

Study on the Phytochemical Constituents, Elemental Analysis and Antimicrobial Activities from the Leaves of *Coleus amboinicus* Lour. (Zi Yar)

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Abstract

The main aim of the present study was to analyse the chemical composition of *Coleus amboinicus* Lour. (Zi Yar). The preliminary investigation of phytochemical compounds present in the leaves of *Coleus amboinicus* Lour. was carried out using the test tube method which resulted in the discovery of compounds such as alkaloids, phenolic compounds, flavonoids, glycosides, polyphenols, tannins, saponins, carbohydrates and α -amino acid. Moreover, some elements in *Coleus amboinicus* Lour. were analysed by the EDXRF method. According to the qualitative EDXRF measurement, K (1.954%), Ca (0.811 %), Si (0.339 %), S (0.077 %), Fe (0.029 %), Ti (0.008 %), Mn (0.002 %), Zn (0.002 %) Cu (0.002 %), Sr (0.001 %) and Cr (0.001 %) were also contained in *Coleus amboinicus* Lour.. The antimicrobial activity of *Coleus amboinicus* Lour. was performed by the agar-well diffusion method. Among them, the ethyl acetate extract of *Coleus amboinicus* Lour. showed higher activity (38.53 mm) against six tested organisms than the other extracts. The MICs value of ethyl acetate extract of *Coleus amboinicus* Lour. was 5 mg in 2 mL against *Bacillus subtilis* and *Escherichia coli*. in the investigation of minimum inhibitory concentration. *Coleus amboinicus* Lour. leaves have various antimicrobial activities. Therefore, the leaves of *Coleus amboinicus* Lour. should be used in different traditional medicine from local source.

Keywords: phytochemical; antimicrobial activity; EDXRF; agar-well diffusion method

Introduction

Herb plants are usually used in foods and in traditional medicines as well. They have been used to cure the disease for a long time. They are popular remedies because of their bioactive compounds which can be helpful for many health problems. *Coleus amboinicus* Lour. is a semi succulent perennial plant belonging to the family Lamiaceae (Data Sheet, 2017) with a pungent oregano-like flavor and odour. The Leaves of *Coleus amboinicus* Lour. are traditionally used for the treatment of coughs, sore throats and nasal congestion and other problems such as infections, rheumatism and flatulence, malaria fever, hepatopathy, renal calculi, asthma and hiccoughs (kirtikar and Basu 2005 and Kaliappan *et al.*, 2008 and Shenoy and Smita, 2012). Its insect repellent properties have been tested (Kathiresan, 2000).

In Myanmar *Coleus amboinicus* Lour. (Zi Yar) is used as a drink for cough and sore throat and its essential oil is also applied on skin to protect insects and on chest for nasal congestion. It is also popular as traditional remedy in Myanmar. Hence, the plant is also known to contain the constituents responsible for cytotoxicity, anti- bacterial activity (Da Costa *et al.*, 2010) and antioxidant activity(khanum *et al.*, 2011). The present study is focused to investigate the phytochemical properties, minerals and antimicrobial activities of leaves of *Coleus amboinicus* Lour.

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Materials and Methods

Sample Collection and Preparation

During September-October 2022, the freshly harvested Leaves of *Coleus amboinicus* Lour. (Zi Yar) were collected from Daw Ta Ma quarter, Loikaw Region, Kayah State (N 19° 40' 50.700" E 97° 12' 47.525") . All samples were washed with distilled water, chopped and dried at room temperature for two weeks. The dried material was made into powder by using a grinding machine and stored in an airtight glass bottle and used throughout the experiment.

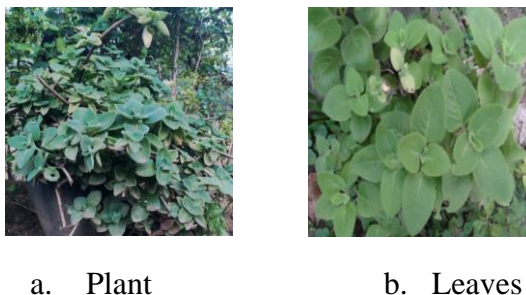


Figure 1. The plant and leaves of *Coleus amboinicus* Lour.

Botanical Description of *Coleus amboinicus* Lour.

Family : Lamiaceae
 Genus : *Coleus*
 Species : *amboinicus* Lour.
 Scientific name : *Coleus amboinicus* Lour.
 English Name : Indian borage
 Myanmar Name: Zi Yar
 Part used : Leaves

Preparation of Crude Extracts

The solvent extraction process of *Coleus amboinicus* Lour. was carried out by immersing in water, ethanol, ethyl acetate and n-hexane. The solvent composition of each formula is 1:10 (w/v), sample: solvent for 10 days. Afterward, the mixtures were filtered through Whatman No.1 filter paper and the residual solvent was evaporated in a water bath at 70 °C. Water extraction was performed and 10 g of *Coleus amboinicus* Lour. was added into a 500 mL conical flask. 100 mL of boiling water was poured over the sample and stirred. After sealing the mouth of the flask with foil and parafilm to avoid contamination, it was placed in a shaker incubator for continuous swirling at 120 rpm, 30 °C for 12 hours and allowed to cool throughout the extraction process. Afterward, the mixture was filtered through Whatman No.1 filter paper and the residual water extract was evaporated in the water bath at 80 °C. Finally, a crude extract of *Coleus amboinicus* Lour. was obtained and stored in the freezer for the next experiments.

Test Organisms

Bacillus pumilus (fever), *Bacillus subtilis* (fever) , *Candida albicans* NITE 09542 (skin disease) , *Escherichia Coli* AUH 5436 (diarrhea, abdominal pain,) *Pseudomonas aeruginosa* (Pneumonia) and *Salmonella typhi* AHU 7943 (Typhoid fever) were used for the agar-well diffusion method in the Laboratory of Department of Chemistry, Loikaw University, Myanmar. These tests organisms were obtained

from the National Institute of Technology and Evaluation (NITE), Japan and the Pharmaceutical Research Department (PRD), Yangon, Myanmar.

Phytochemical Screening of *Coleus amboinicus* Lour.

Phytochemical investigation of *Coleus amboinicus* Lour. was performed using the standard test tube method (Harbone, 2015).

Determination of Elemental Analysis of *Coleus amboinicus* Lour.

Elemental analysis of *Coleus amboinicus* Lour. was performed using an EDXRF spectrometer.

Determination of Antimicrobial Activity of *Coleus amboinicus* Lour.

In a 250 mL sterile conical flask, glucose (0.5 g), yeast (0.3 g), peptone (0.3 g), agar (1.7 g) and 100 mL of distilled water were heated on a hot plate until they reached a 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, 0.1 mL of test organisms were inoculated into 20 mL of medium agar at room temperature and were poured into the sterile Petri dishes under aseptic conditions. After the agar became solid, a cork borer was used to make the well (8 mm in diameter). Then the extracts samples (20 µL) were introduced in the well and were incubated at room temperature for 24 hours. After 24 hours of incubation, the clear zones were measured. The clear zone surrounding the wells indicated the presence of the antimicrobial active compounds in the extracts which inhibit the growth of the test organisms (Dubey and Maheshwari, 2002).

Determination of the Minimum Inhibitory Concentration (MIC) of the Crude Extracts

Minimum Inhibitory concentrations (MICs) were carried out by the two-fold serial dilution Method (Demain, 1986 and Phay, 1997). *Bacillus subtilis* and *Escherichia coli* were used as test organisms. GYP broth (glucose 1.0 g, yeast extract 0.3 g, peptone 0.3 g, distilled water 100 mL) was prepared and labeled A-F. Two-fold serial dilutions of the crude extract were prepared to give a decrease in concentrations ranging from 5, 2.5, 1.25, 0.625, 0.313 and 0.156 mg in 2 mL respectively. This concentration was achieved by weighting and dissolving 10 mg of crude extract in a test tube labeled T containing 1:1 (v/v) (EtOAc: H₂O). One milliliter (1 mL) was transferred from test tube T to test tube A which contained 1 mL of GYP to give 5 mg in 2 mL. This procedure continued until a concentration of 0.156 mg in 2 mL was obtained in the sixth test tube labeled F (0.156 mg in 2 mL). A homogenous mixture was obtained by vigorously shaking each tube for at least 5 seconds . And , 0.2 mL of test organisms were inoculated in each test plate. A total of 20 µL of different dilutions of the compound were dropped into the agar-well. After incubation for 24 hours, the MICs were determined by selecting the lowest concentration of metabolite that caused complete inhibition of test (bacterial) growth. The experiments were done in triplicate. The MICs were determined by the lowest concentration of the compounds that prevented visible growth (Andrews, 2005 and Ewanshiha *et al.*, 2016).

Results and Discussion

Phytochemical Screening of *Coleus amboinicus* Lour.

The phytochemical study was carried out to learning about the different types of phytochemical organic compounds found in selected *Coleus amboinicus* Lour. It was found that alkaloids, α -amino acids, phenolic compounds, carbohydrate, saponins, flavonoids, glycosides, tannins and polyphenols were all involved. These results are shown in Table 1 and Figure 2. Alkaloids and flavonoids, for example, are known to promote the wound healing due to their antimicrobial properties (Lui *et al.*, 2015).

Table 1. Preliminary Phytochemical Screening of *Coleus amboinicus* Lour.

Tests	Extracts	Test reagents	Observations	Results
Alkaloids	1%HCL	Wagner's reagent	Brown	+
		Dragendorff's reagent	Reddish brown	+
		Mayer's Reagent	Pale yellow	+
		Hayer's Reagent	Yellow	+
α -amino acids	H ₂ O	Ninhydrin Reagent	Purple color	+
Phenolic	H ₂ O	10%FeCl ₃ , K ₃ Fe(CN) ₆	Darkblue color	+
Saponins	H ₂ O	Distilled Water	Foaming	+
Flavonoids	EtOH	Conc:HCL, Mg ribbon	Brown	+
Glycosides	H ₂ O	10% lead Acetate	White ppt	+
Tannins	EtOH	10%FeCl ₃	Green	+
Polyphenol	EtOH	10%FeCl ₃ , K ₃ Fe(CN) ₆	Blue	+
Lipophilic	H ₂ O	0.5M KOH	Brown	-
Carbohydrate	H ₂ O	α -naphthol, H ₂ SO ₄ (conc)	Brown Ring	+

(+) = presence

(-) = absence

(ppt) = precipitate

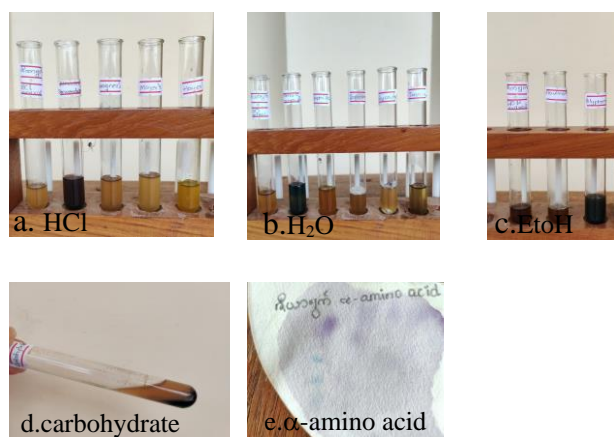


Figure 2. Phytochemical investigation of various crude extracts of *Coleus amboinicus* Lour.

Elemental Analysis of *Coleus amboinicus* Lour.

According to the EDXRF spectrum analysis, it was found that K (1.954 %), Ca (0.811 %), Si (0.339 %), S (0.077 %), Fe (0.029 %), Ti (0.008 %), Mn (0.002 %), Zn (0.002 %) Cu (0.002 %), Sr (0.001 %) and Cr (0.001 %) were also contained in the sample. Therefore, Potassium is an essential nutrient needed for the maintenance of total body fluid volume, acid, electrolyte balance and normal cell function (Young, 2001). Iron builds up the quality of blood and increases resistance to stress and diseases. Besides, Iron (Fe) can bind, transport and release oxygen in the body's health (Nielsen and Hunt, 1989). The resultant EDXRF spectrum of *Coleus amboinicus* Lour. was shown in Table 2 and Figure 3.

Table 2. Relative Abundance of Some Elements In *Coleus amboinicus* Lour.

Elements	Relative abundance (%) Plants
K	1.954
Ca	0.811
Si	0.339
S	0.077
Fe	0.029
Ti	0.008
Cu	0.002
Zn	0.002
Mn	0.002
Cr	0.001
Sr	0.001

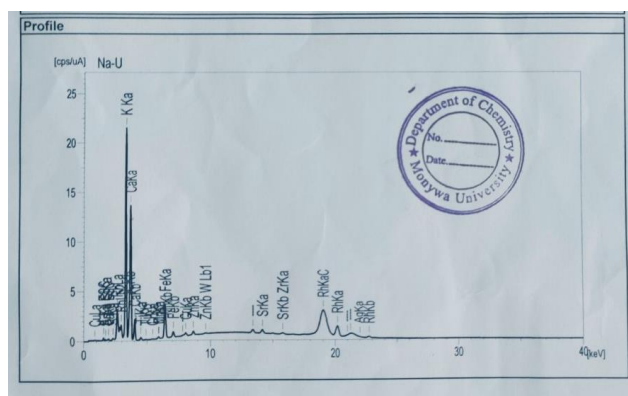


Figure 3. EDXRF spectrum of *Coleus amboinicus* Lour.

Investigation of Antimicrobial Activity of Crude Extracts of *Coleus amboinicus* Lour.

In this study, an ethyl acetate extract of *Coleus amboinicus* Lour. demonstrated higher activity including against six test organisms *Bacillus pumilus* (30.80 mm), *Bacillus subtilis* (34.72 mm), *Candida albicans* (35.10 mm), *Escherichia coli* (38.53 mm), *Pseudomonas aeruginosa* (32.36 mm) and *Salmonella typhi* (31.81 mm) than other extracts such as ethanol extract, water extract and n-hexane extract.

Coleus amboinicus Lour. has antibacterial activity due to the presence of several phytochemical compounds with known antimicrobial properties (Oliveira *et al.*, 2005). These results are shown in Table 3 and Figure 4.

Table 3. Result of an Investigation into the Antimicrobial Activity of *Coleus amboinicus* Lour.

Organism	Diameter of the inhibition zone (mm)			
	H ₂ O extract	EtOH extract	EtOAc extract	n-Hex extract
<i>Bacillus pumilus</i>	–	–	30.80 (+++)	–
<i>Bacillus subtilis</i>	–	–	34.72 (+++)	–
<i>Candida albicans</i>	–	–	35.10 (+++)	–
<i>Escherichia coli</i>	–	–	38.53 (+++)	–
<i>Pseudomonas aeruginosa</i>	–	–	32.36 (+++)	–
<i>Salmonella typhi</i>	–	–	31.81 (+++)	–

Agar-well = 8 mm

10-14 mm (+), 15-20 mm (++), 21 mm and above (+++)

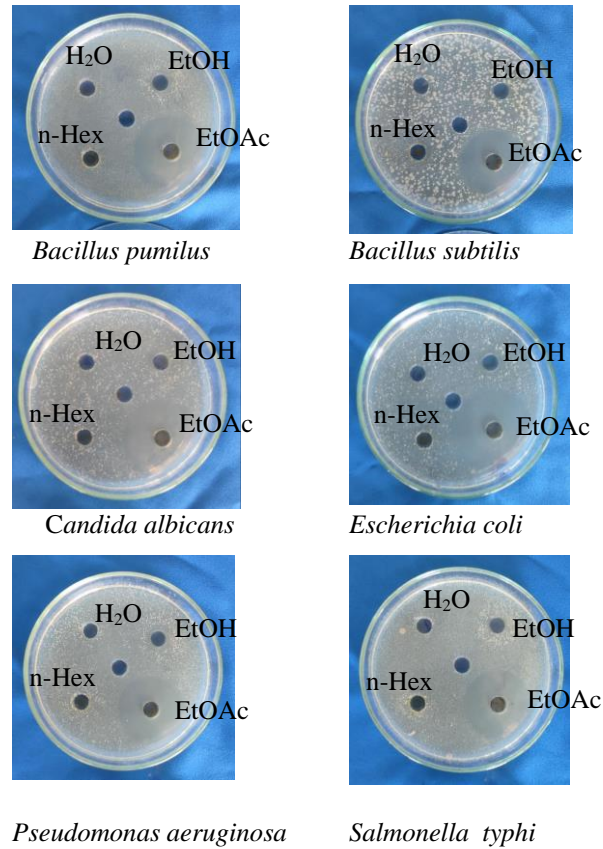


Figure 4. Investigation of antimicrobial activity of *Coleus amboinicus* Lour.

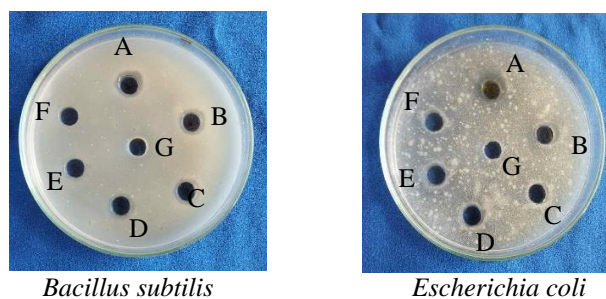
Investigation of the Minimum Inhibitory Concentration (MIC) of the Crude Extracts

In this investigation, the MICs value of ethyl acetate extract of *Coleus amboinicus* Lour. was 5 mg in 2 mL which can inhibit the growth of *Bacillus subtilis* and can also inhibit the *Escherichia coli* at 5 mg in 2mL. According to the above results, ethyl acetate extract of *Coleus amboinicus* Lour. showed as an antibacterial substance and a wide spectrum of antibacterial activity against human pathogenic bacteria. These results are shown in Table 4 and Figure 5.

Table 4. Result of an Investigation of the MIC of *Coleus amboinicus* Lour.

Test organisms	Concentration of extracts (mg/ml)	Size of well in mm (A)	Size of well + area (B)	Zone of inhibition (B-A)	
<i>Bacillus subtilis</i>	A	5	8	11.50	3.5
	B	2.5	8	8	0
	C	1.25	8	8	0
	D	0.625	8	8	0
	E	0.313	8	8	0
	F	0.156	8	8	0
	G	* C	8	8	0
<i>Escherichia coli</i>	A	5	8	12.20	4.2
	B	2.5	8	8	0
	C	1.25	8	8	0
	D	0.625	8	8	0
	E	0.313	8	8	0
	F	0.156	8	8	0
	G	* C	8	8	0

*C = control

Figure 5. Investigation of the MIC of *Coleus amboinicus* Lour.

Conclusion

The present research work, the preliminary phytochemical investigation revealed the presence of alkaloids, α -amino acids, phenolic compounds, saponins, flavonoids, carbohydrates, glycosides, tannins and polyphenols. Besides, these compounds from *Coleus amboinicus* Lour. give many medicinal benefits like disease prevention, health promotion and aesthetic properties. In addition, potassium was more abundant in *Coleus amboinicus* Lour. than the other elements (such as Ca, Mn, S and Cr) and these elements play a vital role in human beings. They can also aid in the maintenance of good health. The ethyl acetate extracts of *Coleus amboinicus* Lour. possesses greater antimicrobial activity against six human pathogens than other extracts. The MIC value of an ethyl acetate extract of *Coleus amboinicus* Lour. has an inhibitory effect on the growth of *Bacillus subtilis* and *Escherichia coli*. Thus, *Coleus amboinicus* Lour. is confirmed as a useful antimicrobial agent. As a result, it suggests that *Coleus amboinicus* Lour. could be a source of drugs used to treat infection caused by bacterial and fungi (human pathogens).

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References

- Andrews, J.M., "BSAC standardized disc susceptibility testing method", (Version 4). *Journal of Antimicrobial Chemotherapy*, vol.56(1), 2005, pp-60-76.
- Da Costa, JGM. Canpos AR. Brito SA. Pereira CKB. Eranio O Souza. Rodrigues FFG. Biological screening of araripe basin medicinal plants using *Artemia salina* Leach and pathogenic bacterial. *Ph Cog Mag*.2010; 6(24):331-334.
- Datasheet, Invasive Species Compendium (2017). *Plectranthus amboinicus* (Indian borage), Centre for Agriculture and Biosciences International.
- Demain, A.L. , "Regulation of secondary metabolism in fungi", *Pure Application Chemistry*, vol. 58, 1986, pp.219-226.
- Dubey, R.C and Maheshwari, D.K. 2002. **Practical Microbiology**. 5. chand and Company Ltd. Ram Nagar, New Dehli. 110-155 ELBS and E. and S. living stone Ltd.
- Ewanshiha, J.U. S.A. , Garba, G. , Musual, S.Y. , Busari, M.B. , Innalegwu,A. , and Doughari, J.H. , "Preliminary Phytochemical and Antimicrobial Activity of Citrus and Limon) Leaf Extract against some pathogenic Microorganisms", *Journal of Applied Life Sciences International*, vol.6 (4) , 2016, pp-1-10.
- Harbone, J.B. , "Phytochemical Method and Analysis", New York -2nd Ed , Chapman and Hall, 2015, pp.120-126.
- Lui, J.C. , Hsu, F.L., Tsai, J.C. , Chan, P and Liu, J.Y.H. , "Antihypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitors of angiotensin converting enzyme", *Life Sciences*. vol. 73, 2015, pp.1543-1555.
- Nielsen, F.H. , Hunt, J. R. , "Trace elements emerging as important in human nutrition", In; P.J. Stumbo, (Ed), *Proceedings of the Fourteenth National Datapank Conference*, Iowa City; University of Iowa, 1989, pp.135-143.
- Oliveira, R.A.G. , Torres, M.F.F.M. , Diniz and Araujo, E.C. , "Estudo sobre o uso de plantas medicinais em crianças hospitalizadas da cidade de Joao Pessoa: riscose beneficios " , *Brazil.J. Pharmacogn*, 2005, 15 : 373-80.
- Kaliappal, Nirmala Devi and Periyanyagam Kasi Viswanathan, (2008). Pharmacognostical studies on the leaves of *Plectranthus amboinicus* (Lour) Spreng. *International Journal of Green Pharmacy*, 2(3):182-184.
- Kathiresan R.M. Allelopathic potential of native plants against water hyacinth. *Crop Protection*. 2000; 19(8-10):705-708.
- Khanum, H., Ramalakshmi, K., Srinivas, P., Borse, B.B. (2011). Synergistic antioxidant action of Oregano, Ajowan and Borage extracts. *Food Nutr. Sci.*, 2:387-392.
- Kirtikar, K.R., Basu B.D. *Indian medicinal plants*, International Book Distributors, Dehradun, vol-III, (2005) 1971.
- Phay, N. , "*Highly selective Antibiotics*, Faculty of Agriculture, Doctoral Thesis", Hokkaido University, Japan, 1997.
- Shenoy, P and k. Smita. (2012). Hepatoprotective activity of *Plectranthus amboinicus* (Lour.) against paracetamol hepatotoxicity in rats. *International Journal of Pharmacology and Clinical Sciences*, 1(2): 32-38.
- Young, D. B. , "*Role of potassium in preventive cardiovascular medicine*", Kluwar Academic Publisher, Boston, MA, USA, vol.8, 2001.