *et al*.,2007). *Leea macrophylla* Roxb. is one of the medicinal plants of immense pharmaceutical value, therefore, in this present research work, *Leea macrophylla* Roxb. was chosen to study for their possible pharmacological values. The plant has various ethno-pharmacological uses and almost all parts of the plants possess potential curative properties. Roots are used in a number of disorders like fracture and cut injury. Roots also possess anthelmintic, astringent, styptic and antiseptic properties (Nizam *et al*., 2012).

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Botanical Description

(a) Habit

(b) Inflorescence

(c) Root

Figure 1. *Leea macrophylla* Roxb. ex Hornem.

Scientific name	:	*Leea macrophylla* Roxb.ex Hornem.
Family	:	Vitaceae
English name	:	Large-leaf Leea; Hathikana; Leea
Myanmar name	:	Kya hpetgyi
Part used	:	Root
Medicinal uses	:	goiter, gastric tumor, lipoma, anthelmintic, anesthetic (Aliev G *et al*., 2009)

Preliminary Phytochemical Screening of the Root of *Leea macrophylla* Roxb.

The preliminary phytochemical screening for alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, steroids, reducing sugars, terpenes and tannins were carried out by standard method. (Harbones, 1998)

Antimicrobial Activity from the Root of *Leea macrophylla* Roxb.

Antimicrobial activity of ethyl acetate, ethanol, *n*-hexane and water extracts of *Leea macrophylla* Roxb., was determined by using paper disc diffusion assay method. All of these extracts were tested on six selected organisms at Department of Chemistry, Pakokku University.

Screening of Antioxidant Activity by DPPH Free Radical Scavenging Assay Method

Screening of antioxidant activity of the ethyl acetate and *n*-hexane crude extracts of *Leea macrophylla* Roxb., was measured by using DPPH free radical scavenging assay method (Brand-Williams *et al*., 1995). Absorbance measurements were done in triplicate for each sample solution. Absorbance values obtained were used to calculate % inhibition and 50 % inhibitory concentration (IC_{50}). The lower the IC_{50} value indicates the higher the free radical scavenging activity.

Measurement of DPPH radical scavenging activity by spectrophotometric method

The control solution was prepared by mixing 2.0 mL of 60 μM DPPH solution and 2.0 mL of ethanol. Then, the test sample solutions were also prepared by mixing 2.0 mL of test solution and 2.0 mL of 60 μM DPPH solution with various concentrations. The blank solution was also prepared by mixing 2.0 mL of test sample solution and 2.0 mL of 95 % ethanol. These solutions were allowed to stand at room temperature for 30 minutes. Moreover, the absorbance of each solution was measured at 519 nm by using UV-Vis spectrophotometer. Ascorbic acid was used as standard compound and the amount of antioxidant activity was calculated from ascorbic acid standard curve.

Determination of Anti-inflammatory Activity from the Root of *Leea macrophylla* Roxb. by the Croton Oil-induced Ear Edema Method

The anti-inflammatory activity of the ethanol extract of this sample was tested by the croton oil-induced ear edema method (Manga, *et al*., 2004), at the Department of Biotechnology, Mandalay Technological University, Patheingyi Township, Mandalay Region, Myanmar.

The internal surface of the right ear of five groups of mice with weight of 30 g was treated with 20 µL of 5 % croton oil as an irritant to induce skin inflammation. The left ear remained untreated. The experimental mice were topically applied 0.75 mg/ear, 1.5 mg/ear and 2.25 mg/ear of ethanol extract prior to 30 min croton oil. The positive control group was received 0.50 mg/ear aspirin. Ear thickness was evaluated with clipper 6 hours after edema induction.

The mice were randomized into five groups, each of which included five mice.

1. Negative control group: 5 % croton oil only.
2. Positive control group: Received apirin (0.50 mg/ear).
3. Group A, Received 0.75 mg/ear of ethanol extract of test sample
4. Group B, Received 1.5 mg/ear of ethanol extract of test sample
5. Group C, Received 2.25 mg/ear of ethanol extract of test sample

The percentage of edema inhibition was defined in relation to the control group, which received the croton oil solution, according to the following formula:

Inhibition % = (DControl - DTreated / DControl) × 100

where DControl is the difference in edema thickness in the control group and DTreatment is the difference in edema thickness for the treated groups.

Results and Discussion

Preliminary Phytochemical Screening of the Root of *Leea macrophylla* Roxb.

The results of phytochemical screening of the root of *Leea macrophylla* Roxb.*, was* shown *in* Table 1.

Table 1. Results of Phytochemical Screening on the Root of *Leea macrophylla* Roxb.

No	Test of compounds	Extract solvent	Test reagent	Observation	Results
1	Alkaloids	1 % HCl	Wagner's reagent	No Brown ppt	-
			Dragendorff's reagent	Orange ppt	+
2	Flavonoids	95 % EtOH	Small pieces of Mg ribbon, HCl (conc:)	Red colour solution	+
3	Glycosides	Distilled water	10% lead acetate solution	White ppt	+
4	Phenolic compounds	Distilled water	10% ferric chloride solution	Dark green colour solution	+
5	Polyphenols	95 % EtOH	1% $FeCl_3$, 1 % $K_3[Fe(CN)_6]$	Dark green colour solution	+
6	Saponins	Distilled water	Distilled water, shake	Forthing	+
7	Steroids	Pet-ether	Acetic anhydride, conc. H_2SO_4, chloroform	Green colour solution	+
8	Reducing sugars	Distilled water	Benedict's Reagent	Brick-red ppt	+
9	Terpenes	95 % EtOH	acetic anhydride, chloroform, conc. H_2SO_4	Red colour solution	+
10	Tannins	95 % EtOH	10 % $FeCl_3$, dil., H_2SO_4 solution	White ppt	+

According to the phytochemical results, the root of *Leea macrophylla* Roxb. consists of alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, steroids, reducing sugars, terpenes and tannins respectively.

Antimicrobial Activity of the Root of *Leea macrophylla* Roxb.

The antimicrobial activity of ethyl acetate, ethanol, *n*-Hexane and water extracts of *Leea macrophylla* Roxb., was determined by using paper disc diffusion assay method on six selected organisms. The results are shown in Table 2 and Figure 3.

Table 2. Results of Antimicrobial Activity of Root of *Leea macrophylla* Roxb.

Extract	Test Organisms (mm)					
	Bacillus subtilis	***Bacillus pumilis***	***Salmonella typhi***	***Staphylococcus aureus***	***Escherichia coli***	***Candida albicans***
EtOAc	22	22	21	22	22	19
EtOH	21	22	20	21	22	18
n- Hexane	13	13	14	12	12	-
Water	-	-	-	-	-	-

Paper disc diameter 8mm 15 mm- 19 mm Medium
10 mm - 14 mm Low 20 mm above High

The results of the antimicrobial activity tests showed that both the ethyl acetate and ethanol extracts exhibited the highest activity against *Bacillus subtilis*, *Bacillus pumilus*, *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*, and moderate activity against *Candida albicans*. In contrast, the *n*-hexane extract showed low activity against all tested organisms except *Candida albicans*. The aqueous (water) extract did not exhibit any antimicrobial activity against any of the tested organisms. Based on these results, the ethyl acetate and ethanol extracts demonstrated stronger antimicrobial activity compared to the other extracts. These antimicrobial agents may play an essential role in the prevention, treatment, and control of foodborne diseases in animals caused by pathogenic microorganisms. (Chain, 1958).

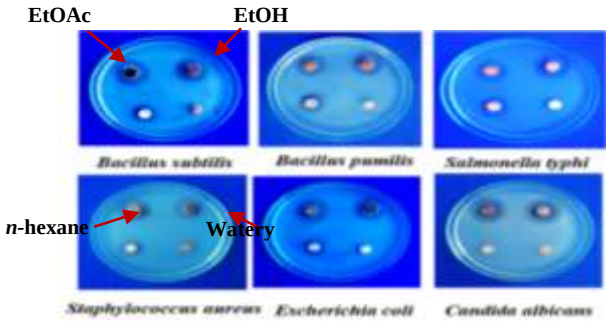

Figure 2. Antimicrobial activity of the root of *Leea macrophylla* Roxb.

Determination of Antioxidant Activity for Crude Extracts of the Root of *Leea macrophylla* Roxb.

The antioxidant activity of ethyl acetate crude extract and *n*-hexane crude extract of selected sample was determined by DPPH free radical scavenging assay method. The results of the antioxidant activity for standard ascorbic acid and crude extracts are shown in Table 3, 4, 5 and Figure 4, 5, 6.

Table 3. % Inhibition in Different Concentrations and IC_{50} Value of Standard Ascorbic Acid

Sample	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Ascorbic acid	1	0.228	34.83	4.41
	2	0.166	40.91	
	4	0.129	49.74	
	8	0.109	65.62	
	16	0.086	92.91	

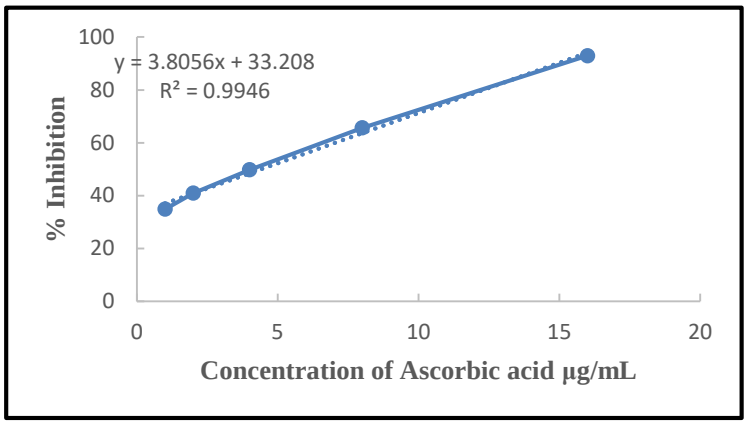

Figure 3. % Inhibition vs different concentration of standard ascorbic acid

Table 4. % Inhibition in Different Concentrations and IC_{50} Value of Ethyl Acetate Extract from the Root of *Leea macrophylla* Roxb.

Extract	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Ethyl Acetate	15.625	0.212	47.303	20.34
	31.25	0.202	54.331	
	62.5	0.197	56.685	
	125	0.125	63.027	
	250	0.031	83.589	

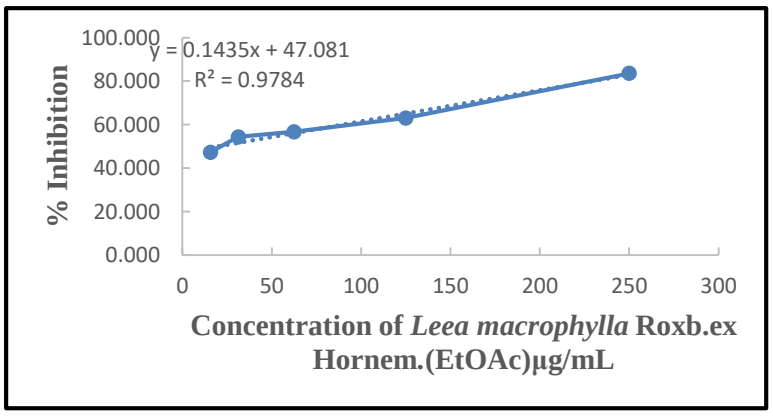

Figure 4. % Inhibition vs different concentration of ethyl acetate crude extract

Table 5. % Inhibition in Different Concentration and IC_{50} Value of *n*-Hexane Extract from the Root of *Leea macrophylla* Roxb.

xtract	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
n-hexane extract	25	0.232	45.412	31.31
	50	0.197	53.647	
	100	0.164	61.412	
	200	0.127	70.118	
	400	0.028	93.412	

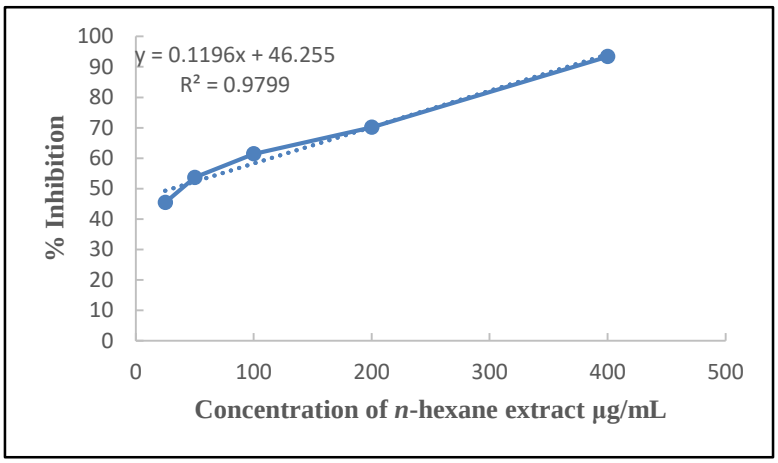

Figure 5. % Inhibition vs different concentration of *n*-hexane crude extract

The IC_{50} value is a commonly used parameter to evaluate antioxidant activity. Radical scavenging activity is expressed in terms of IC_{50}, which represents the concentration required to inhibit 50 % of free radicals. A lower IC_{50} value indicates higher antioxidant activity. Based on the results, the ethyl acetate extract of the root of *Leea macrophylla* Roxb. exhibited stronger antioxidant activity (IC_{50} = 20.341 µg/mL) compared to the *n*-hexane extract (IC_{50} = 31.31 µg/mL). Antioxidants play a protective role in the prevention of blood vessel diseases, atherosclerosis, cancer, and other conditions in the human body. (Fakruddin, 2012)

Anti-inflammatory Activities from Ethanol Extract of the Root of *Leea macrophylla* Roxb. by the Croton Oil-Induced Ear Edema Method

Anti- inflammatory activities from the root of selected sample were determined in ethanol solvent extracts by using croton oil-induced ear edema test at the Department of Biotechnology, Mandalay Technological University, Patheingyi Township, Mandalay Region, Myanmar.The results are shown in Table 6.

Table 6. The Results of Anti-Inflammatory Activity of the Root of *Leea macrophylla* Roxb.

Treatment	Dose µL	Differences (Thickness) mm	Inhibition %
Negative Control (Croton oil only)	20	1.352	-
Aspirin (Positive Control)	0.5	0.194	86
Group A	0.75	0.868	36
Group B	1.15	0.738	45
Group C	2.25	0.698	48

The anti-inflammatory activity of the root of *Leea macrophylla* was evaluated based on ear thickness reduction and the corresponding percentage inhibition of inflammation. The results were compared with a control group, where the positive control showed 86 % inhibition at 0.5 mg/ear. The treatment groups A, B, and C demonstrated 36 % inhibition at 0.75 mg/ear, 45 % at 1.15 mg/ear, and 48 % at 2.25 mg/ear, respectively. These findings indicate that the selected sample exhibits moderate anti-inflammatory activity.

Conclusion

In this research, *Leea macrophylla* Roxb., a medicinal plant from Myanmar, was selected to investigate its phytochemical constituents, as well as its antimicrobial, antioxidant, and anti-inflammatory activities. Phytochemical screening of the root revealed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, steroids, reducing sugars, terpenes, and tannins. The antimicrobial activity of the ethyl acetate and ethanol extracts showed the highest effectiveness against *Bacillus subtilis*, *Bacillus pumilus*, *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*, with moderate activity against *Candida albicans*. In contrast, the *n*-hexane extract exhibited low activity against all tested organisms except *Candida albicans*, while the aqueous extract did not show any antimicrobial activity. Based on these findings, the ethyl acetate and ethanol extracts demonstrated stronger antimicrobial activity than the other extracts. Furthermore, the antioxidant activities of the ethyl acetate and n-hexane extracts were evaluated using the DPPH free radical scavenging assay. The ethyl acetate extract exhibited higher antioxidant activity, with an IC_{50} value of 20.341 μg/mL, compared to the n-hexane extract, which showed an IC_{50} value of 31.313 μg/mL. In addition, the anti-inflammatory activity of the ethanol crude extract was assessed using the croton oil-induced ear edema method. The treatment groups A, B, and C showed inhibition rates of 36 % (0.75 mg/ear), 45 % (1.15 mg/ear), and 48 % (2.25 mg/ear), respectively. These results indicate that the root extract of *Leea macrophylla* exhibits moderate anti-inflammatory activity.

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