

Phytochemical Screening, Elemental Analysis and Antimicrobial Activity from Leaves of *Heliotropium indicum* L.

HlaingMyint Thu
hlaingmyintthu753@gmail.com

ZarZar Yin
zzy.microbio@gmail.com

Su Su Latt
sslatt1997@gmail.com

Abstract

Heliotropium indicum L. is used in folkloric medicine for many diseases. In the present project study, the phytochemical screening of plants of *Heliotropium indicum* L. extracts gave rise to some compounds such as Alkaloids, Flavonoids, Polyphenols, phenolic, steroids, Saponins, respectively. Moreover, some elements in *Heliotropium indicum* L. were analyzed by EDXRF method. According to qualitative EDXRF measurement, Ca (55.450 %), K (21.259 %), CL (11.163 %), Fe (6.376%), S (2.419 %) and trace amount of elements Mn (0.978%), Zn (0.387 %), Cu(0.175%), were contained in *Heliotropium indicum* L.. The antimicrobial activities of *Heliotropium indicum* L. was performed by agar well diffusion method. Water, ethanol and petroleum ether extracts of *Heliotropium indicum* L. did not show antimicrobial activity. But, ethyl acetate extracts of *Heliotropium indicum* L. against seven test organisms. In leaves, EtoAc extract of *Heliotropium indicum* L. showed strong activity (22.92-32.46mm) against seven test organisms. According to the above results, *Heliotropium indicum* L. could be used in different traditional and play important roles in maintaining healthy life style.

Keywords: phytochemical screening, phenolic, antimicrobial activities, test organisms,

1. Introduction

Plants have been considered as valuable resource of natural products and bioactive compounds which could play important roles in maintaining healthy life style [4]. In folkloric medicine, *Heliotropium indicum* L. is highly valued in treating dermatitis, venereal diseases, insect bites, scabies, skin pruritis, menstrual disorder, malaria, abdominal pain, fever, urticaria, and sore throat. Its crude extract is applied to cure rheumatism and skin infections while its decoction is used as diuretic and kidney stone remedy [8,6].

2. Materials and Methods

2.1 Sample Collection

Heliotropium indicum L. was collected from Za-Ga-War road in Patheingyi Township (N 16° 47' 49.073" E 94° 44' 12.594" during June- July 2018), Ayeyarwady Region. All samples were washed with water, and were cut into small pieces and allowed to air dry for two weeks. The air dried samples were stored in well stopper bottle and used throughout the experiment. The

antimicrobial activity of *Heliotropium indicum* L. was performed in the laboratory of Biological Resources and Biotechnology Development Center at Patheingyi University.



Figure 1. The habit of *Heliotropium indicum* Linn.

2.2. Test Organisms

The test organisms used for this experiment were *Salmonella typhi*, *Bacillus pumilus*, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542, *Escherichia coli* AHU5436, *Pseudomonas fluorescens* IFO94307, *Staphylococcus aureus* AHU8465. The organisms were obtained from National Institute of Technology and Evaluation (NITE, Japan), and Pharmaceutical Research Department, Yangon, Myanmar.

2.3. Screening of Antimicrobial Activity by Using Agar Well Diffusion Method

Glucose 0.5g, Yeast extract 0.3g, Peptone 0.3g, Agar 1.7g, 100ml of distilled water were added in a 250mL sterile conical flask and heated on hot plate until boil medium. Then, the mouth of the flask was plugged with a piece of cotton wool. This medium was sterilized in an autoclave at 121 ° C for 45 minutes. After 45 minutes, a 0.1mL test organisms were inoculated into 20mL of medium agar at about 40 ° C and were poured into the sterile petri- dishes at aseptic condition. After the agar become solid, cork borer was used to make the wells (8 mm in diameter). Then extract samples (20 µL) were introduced into the well and they were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Clear zone surrounding the wells indicated the presence of antimicrobial active compound in the extracts which inhibit the growth of the test organisms. The antimicrobial activity of *Heliotropium indicum* L. was performed by agar well diffusion method [3] in the Laboratory of Biological Resources and Biotechnology Development Center at Patheingyi University.

3. Results and Discussion

3.1. Phytochemical Screening of *Heliotropium Indicum* L.

The preliminary phytochemical screening of plants of *Heliotropium indicum* L. extract showed the presence of alkaloids, flavonoids, polyphenols, phenolic compounds, steroids, saponins (Table 1). Some alkaloids were known to have muscarinic activity [5] and Saponin is one of the major constituents of *Heliotropium Indicum* L. is known to elicit serum cholesterol lowering activity and may be classified as a direct antidyslipidemic agent, in contrast indirect antidyslipidemic agents like the sulphonylureas that act through the stimulation of pancreatic beta cells increase insulin release [1]. Plant phenolics are very important constituents because of their free radical scavenging activity resulting from their hydroxyl group. The phenolic compounds may contribute directly to the antioxidative action [2].

Table 1. Preliminary phytochemical screening of *Heliotropium indicum* L.

Tests	Solvent Extract	Reagents	Observation	Result
1 Alkaloids	1% HCl	Dragendroff's reagent	Red ppt	+
		Wagner's reagent	Reddish-Brown	+
		Mayer's reagent	Yellow	+
		Hager's test	Yellow ppt	+
2 Flavonoids	95% EtOH	Mg, conc:HCl	Yellow colour	+
		1% KOH	Yellow colour	+
		1% FeCl ₃	Blue green	+
1% K ₃ Fe(CN) ₆				
3 Polyphenol	EtOH	1% FeCl ₃	Bluish black	+
4 Phenolics	Distilled water	1% K ₃ Fe(CN) ₆		
5 Steroids	PE,	(CH ₃ CO) ₂ O,	Blue ppt	+
		H ₂ SO ₄	Golden Yellow	+
6 Saponins	CHCl ₃	Conc:H ₂ SO ₄	foams	+
		Distilled water		

(+) Presence of constituents, (-) Absence of constituents

3.2. Qualitative Elemental analysis of *Heliotropium Indicum* L.

The elements present in ash obtained from *Heliotropium indicum* L.. Plants were quantitatively determined by EDXRF spectrometer. The resultant EDXRF spectrum of *Heliotropium indicum* L. is illustrated in figure 2 and Table 2. According to this experiment, it was found that Ca, K, Cl, S, Fe, Mn, Zn, and Cu, were present in *Heliotropium indicum* L.. Ca (55.450%) was found to be the most abundant element, followed by K (21.259%), Cl (11.163%), Fe (6.376%), S (2.419%), and trace amount of Mn, Zn, Cu, were also contained in samples. Therefore, calcium is builds and maintains bones in teeth; regulates heart rhythm; eases ansomina; helps regulate the passage of nutrients in and out of the cell walls; exists in normal blood clotting; helps maintain proper never and muscle function important to normal kidney function. Potassium is one of the essential elements and every significant body mineral, important to both cellular and electrical function. Potassium is essential for the body's growth and maintenance, needed for the heart function, kidneys and hypertension. Sulfur can kill insects, mites, fungi, and rodents. Sulfur has been registered for use in pesticides in the united stated since the 1920s [7]. Iron builds up the quality of the blood and increases resistance to stress and diseases. Thus, it can be suggested that these essential elements necessary for human beings can be supplied from *Heliotropium indicum* L..

Table. 2 Relative Abundance of Some Elements in *Heliotropium idicum* L.

No	Elements	Relative abundance (%)
Leaves		
1	Ca	55.450
2	K	21.259
3	Cl	11.163
4	Fe	6.376
5	S	2.419
6	Mn	0.978
7	Zn	0.387
10	Cu	0.175

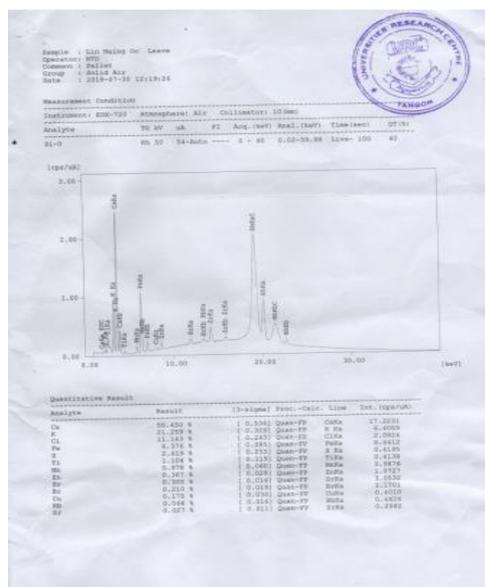


Figure 2. EDXRF spectrum of *Heliotropium indicum*L.

3.3. Screening on Antimicrobial Activity of *Heliotropium indicum* L.

In a leaves of *Heliotropium indicum* Linn, water, petroleum ether and ethanol extracts did not show activity on seven test organisms but ethyl acetate extracts showed highest activity 32.46mm against *Salmonella typhi*, 24.06mm against *Bacillus pumilus*, 30.34mm against *Bacillus subtilis*, 30.01mm against *Candida albicans*, 25.65 mm against *E.coli*, 23.72mm against *P.fluorescens* and 22.92mm against *S. aureus* (Table 3 and Figure 3).

Table 3. Antimicrobial activity of *Heliotropium Indicum*L.(leaves) against seven tests organisms

Extracts	1	2	3	4	5	6	7
H ₂ O extract	-	-	-	-	-	-	-
PE extract	-	-	-	-	-	-	-
EtOH extract	-	-	-	-	-	-	-
EtOAc extract	32.46	24.06	30.34	30.00	25.64	23.72	22.92

(+) = Activity (-) = No activity Agar well = 8
 1. *Salmonella typhi* 2. *B.Pumilus* 3. *B.subtilis* 4. *C.albicans*
 5. *E. Coli* 6. *P.Fluorescens* 7. *S.aureus*

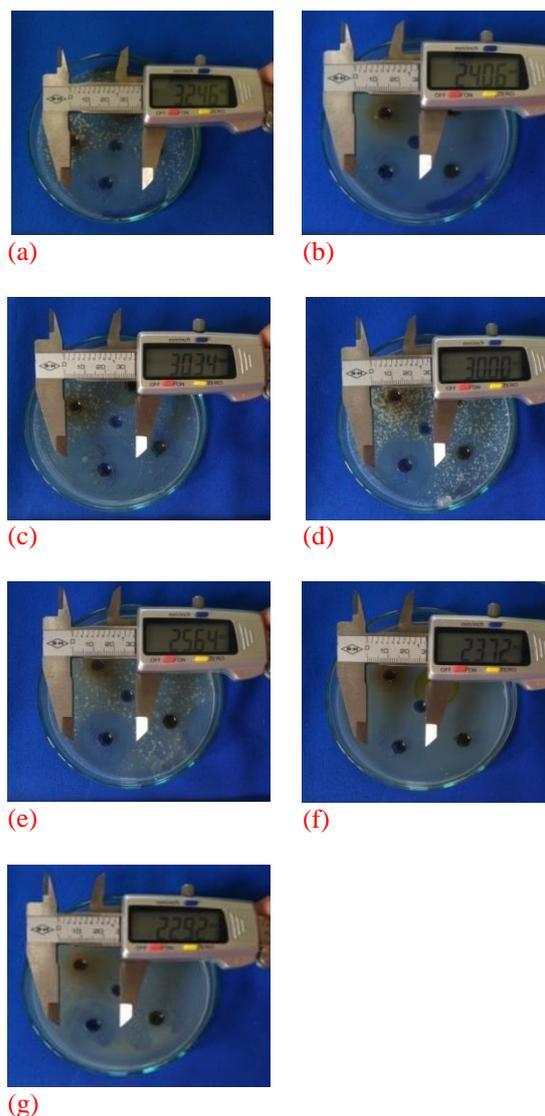


Figure 3. Antimicrobial activity of crude extracts of *Heliotropium Indicum* L. (leaves) against (a) *Salmonella typhi*, (b) *B.pumilus*, (c) *B.subtilis*, (d) *Candida albicans*, (e) *E.coli*, (f) *P.fluorescens*, (g) *S. aureus*

Conclusion

From the present investigation, the results of phytochemical examination indicated that *Heliotropium indicum*L. consist of alkaloids, flavonoids, polyphenols, phenolic compounds, steroids, saponins, carbohydrates, α- amino acids, glycosides, starch, and reducing sugars. Therefore, these Phyto compounds from *Heliotropium indicum* L. plants provide the many medicinal benefits.

Furthermore, during the Elemental analysis by EDXRF spectrometer, calcium was found to be the most abundant element followed by K (21.259%), Cl (11.163%), Fe (6.376%), S (2.419%), and trace amount of Mn, Zn, Cu, were also contained in samples. The principal minerals elements are required in the human body and

small quantities of trace elements may also be essential to life. *Heliotropium indicum* L. possessed strong antimicrobial compounds and may act against broad range of disease causing microorganisms. Therefore, it was concluded that *Heliotropium indicum* L. can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

Acknowledgements

We would like to thank to the Department of Higher Education, Ministry of Education in Myanmar, for giving us the opportunity to do this research. Our deepest gratitude is expressed to Dr. Zar Zar Yin, Associate Professor, Department of Botany, and University of Bago for her encouragement, kind guidance, and kind help to do this research. We wish to thank the University journal of Creativity and Innovative Research (Banmaw, University of Computer) for allowing to present this paper.

References

- [1] Abdel-Hassan I.A, Abdel-Barry J.A, Mohammad S.T. "The hypoglycaemic and Antihyperglycaemic effect of citrulluscolocynthis fruit aqueous extract in Normal and alloxan diabetic rabbits". *Journal of Ethnopharmacology*,2000, 71:325-330
- [2] Bidchol A.M, AWilfred, PAdhijna, R.Harish,"Food and Bioprocess Technology", 2011,4, 1137-1143
- [3] Collins, C.H.,Microbiological Methods. Landon: 5th Edition, Butterworth and Co., Publishers Ltd., 1965.
- [4] Kumar B.M.V, R.R, Govinda, G.P. Pushpan"Ethnopharmacological approaches to wound healing-Exploring medicinal plants of India".*J Ethnopharmacol*, 2007, 144: 103-113
- [5] Lui JC, FLHsu, JCTsai, PChan, JYHLiu, "Anthypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitor of angiotensin converting enzyme". *Life Sciences*. 2003,73:1543-1555
- [6] Muthu C, MAyyanar, NRaja, SIgnacimuthu,*Journal of Ethnobiology and Ethnomedicine*, 2006,2, 43
- [7] NPRO.NPIC Product Research Online: Sulfur, National pesticide information Center: Corvallis,OR., 2017,(11.8.18)
- [8] Togola A, D. Diallo, S. Dembélé, H. Barsett and B. S. Paulsen, "Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions. "Doila, Kolokani and Siby. *J EthnoboilEthnomedicine*.2015,1(1): 7.