PHYTOCHEMICAL INVESTIGATION, NUTRITIONAL VALUES, TRACE METAL CONTENTS AND ANTIMICROBIAL ACTIVITY OF *VINCETOXICUM FLEXUOSUM* VAR. TENUIS (BLUME) SCHNEIDT, MEVE & LIEDE

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Abstract

The medicinal plant *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede (Ma-Line-Twat) belongs to the family Apocynaceae. The specimens were collected from Yae Chan Pyin Village, Sittwe Area, during the flowering and fruiting period, from May to July, 2023. In this research, morphological characters, preliminary phytochemical tests, nutritional values, trace metal contents analysis and antimicrobial activity tests were carried out. In the morphological study, the plant was climber and milky sap present. The preliminary phytochemical tests revealed that the presence of alkaloid, glycoside, carbohydrate, reducing sugar, saponin and tannin were found; the α-amino acid, starch, phenolic compound and flavonoid were absent. In a nutritional study, it was significantly found that 38.13 % of fibres in leaves. According to the Energy Dispersive X-Rays Fluorescence Spectrophotometer (EDXRF) analysis results, chlorine was found more than other elements in leaves. The antimicrobial activity was carried out by agar well diffusion method on six types of test microorganisms. Ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on all other test microorganisms. The watery and pet-ether extracts showed the negative results on all types of test microorganisms.

Keywords: V-Flexuosum, Apocynaceae, antimicrobial activities, test microorganisms

Introduction

Plant is an important source of medicine and plays a key role in world health. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintain good health and conditions. Two-third of the world's population depends on herbal medicine for primary health care in developing countries. The reasons for this is because of their better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects (Sandberg and Corrigan, 2001).

The Apocynaceae is one of the largest and important families in angiosperm. Characteristic features of the family are almost all species produce milky sap (Wiart, 2006). Leaves are simple, opposite or whorled; flowers are large, colourful and slightly fragrant with five contorted lobes; and fruits are in pairs. Members of family Apocynaceae are rich in alkaloids, terpenoids, steroids, flavonoids, glycosides, simple phenols, lactones and hydrocarbons. Several members of this family have medicinal properties and have been in the treatment of various ailments. Most of them are consumed as food by tribal people whereas a few plants are used as source of poison.

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The native range of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede is Tropical & Subtropical Asia to Caroline Islands, China, Bangladesh, India and Myanmar etc. (Website.1).

The plants are alexipharmic; used for the treatment of urticaria, smallpox, excessive perspiration, bilious swellings, as an antidote to arsenic poisoning. Leaves are used for scabies. The leaves and roots of this plant have been used to treat jaundice and symptomatic range of bioactivities, including immune modulatory effects, free-radical scavenging (anti-oxidant), hepato-protective, anti-convulsant, anti-anxiety, anti-bacterial, anti-asthmatic, anti-inflammatory, anti-cancer, anti-amoebic, anti-psoriasis, seborrhic, and anaphylactic effects, and they are used for the treatment of leucopenia and the Schultz-Dale reaction (Mostafa, *et al*., 2023).

The aim and objectives of present research were to know the morphological characters of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede, chemical constituents, nutritional values, trace metal contents by EDXRF and antimicrobial activities of this medicinal plant.

Materials and Methods

Collection and preparation of samples

The specimens of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede were collected from Yae-Chan-Pyin Village, Sittwe Township, Rakhine State. They were collected especially during the flowering and fruiting period from May to July in 2023. After the plant collection, both vegetative and reproductive parts of the plants were identified by using the literatures of Lawrences, 1964; Backer and Brisk 1965; Hundley and Chit Ko Ko, 1987; Dassanayake, 2000 and Kress *et al*., 2003.

Preparation of Powdered Leaves Samples

The leaves of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede were collected from Rakhine State. The plant samples were washed with water thoroughly and dried at room temperature for 2-3 weeks. The dried samples were pulverized by grinding with a blender to get fine powdered and stored in air tight container to prevent it from moisture and air-borne contamination.

Preliminary Phytochemical Investigation from Leaves of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

For Preliminary Phytochemical test, the air-dried powdered of the leaves were tested for alkaloids, α-amino acid, glycoside, carbohydrates, reducing sugar, starch, saponin, tannin, phenolic compound and flavonoid were carried out.

Determination of nutritional values of leaves

The nutritional values of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve &Liede leaves were analyzed at the SmallScale Industries Department, Ministry of Cooperatives and Rural Development, Nay Pyi Taw. The nutritional values were determined by the method of Pearson, 1976.

Determination of elemental composition of leaves

The concentrations of elements in *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede powdered leaves were analyzed using an Energy Dispersive X-ray Florescence (EDXRF) spectrophotometer at the Universities' Research Center in University of Yangon.

Screening the Antimicrobial Activities of Different Solvent Extracts from Leaves of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

The extraction of crude drugs

For the extraction of crude drugs, the leaves powder were soaked in different solvents, such as ethyl acetate, methanol, ethanol, acetone, water and pet-ether for about three days and thoroughly shaked. Then, these solutions were separately filtered and evaporated on the water bath.

Cultivation of Test Organisms

Bacillus pumilus (IFO 905771), *Bacillus subtilis* (JAP- 0225025), *Candida albicans* (IFO- 1060), *Micrococcus luteus* (NITE 83297), *Pseudomonas* sp. (IFO 94307), and *Staphylococcus aureus* (ATCC 12877) were used for the antimicrobial activities. They were inoculated into the nutrient broth and transferred into nutrient agar media.

Preparation of Plates for Antimicrobial Activities Test

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to method described by Cruickshank, 1975. The ingredients were dissolved in 100 mL of distilled water by heating in flat bottomed flask and poured into separate conical flasks. The flasks were sterilized by autoclaving at 121℃ for 15 min. After autoclaving, the media were cool down to 45℃ and then test organisms were added to the nutrient agar medium.

This medium poured into petridishes and solidified. After solidification, the holes were made on medium by means of a cork borer having a diameter of 6 mm. The loose agar blocks were removed from the wholes by using the sterilized wire needle.

After that about 0.01 mL of sample was introduced into agar well and incubated at 37℃ for 24 hours. The inhibition zones appeared around the agar well indicating the presence of antimicrobial activities. The extracts of antimicrobial activities were measured from the zones of inhibition diameter.

Results

Morphological Characters of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

Scientific name	-	*Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede
Myanmar name	-	Unknown
English name	-	Snakeweed
Rakhine Name	-	Ma-line-twat
Family	-	Apocynaceae
Flowering and fruiting period	-	May to July
Parts used	-	Leaves

The plants are climbing herbs with milky latex. Leaves simple, opposite or whorled, exstipulate, petiolate. Inflorescences umbels in branched racemes, 3-7 flowered. Flowers brownish yellow, about 0.3 cm in diameter, complete, bisexual, actinomorphic, cyclic, hypogynous. Sepals 5 deeply lobed, divided almost to the base. Petals 5, united, salverform. Stamens 5, alternating with corolla lobes, epipetalous; filaments very short; anthers dithecous, sagittate, introrse, free or adherent to the stigmatic surface by a viscid fluid, connective often prolonged apically. Ovary superior, carpels 2, apocarpous; style usually 1(with a thickened head); stigma umbel-shaped. The results were shown in Figure (1-6).

Specimen examined: Yae-Chan-Pyin Village, Sittwe Township, Rakhine State

Figure.1 Habit

Figure.2 Ventral surface of leaves

Figure.3 Dorsal surface of leaves

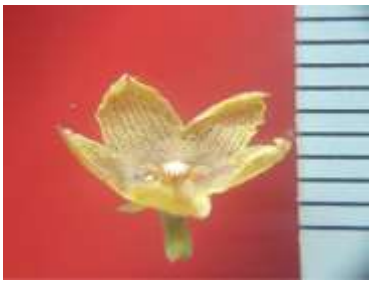


Figure. 4 Flower

Figure. 5 Calyx

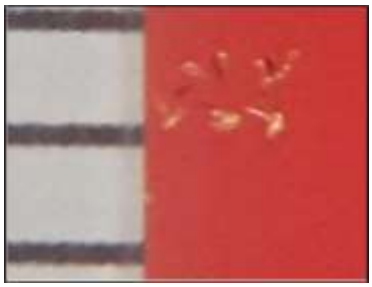


Figure. 6 Androecium

Preliminary Phytochemical Test of Leaves from *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

The results of preliminary phytochemical test of air-dried powdered of leaves indicated that alkaloid, glycoside, carbohydrate, reducing sugar, saponin and tannin were found to be present. The α-amino acid, starch, flavonoids and phenolic compound were not present. The results were shown in Figure (7) and Table (1).

Figure 7. Phytochemical test of leaves from *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

Table 1 Preliminary Phytochemical Test of Leaves from *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

No.	Test	Extract	Test Reagent	Observation	Results
1.	Alkaloid	10% HCl	Wagner's reagent	White ppt	+
2.	α-amino acid	H_2O	Ninhydrin reagent	White	-
3.	Glycoside	H_2O	10% lead acetate solution	Pale yellow ppt	+
4.	Carbohydrates	H_2O	10% α-napthol + H_2SO_4 (Con:)	Red ring	+
5.	Reducing sugar	H_2O	Benedict's solution	Brick red ppt	+
6.	Starch	H_2O	Iodine solution	Brown ppt	-
7.	Saponin	H_2O	Distilled water	Foaming	+
8.	Tannin	H_2O	1% $FeCL_3$ solution	Bluish black	+
9.	Phenolic compound	H_2O	5% $FeCL_3$ solution	Brown	-
10.	Flavonoid	MeOH	H_2SO_4 (Con:) + Magnesium	Dark green ppt	-

Nutritional Values and Trace Metal Contents of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

The nutritional values, such as protein, fat, fiber, ash, and carbohydrate of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede leaves were observed. The results were shown in Figure (8) and Table (2).

REPUBLIC OF THE UNION OF MYANMAR
Ministry of Cooperatives and Rural Development
Small-Scale Industries Department
Hay Pyi Taw

LABORATORY REPORT

1. Reference: Letter of Dr.Kay Thi Wynt, Dated: 21. November.2023
2. Sample: *Tylophora tenuissima*
3. Sender:
4. Objective: To analyze the quality of sample by Chemical Test.
5. Date of receive: 23.11.2023

RESULTS

SR	Experiment	Present Chemical Analysis Result
1.	Moisture (%)	13.23
2.	Ash (%)	18.77
3.	Protein (%)	18.47
4.	Fat (%)	5.531
5.	Fiber (%)	38.13
6.	Carbohydrate (%)	5.869

Remark: Result valid only for sample tested.
Method employed: standard method for the chemical Analysis of food by David Pearson.

Tested by: Thazin Zar Lin (Chemist)
Checked by: Htay Htay Wai (Assistant Director)
(For the Director General): Khine Khine Zan (Director)

Our Reference - SI Man (Thu) 5/2023

Figure 8 Nutritional values of the leaves of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

Table 2 Nutritional Values of the Leaves of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

Sr.	Nutritional Parameter	Content (%)
1	Moisture	13.23
2	Proteins	18.47
3	Fat	5.531
4	Fiber	38.13
5	Ash	18.77
6	Carbohydrate	5.869

Elements Composition of Leaves by EDXRF Spectrometry

The content of elements in the leaves of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede was analyzed using Energy Dispersive X-ray Florescence (EDXRF) spectrophotometer analysis. It was found that chlorine was significantly present than other elements in leaves. The spectrum and spectral data were shown in Figure (9) and Table (3).

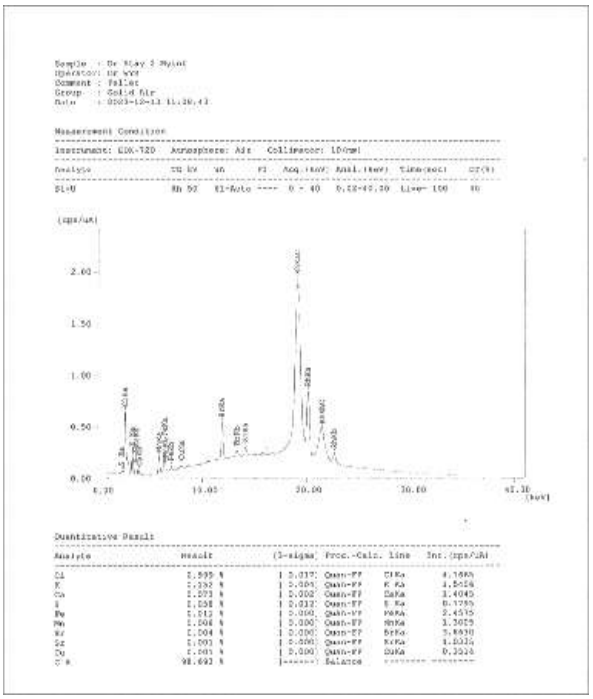


Figure.9 EDXRF spectrum of leaves

Table 3 Relative Abundance of Elements in Sample by using EDXRF

No	Element	Relative Abundance %
1	Cl	0.999
2	K	0.152
3	Ca	0.073
4	S	0.058
5	Fe	0.011
6	Mn	0.008
7	Br	0.004
8	Sr	0.001
9	Cu	0.001
10	CH	98.693

Antimicrobial Activities of Different Solvent Extracts of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

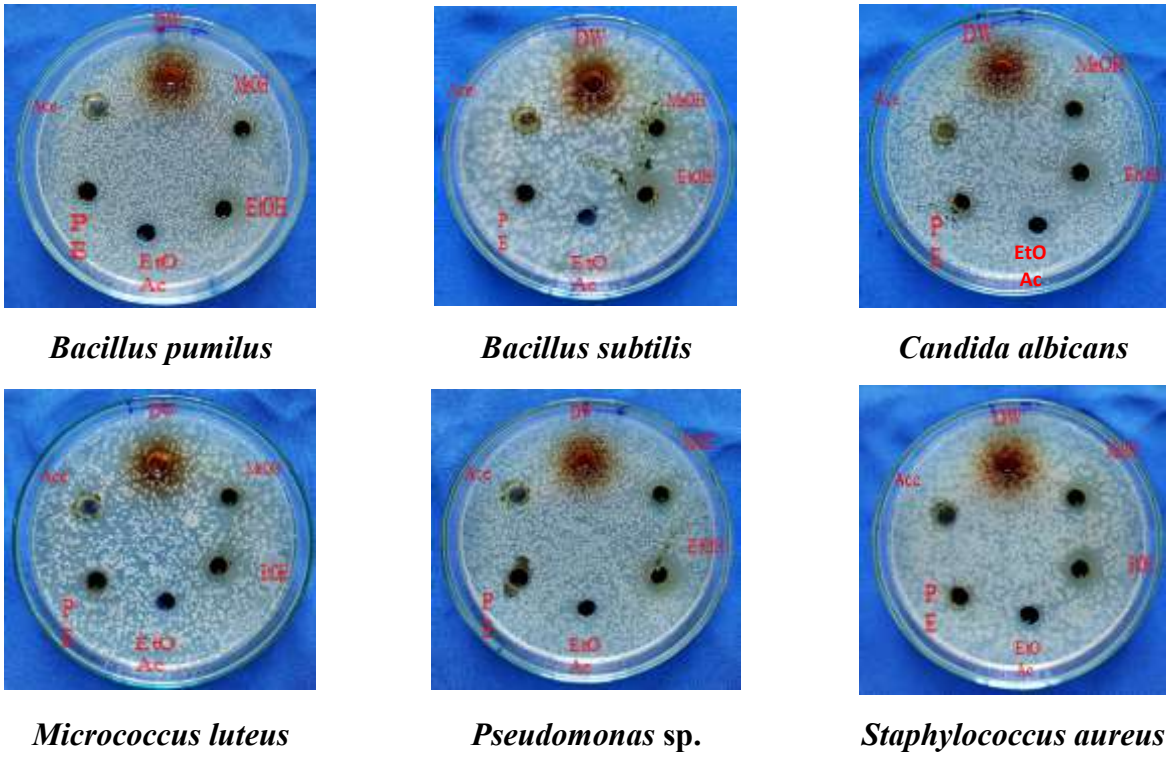
In this study, Ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on *Bacillus pumilus* and least against on *Micrococcus luteus.* The watery and pet-ether extract showed the negative results on all types of test microorganisms. The results were shown in Figure (10) and Table (4).

Table 4. Antimicrobial Activities of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

No.	Microorganisms	Size Diameter of Inhibition Zone (mm)					
		H_2O	MeOH	EtOH	EtOAc	Ace	P. E
1	*Bacillus pumilus*	-	11.7	13.6	-	11.4	-
2	*Bacillus subtilis*	-	10.0	19.0	-	-	-
3	*Candida albicans*	-	13.7	15.0	9.5	9.0	-
4	*Micrococcus luteus*	-	9.0	11.0	-	-	-
5	*Pseudomonas* sp.	-	9.0	12.8	-	-	-
6	*Staphylococcus aureus*	-	10.9	12.5	-	8.7	-

Key to table, 10 -12 mm = (+) weak activity, 13 -17 = (++) medium activity,

18 - 25 = (+++) high activity, 6 mm = Agar well



Bacillus pumilus ***Bacillus subtilis*** ***Candida albicans***

Micrococcus luteus ***Pseudomonas* sp.** ***Staphylococcus aureus***

Figure.10 Antimicrobial activities of leaves of *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede

Discussion and Conclusion

Medicinal plants are globally valuable sources of new drugs. In developing countries, 80 % of people are totally dependent on herbal drugs for their primary healthcare and over 25% of prescribed medicines in developed countries are derived from wild plant species (Hamilton, 2000). The secondary plant metabolites like alkaloids, flavonoids, terpenoids, glycosides, tannins etc. are biosynthesized by plants. These are active constituents in the plant and have marked pharmacological activity. These are very potent in process. Secondary metabolites are small biomolecules regarded as non-essential for the life of the producer organism (Agostini *et al*., 2012).

The *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede plants are climbing herbs with milky latex, simple leaves, 3-7 flowered umbels racemes, brownish yellow flowers, 5-stamens and superior ovary. These characters were agreed with the Backer and Brink 1965.

People take *Vincetoxicum flexuosum* var. tenuis (Blume) Schneidt, Meve & Liede by mouth for allergies, asthma, cancer, congestion, constipation, cough, inflamed skin, diarrhea, bloody diarrhea, hemorrhoids, tender joints, jaundice, rheumatoid arthritis, whooping cough, vomit, to cause sweating (Website.2).

Jayakumar and Karuppusamy, (2015) stated that *Tylophora* has alkaloids, flavonoids, phenols and glycosides. In this study, the preliminary phytochemical tests revealed the presence of alkaloids, glycosides, carbohydrates, reducing sugar, saponin and tannin. As exceptional α-amino acid, starch, phenolic compound and flavonoid were absent.

Ranemma *et al*., (2017) stated that the antibacterial screening of the *T. indica* leaves extract were performed against gram positive (*S.aureus*) and gram-negative bacteria (*E. coli, P. aeruginosa* and *K. pneumoniae*) by the disk diffusion method. In this study, ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on *Bacillus pumilus* and least against on *Micrococcus luteus*. The watery and pet-ether extract showed the negative results on all types of test microorganisms.

In the nutritional property analysis, moisture 13.23 %, proteins 18.47 %, fat 5.531%, fiber 38.13%, ash 18.77% and carbohydrate 5.869 % were obtained from the powdered leaves.

Moisture content refers to the number of water molecules that become incorporated into a food product. Moisture can enter into a product in a number of ways, it could be related to the production method of the product, the atmospheric moisture in the food production area, the packaging method of the product, or it can be related to the method of food storage. (Website.3)

Fibers appear to the lower risk of developing various conditions, including heart disease, diabetes, diverticular disease, and constipation. Proteins are need to build up muscle, blood, skin, bones, tissues and organs of the human body. A high protein intake can help with weight loss, increase muscle mass besides improve both mental and physical health. Fats are nutrients in food that the body uses to build cell membranes, nerve tissue and hormones. The body also uses fat as fuel. (Website.4)

The ash content in food refers to the minerals and inorganics left after the food sample has been heated to a very high temperature removing moisture, volatiles, and organics. Fats are

nutrients in food that the body uses to build cell membranes, nerve tissue and hormones. The body also uses fat as fuel. (Website.5)

According to the Energy Dispersive X-Rays Fluorescence Spectrophotometer analysis (EDXRF) results, chlorine was significantly present than other elements in leaves.

Chlorine is usually used to kill bacteria and other microbes in drinking water supplies and public swimming pools. Chlorine is a component of all body secretions and excretions resulting from processes of building (anabolism) and breaking down (catabolism) body tissues. Chlorine is stored to a limited extent in the skin, subcutaneous tissues, and skeleton and constitutes two-thirds of the negatively charged ions (anions) in the blood. Chlorine is readily absorbed during digestion, and similarly its rate of excretion through sweat, kidney excretion, and intestinal expulsion is high (Website.6). Potassium takes part in the ionic balance of the human body and maintains tissue excitability. Potassium is important for its diuretic nature (WHO, 2006).

In this study, ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on *Bacillus pumilus and least against on Micrococcus luteus.* The watery and pet-ether extract showed the negative results on all types of test microorganisms. Ranemma *et al.,* (2017) stated that the antibacterial screening of the *T. indica* leaf extracts were performed against gram positive *(S. aureus*) and gram-negative bacteria (*E.coli, P. aeruginosa and K. pneumoniae*) by the disk diffusion method.

The present research showed the significant properties of secondary metabolites, nutritional values, elemental analysis and antimicrobial activities. It was beneficial to the human health-care system and will promote natural traditional products research.

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