COMPARATIVE STUDY ON HISTOLOGICAL CHARACTERS, PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *ANDROGRAPHIS PANICULATA* (BURM.F.) WALL. EX NEES AND *ANDROGRAPHIS ECHIOIDES* (L.) NEES

Moe Moe[1], Ei Ei Phyu[2], Than Than Soe[3], Thet Thet Zin[4]

Abstract

Andrographis paniculata (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees belong to the family Acanthaceae. These species were collected from Patheingyi Township, Mandalay Region, during August 2022 to March 2023. They are known as traditional medicinal plants not only in Myanmar but also in Asia. The present studies were undertaken to establish a comparative study of morphological, histological characters, phytochemicals analysis, antioxidant and antimicrobial activities of two species. The morphological studies of two species have annual erect herbs. The leaves of *A. paniculata* (Burm. f.) Wall. ex Nees was lanceolate, glabrous on both surfaces. *A. echioides* (L.) Nees was oblong, pubescent on both surfaces. In histological characters, diacytic types of stomata found only on abaxial surface and stomata index were 13.3-21.43 in *A. paniculata* (Burm. f.) Wall. ex Nees. *A. echioides* (L.) Nees observed on both surfaces and stomata index were 14.28-25.0, 20.0-33.33. The phytochemical analysis of aqueous leaves extract showed the presence of high number phytocompounds when compared with ethanol and methanol. It showed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, proteins and reducing sugar in both species. The free radicals scavenging of leaves of two species have excellent antioxidant activity showing IC_{50} value 5.98 and 3.44 mg/l**.** Antimicrobial activities of the different solvent extracts of leaves were tested on five pathogenic microorganisms. The antibacterial activity of the chloroform extracts of *A. paniculata* (Burm.f.) Wall. ex Nees were tested against *Micrococcus luteus*. In *A. echioides* (L.) Nees, ethyl-acetate extract was found active against the strains of *Pseudomonas albicans*. The results of present studies can serve as a useful tool in the identification, authentication, and efficacy of the plants, which leads to pharmaceutical application.

Keywords: Morphology, histology, phytochemical, antioxidant, pathogenic microorganisms

Introduction

Acanthaceae represent about 229 genera and 3450 species, distributed in the tropical regions, Central America, Brazil and Africa. Plants are herbs, shrubs, small trees with cystoliths. Leaves are simple, opposite, and exstipulate. Flowers bisexual, hypogynous with bracts and bracteoles. Fruit elastically dehiscent bivalve capsule. Seeds are often on hook like outgrowths the so called retinacula or jaculators (Ankush and Sicjamdra. 2023).

The genus *Andrographis* comprising of about 40 species, is essentially distributed in southern India and in tropical Asia. *Andrographis paniculat*a (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees plants have mostly in dry places, such as India, Sri Lanka and South Asian countries. In Myanmar, *A. paniculata* (Burm.f.) all. ex Nees is commonly known as Se ga gyi or Shan Se ga gyi. It has various pharmacological effects and used as medicinal herb

[1] Department of Botany, University of Mandalay
[2] Department of Botany, University of Mandalay
[3] Department of Botany, University of Mandalay
[4] Department of Botany, University of Mandalay

for treating upper gastrointestinal tract, upper respiratory infections, herpes, other chronic diseases and its constituents relating to Covid-19 (Nyeem *et al*. 2017, Hossain *et al*. 2021).

A. echioides (L.) Nees is commonly known as Se ga gyi hmwe htu. It is highly medicinal important and used in liver diseases, bacterial malaria, fungal disease and antioxidant, and anti-inflammatory (Pande *et al.* 2018). The leaf juice of this plant is used to treating fever (Elaiyaraja *et al*. 2015). Both species are widely distributed in Mandalay Region. Medicinal plants have been a long history of usage in traditional medicine. The authentic knowledge of the usage of medicinal plants passed from one generation to another, after refining and addition (Haq *et al*. 2011).

Medicinal plants are an important source of valuable therapeutic agents, both in modern and in traditional medicine. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid and phenolic compounds. Plants act as antimicrobial compounds due to the presence of secondary metabolites. The medicinal properties and capabilities of the plant, the chemical analysis of phytochemical content and antioxidant activity assay. Antioxidants help in preventing degenerative diseases such as cancer, heart disease and even altitude sickness (Keita *et al*. 2022). Both species are widely used as traditional medicine. In Mandalay Region, almost every household cultivated *A. paniculat*a (Burm.f.) Wall. ex Nees in the style of homegardern.

In this study, general aim and objectives to promote green economy and green growth through economic scale cultivation of these species. Specific aim and objectives to use as tool in the identification, authentication and standardization of the plant material in pharmaceutical application because of having potential for manufacturing pallet for traditional medicine.

Materials and Methods

Plant Sample collection

Andrographis paniculata (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees were collected from Patheingyi Township, Mandalay Region, during August 2022 to March 2023. The collected specimens were studied at the Department of Botany, University of Mandalay. The morphological studies of the plants were identified with the help of literatures; Backer *et al* (1965), Hooker (1885), Dassanayake (1998) and Kress *et al*. (2003).

Histological Studies

According to Johnsen's method (1940), killing and fixation, dehydration, infiltration, embedding, staining and mounting were made. Free hand section was made by using chloral hydrate solution and a new razor blade and stomatal index was calculated according to Kokate (2000), Metcalfe & Chalk (1957). Histological studied were carried out Department of Botany, University of Mandalay.

Phytochemical Screening Analysis

Phytochemical screening analysis of aqueous, ethanol and methanol leaves extract of two species were carried out using standard procedures to identify the presence of secondary metabolites as described by Harbone (1998), Sofowora (1993). Phytochemical analysis were

examined at Department of Botany, University of Mandalay, University Research Center (URC), University of Mandalay.

Determination of Antioxidant Activity Analysis by DPPH Method

The antioxidant activity of leaves extracts of two species were assessed by the ability of the extract to scavenge DPPH free radicals. Ascorbic acid was used as the standard compound. The antioxidant activity of samples were shown as % inhibition of DPPH absorbance reported by Sharma & Bhat (2009) at the U Kar Ka Food Testing Laboratory, Mandalay, Myanmar.

Antimicrobial Activity Analysis

The antimicrobial activity analysis of two species leaves were performed by agar well diffusion method reported by Balouiri *et al*. (2016) at the Microbiology Laboratory in Department of Botany, University of Mandalay. Antimicrobial activities of the different solvents extract of leaves were tested on five pathogenic microorganisms.

Results

Morphological Studies

Table 1. Comparative morphological characters of *Andrographis paniculat*a (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees

Scientific Name	***A. paniculat*a (Burm.f.) Wall. ex Nees**	***A. echioides* (L.) Nees**
Myanmar Name	Se ga gyi	Se ga gyi hmwe tu
English Name	King of bitter	False water willow
Habit	Annual herb	Annual herb
Stem shaped	Quadrangular stem, glabrous	Quadrangular stem with hairy
Leaves	Simple, opposite and decussate, glabrous on both surfaces	Simple, opposite and decussate, pubescent on both surfaces
Leaf base	Cuneate	Cuneate
Leaf apex	Acute	Obtuse
Inflorescence	Terminal or axially paniculate racemes	Terminal or axially paniculate racemes, divericate
Flowers	White with purple spots, glabrous, bisexual, zygomorphic, hypogynous	White or pink with dark purple spots, pubescent, bisexual, zygomorphic, hypogynous
Androecium	Stamens 2, epipetalous, exserted, white, anther dithecous, basifixed	Stamens 2, epipetalous, exserted, white, anther dithecous, basifixed

Scientific Name	***A. paniculata* (Burm.f.) Wall. ex Nees**	***A. echioides* (L.) Nees**
Gynoecium	Carpel 2, fused, Ovary superior, ovoid. many ovule in each locule on the axile placenta, stigma bifid	Carpel 2, fused, Ovary superior, ovoid. many ovule in each locule on the axile placenta, stigma bifid
Fruit type	Loculicidal capsule, linear-oblong acute at both ends, glabrous	Loculicidal capsule, linear-oblong acute at both ends, pubescent
Seed	12 seeded, ovate, nonendospermic	4 seeded, oblongoid, nonendospermic

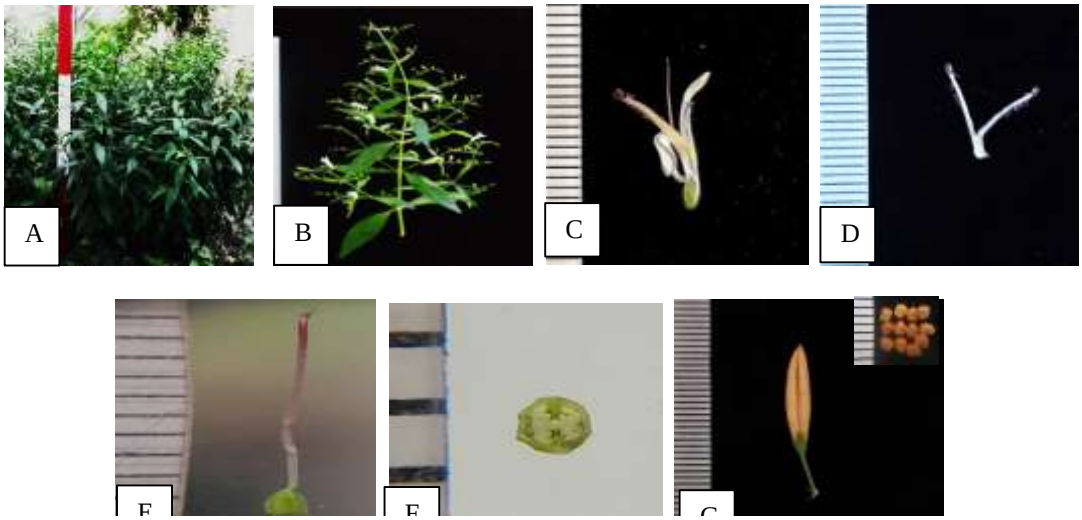

Figure 1. Morphological characters of *Andrographis paniculata* (Burm.f.) Wall. ex Nees
A. Habit B. Inflorescence C. L.S of flower D. Androecium
E. Gynoecium F. T.S of ovary G. Fruit and seeds

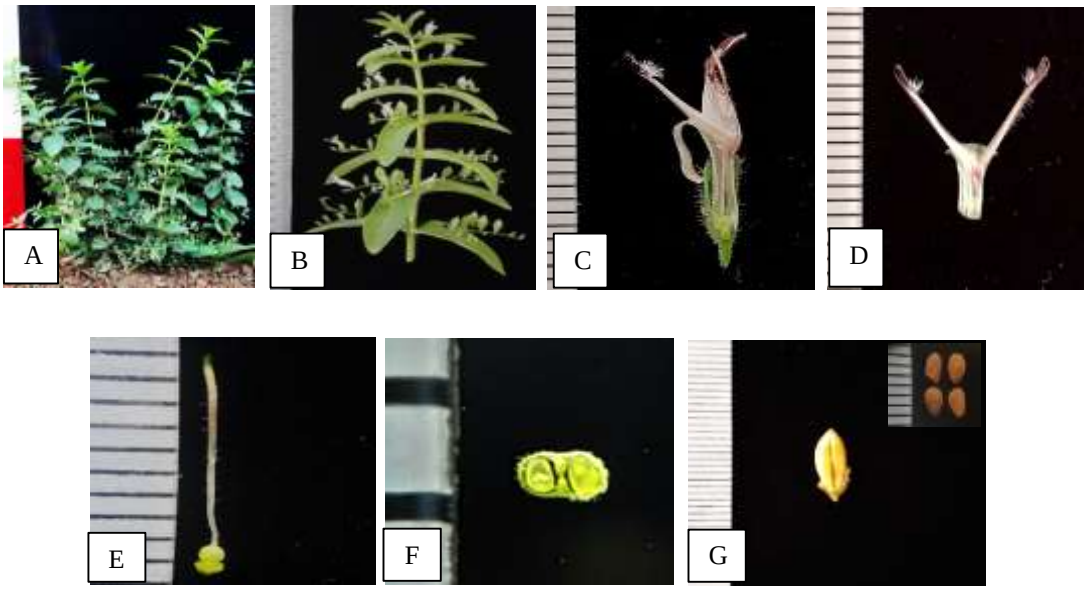

Figure 2. Morphological characters of *Andrographis echioides* (L.) Nees
A. Habit B. Inflorescence C. L.S of flower D. Androecium
E. Gynoecium F. T.S of ovary G. Fruit and seeds

Histological Studies

Table 2. Comparative histological characters of *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees

Plant part	*A. paniculata* (Burm.f.) Wall. ex Nees	*A. echioides* (L.) Nees
Shape of petiole	Rectangular shape	Crescent shape
Shape of midrib	Triangular	Rectangular
Stomatal type & Stomatal Index	Diacytic stomata only in lower surface and 13.3 - 21.43	Diacytic stomata on both surfaces and 14.28 - 25.0, 20.0 - 33.33
Vascular bundle	Crescent shape and collateral	Crescent shape and collateral
Cystoliths	Round	Elongated
Trichomes	Uniseriate unicellular, sessile glandular trichomes	Uniseriate multicellular, non-glandular trichomes
Stem shape	Quadrangular with distinct wing	Quadrangular with slightly distinct wing

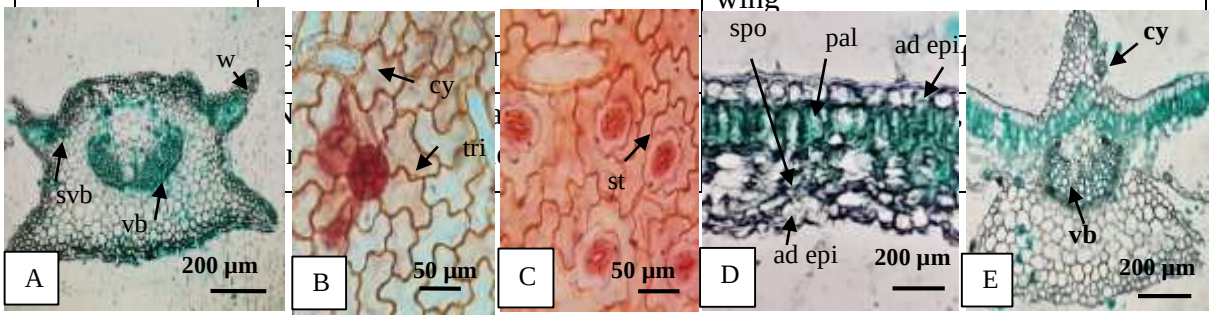

Figure 3. Internal structure of leaf of *Andrographis paniculata* (Burm.f.)Wall. ex Nees
A. T.S of petiole B. Adaxial surface view of lamina C. Abaxial surface view of lamina showing diacytic types of stomata D. T.S of lamina E. T.S of midrib

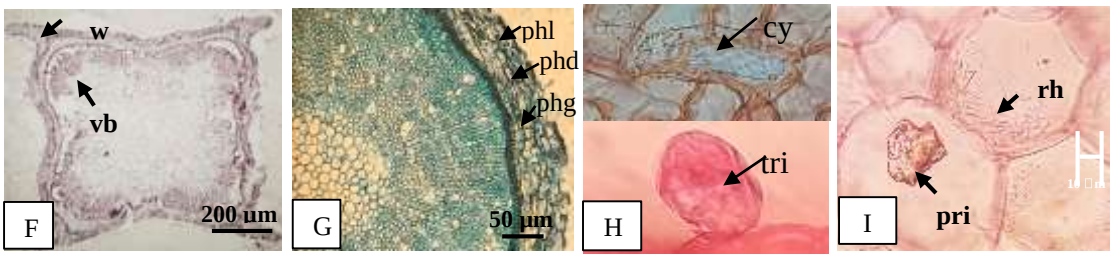

Figure 4. Internal structure of stem & root of *Andrographis paniculata* (Burm.f.) Wall. ex Nees
F. T.S of stem G. T.S of root H. Trichome & cystolith I. Prismatic & needle shaped

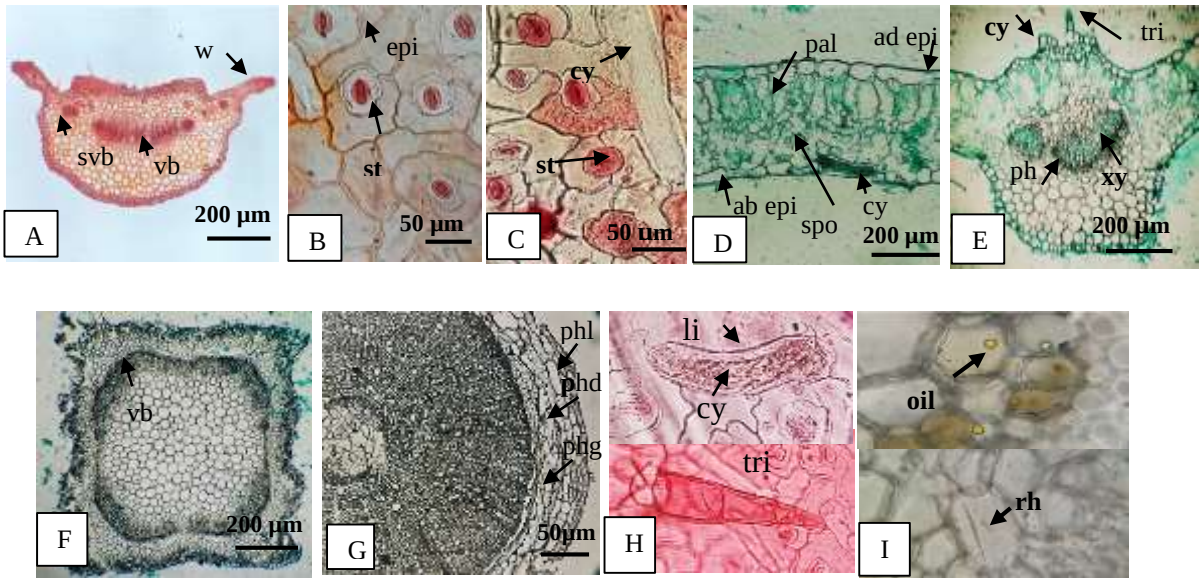

Figure 5. Internal structure of leaf, stem & root of *Andrographis echioides* (L.) Nees
A. T.S of petiole B. Adaxial surface view of lamina C. Abaxial surface view of lamina showing diacytic types of stomata D. T.S of lamina E. T.S of midrib showing a vascular bundle F. T.S of stem G. T.S of root H. Trichome & cystolith I. Needle shaped & oil globule
ad epi= adaxial epidermal cell, ab epi= abaxial epidermal cell, svb= small vascular bundle, pal= palisade parenchyma cell, spo= spongy parenchyma cell, tri= trichome, vb= vascular bundle, ph= phloem xy= xylem, epi= epidermis, w= wing, st= stomata, li= lithocyst, pri=prismatic crystal, rh= single raphide

Preliminary phytochemical Analysis

Table 3. Phytochemical analysis of powder leaves extract were examined by using various chemical reagents for the presence or absence of chemical constituents

No.	Phytochemical	*A. paniculata* (Burm.f.) Wall. ex Nees				*A. echioides* (L.) Nees			
		H_2O	EtOH	MeOH	Observation	H_2O	EtOH	MeOH	Observation
1.	Alkaloids (Mayer's reagent)	+	+	+	Cream ppt.	+	+	+	Cream ppt
	Alkaloids (Wagner's reagent)	+	+	+	Reddish brown ppt.	+	+	+	Reddish brown ppt
	Alkaloids (Hager's reagent's)	+	+	+	Yellow ppt.	+	+	+	Yellow ppt.
2.	Steroids	+	+	-	Upper reddish	+	-	-	Upper reddish
3.	Terpenoids	-	+	-	Lower greenish yellow	-	-	-	No Lower greenish yellow

No.	Phytochemical	*A. paniculata* (Burm.f.)Wall. ex Nees				*A. echioides* (L.) Nees			
		H_2O	EtOH	MeOH	Observation	H_2O	EtOH	MeOH	Observation
4.	Glycosides	+	-	+	Yellow	+	-	+	Yellow
5.	Phenolic compounds	+	+	+	Dark green	+	+	+	Dark green
6.	Flavonoids	+	+	+	Pale green	+	+	+	Yellow
7.	Tannins	+	+	+	Black	+	+	+	Black
8.	Saponins	+	+	-	Frothing	+	-	+	Frothing
9.	α - amino acids	+	+	+	Purple spot	+	+	+	Purple spot
10.	Proteins	+	+	+	Yellow ppt	+	+	+	Yellow ppt
11.	Reducing sugars	+	+	+	Green	+	-	+	Green
12.	Starch	+	+	+	Black	-	+	+	Black
13.	Carbohydrates	+	-	-	Purple ring	+	-	-	Purple ring
14.	Cyanogenic glycosides	-	-	-	No brickish color	-	-	-	No brickish color

(+) = present, (-) = absent, ppt= precipitate, H_2O= Aqueous, EtoH= Ethanol, MeOH= Methanol

Antioxidant capacity determination by 2, 2-diphenyl-1-pricyl hydraylzyl (DPPH) assay

Table 4. Analysis of DPPH radical scavenging of leaves extract of *Andrographis. paniculata* (Burm.f.) Wall. ex Nees and *Andrographis. echioides* (L.) Nees

No.	Extract Name	Sample concentration	Absorbance	% inhibition	IC_{50} Value mg/l
1.	Ascorbic acid	50	0.264	89.41459503	6.75
		25	0.724	70.97032879	
		12.5	1.123	54.97193264	
		6.25	1.390	44.26623897	
		3.125	1.511	39.41459503	
1	Hot water extract of *A. paniculata* (Burm.f.) Wall. ex	100	1.169	53.12751	5.98
		50	1.210	51.48356	
		25	1.217	51.20289	
		12.5	1.231	50.64154	

No.	Extract Name	Sample concentration	Absorbance	% inhibition	IC $_{50}$Value mg/l
	Nees	6.25	1.240	50.28067	
2	Hot water extract of *A. echioides* (L.) Nees	100	1.203	51.95687	3.44
		50	1.217	51.39776	
		25	1.227	50.99840	
		12.5	1.230	50.87879	
		6.25	1.233	50.75879	

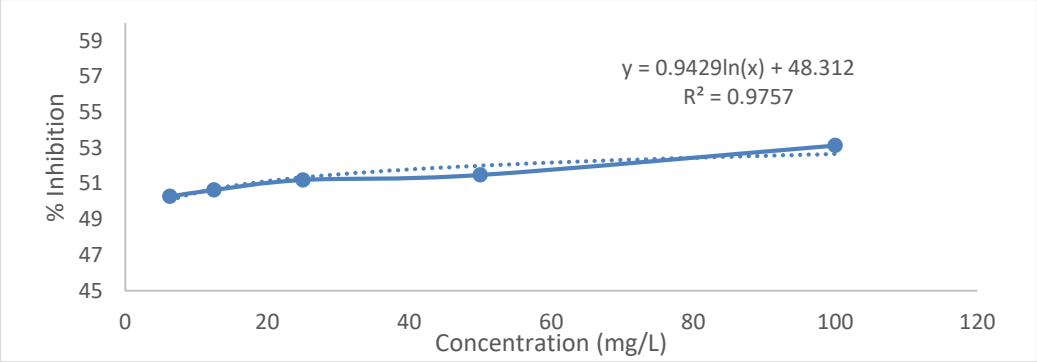

Figure 6. % inhibition & Different concentration of leaves of *A. paniculata* (Burm.f.) Wall. ex Nees

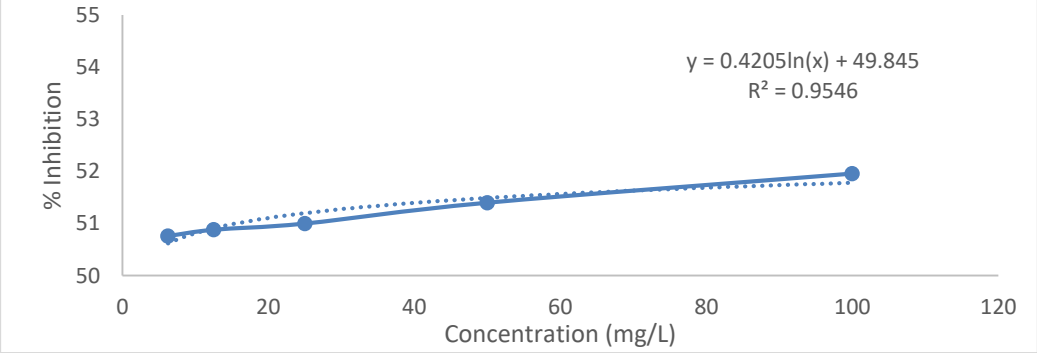

Figure 7. % inhibition & Different concentration of leaves of *A. echioides* (L.) Nees

Antimicrobial Activity Analysis

Table 5. Antimicrobial activity of various solvents from powdered leaves of *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) against five pathogenic microorganisms

Extract	Inhibition zone diameter(mm)									
	***A. paniculata* (Burm.f.) Wall. ex Nees**					***A. echioides* (L.) Nees**				
	Escherichia coli	*Micrococcus luteus*	*Staphylococus aureus*	*Pseudomonas albicans*	*Candida albicans*	*Escherichia coli*	*Micrococcus luteus*	*Staphylococus aureus*	*Pseudomonas albicans*	*Candida albicans*
Chloroform	17.8	21.9	20.0	18.7	18.3	-	10.8	10.8	12.8	13.0
Pet-ether	10.4	9.8	-	10.4	-	-	-	-	-	-
Ethyl-acetate	18.7	17.3	18.3	19.2	19.2	17.9	18.7	17.8	20.1	15.6
Ethanol	18.7	18.7	16.9	19.7	18.9	12.3	14.2	15.2	-	13.5
Methanol	18.2	16.9	15.9	16.9	17.0	9.3	11.7	11.9	11.2	12.1
Water	-	-	-	-	-	-	-	-	-	-

Agar well = 6mm

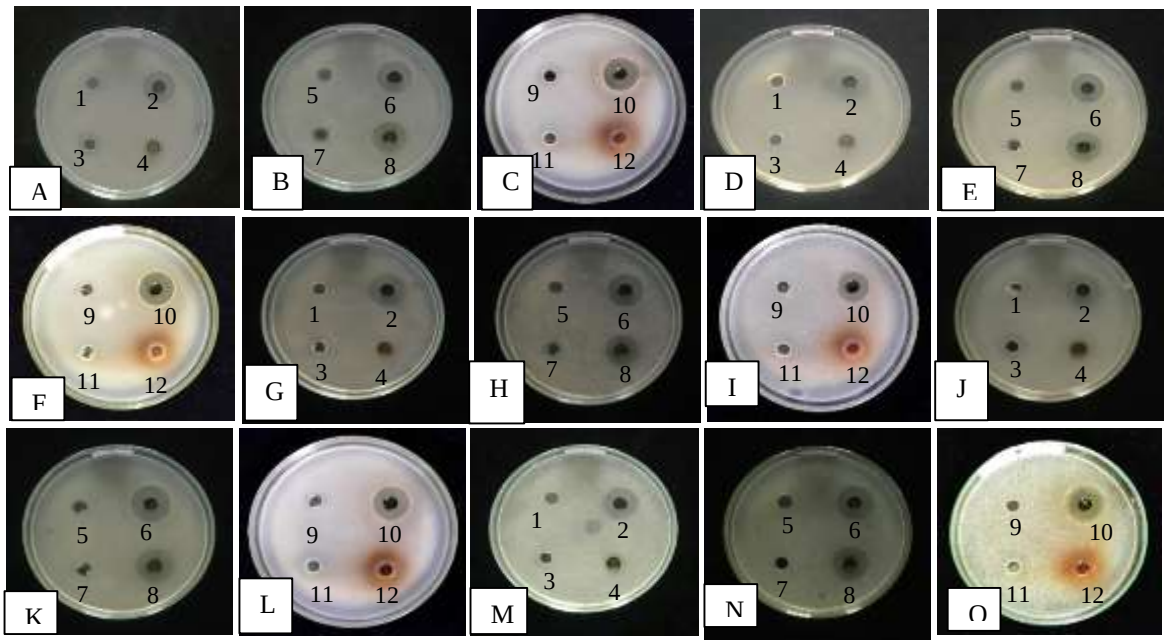

Figure 8. Antimicrobial Activity of different extracts from leaves of *A. paniculata* (Burm.f.) Wall. ex Nees

A, B, C = Test against *Escherichia coli* D, E, F =Test against *Micrococcus luteus* G,H,I. =Test against *Staphylococcus aureus* J, K, I.= Test against *Pseudomonas albican* M, N, O = Test against *Candida albicans* 1, 3,5,7,9,11 = Control 2 = Test for chloroform 4 = Test for pet-ether 6 = Test for ethyl-acetate 8= Test for ethanol 10 =Test for methanol 12 = Test for aqueous

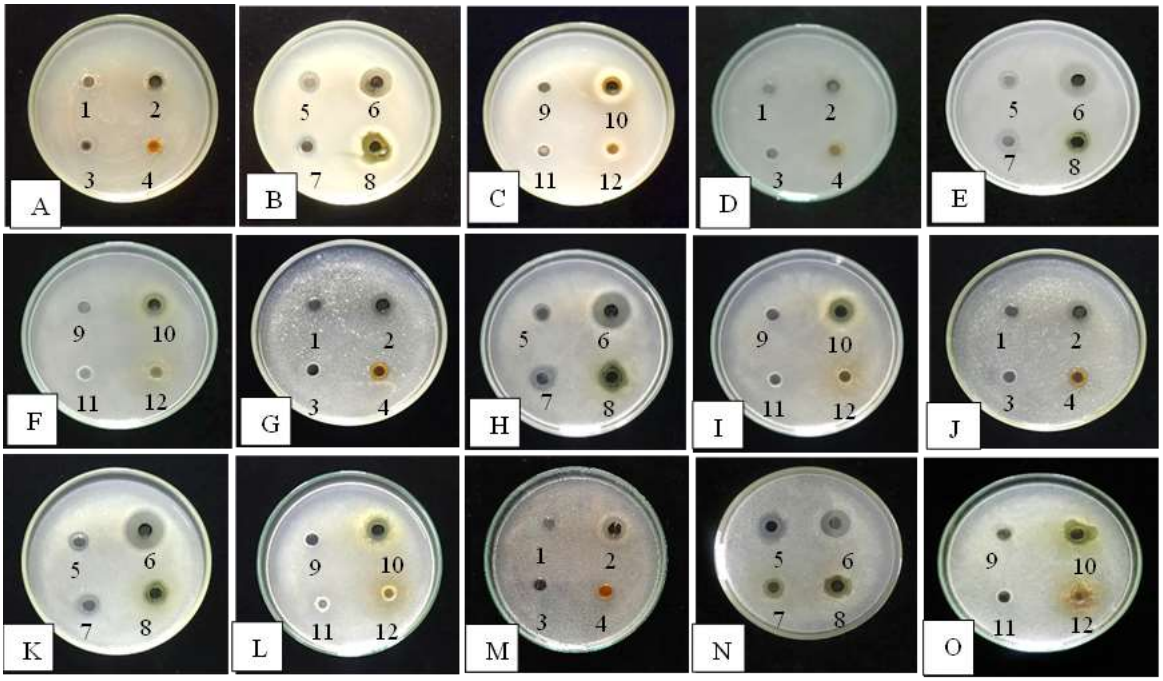

Figure 9. Antimicrobial Activity of different extracts from leaves of *A. echioides* (L.) Nees
A,B,C = Test against *Escherichia coli* D,E,F =Test against *Micrococcus luteus* G,H,I = Test against *Staphylococcus aureus* J,K,I. = Test against *Pseudomonas albicans* M,N,O = Test against *Candida albicans* 1,3,5,7,9,11 = Control 2 = Test for chloroform 4 = Test for pet-ether 6 = Test for ethyl-acetate 8= Test for ethanol 10 = Test for methanol 12 = Test for aqueous

Discussion

In this research, *A. paniculata* (Burm.f.) Wall. ex Nees and *A. echioides* (L.) Nees belong to the family Acanthaceae had been studied. The morphology characters of two species were annual herbs, quadrangular stems, leaves opposite and decussate. Inflorescences were axially or terminal spikes. Flowers were white with purple spots, bisexual, hypogynous and ovary superior. The loculicidal dehiscence fruits were found in two species. These characters were similar to Hooker (1885), Backer *et al* (1965), Dassanayake (1998).

In histology characters of leaves were diacytic types of stomata were found in two species. The stomata found only in abaxial surface and stoma index was 13.3-21.43 in *A. paniculata* (Burm.f.) Wall. ex Nees. *A. echioides* (L.) Nees observed on both surfaces and stoma index were 14.28-25.0, 20.0-33.33. The transverse section of *A. paniculata* (Burm.f.) Wall. ex Nees petiole was rectangular shaped and *A. echioides* (L.) Nees was crescent shaped. In transverse section of midrib, *A. paniculata* (Burm.f.) Wall. ex Nees was triangular and the other one rectangular. The vascular bundles of two species were crescent shaped and collateral types. These characters were agreement with Metcalf & Chalk (1957), Kokate (2000).

The transverse section of stem of two species were found in quadrangular shaped. Vascular bundles of stem were forming a circular ring and collateral type. One of the most outstanding histological features is the widespread occurrence of various shaped cystoliths located in both stem and leaf. The present data were similar to Metcalf & Chalk (1957).

The root of two species found circular in shaped. Periderm formation occurred at the outer part of the cortex. It consists of phellogen, phelloderm and phellem. These characters were in accordance with Metcalf & Chalk (1957).

The phytochemical analysis of the leaves of two species is summarized in Table 3. The aqueous extract of leaves shows the presence of high number of phytocompounds when compared with ethanol and methanol. It shows the presence of alkaloids, glycoside, tannins, phenolic compounds, flavonoids, saponins, α-amino acids, proteins, and reducing sugar whereas cyanogenic glycosides were not presence in both species. Phytochemicals have highest therapeutic efficiency in pharmaceutical field. These resulting were similar to Ankush and Sicgandra (2023) Pande *et al*. (2018).

The free radicals scavenging activity of leaves of two species have been observed that the leaves extracts have excellent antioxidant activity showing IC_{50} value of 5.98 and 3.44 mg/l compared to standard ascorbic acid 6.75 mg/l. The antioxidants activity of the leaves extracts are observed in lower IC_{50}. Antioxidants are natural substance that can help to delay cell damage and defend cells against. These resulting data were similar to those of Jeevanantham and Hussain (2021).

The antimicrobial activity of chloroform extract of *A. paniculata* (Burm.f.) Wall. ex Nees showed against maximum antimicrobial activity of *Micrococcus luteus* and aqueous extract did not showed. In *A. echioides* (L.) Nees, ethyl-acetate of leaves extract were investigated for its antimicrobial properties against *Pseudomonas albicans* whereas aqueous and pet-ether extracts were not effective against. The antimicrobial activity of the medicinal plant extracts depends on the presence of different concentration of secondary metabolites. The present data similar to Pande *et al*. (2018), Keita *et al*. (2022).

Medicinal plants have been a long history of usage in traditional medicine. Plant leaves have been used as herbal medicine for their healing properties since ancient times. Plant natural product have been explored and used in the treatment of countless diseases.

Conclusion

The result of this study revealed the antibacterial and antifungal potential of the studied medicinal plants against drug resistant pathogens. The phytochemical constituents which are responsible for many pharmacological activities. Additionally, these medicinal plants could be used as a natural source of oxidants. *A. paniculata* (Burm.f.) Wall. ex Nees is widely used as traditional medicinal soup. In Mandalay Region almost every household cultivated in the style of homegarden. Traditional medicines have been gaining momentum in popularity, to prevent Covid-19 sickness and demand has been increasing year by year. *A. echioides* (L.) Nees has potential source of medicine as anti-bacterial and antioxidant activity. Therefore**,** two species can be hoped that, homegarden scale cultivation can also be gained the medium scale and commercial cultivation, which would generate green economy in future.

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