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Preliminary Phytochemical Screening, Biochemical Analysis and Antibacterial Activity of Mulberry (Morus spp.), Coleus amboinicus Lour., and Sauropus androgynus (L.)

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Abstract

The present study aims to screen three well-known plants for their biochemical constituents and their antimicrobial activity on gram positive bacteria Staphylococcus aureus. The three botanical extracts chosen for the study are from Morus V1 Variety, Coleus amboinicus and Sauropus androgynus. Mulberry (Morus spp.), plays a vital role in the sericulture industry, as it is rich in bioactive phytochemicals it has got some medicinal properties. Coleus amboinicus are also known as Indian Borage it is a popular medicinal plant which belongs to the family Lamiaceae(Amol et.al 2023). It is distributed in almost all over India. Coleus Lour. is a genus used to treat a wide range of diseases such as disorders of the digestive system, respiratory ailments and various skin conditions (Lukhoba et al., 2006; Waldia et al., 2011, Guilherme Perez Pinheiro et al., 2023) Sauropus androgynus L. Merr is an unexploited medicinal shrub under the family Phyllanthaceae known as a green multivitamin plant and used as a leafy vegetable (Dhaneswar Swain et al., 2024). "Many phytochemicals from the leaf extracts of S. androgynous are reported to act as antioxidant, anti-microbial, wound-healing, anti-inflammatory, anti-diabetic, and anti-obesity agents" (Dhaneswar Swain et al., 2024). Because of the significance of the selected plants in health care, an attempt has been made to investigate the phytochemical profile, biochemical constituents, and antibacterial activity of leaf extracts from Morus spp. (V1 variety), Coleus amboinicus, and Sauropus androgynus on gram +ve bacteria Staphylococcus aureus which is commonly reported to be the main reason for causing majority of human nasal passage, skin and soft tissue infections. In the present investigation phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, steroids, saponins, tannins, lignins, and glycosides in all three species. Biochemical analyses revealed considerable concentrations of carbohydrates, proteins, amino acids, and chlorophyll, supporting their nutritional potential. Antibacterial activity assessed by agar well diffusion showed significant zones of inhibition, particularly in ethanol extracts against Staphylococcus aureus These findings highlight the medicinal value of the selected plants and suggest their applicability in developing affordable, plant-based antimicrobial and nutritional products.

Keywords: *Morus spp., phytochemical screening, antibacterial activity, Coleus amboinicus, Sauropus androgynus, Staphylococcus aureus.*

INTRODUCTION

Plants are known to have therapeutic properties and has a long history of human usage. Botanical plant extracts are the source of drug identification and development(Dias et al., 2012; Newman and Cragg, 2020). In many countries still people depend on plant products that have medicinal values. (Smith-Hall et al., 2012). Although medicinal plant products have many health benefits their potentiality and the qualitative and quantitative properties vary depending on the type of climatic and soil conditions they are growing. (Li et al., 2020), resulting in changes in their bioactive compounds and their efficacy(Dhaneswar Swain et al., 2024). Many botanicals have medicinal properties due to the presence of various secondary metabolites like alkaloids,flavonoids,tanins and saponins. These secondary metabolites have various therapeutic properties like antimicrobial, antioxidantand anti inflammatory properties. (Tiwari et al., 2011; Edeoga et al., 2005). Morus spp., Coleus amboinicus, and Sauropus androgynus are three such plants valued both for their nutritional and pharmacological significance.

Mulberry (Morus alba) is widely cultivated for its use as feed in Sericulture, Fruits and leaf tea for human consumption. Mulberry leaf and fruits are known for their health- benefits. Mulberry leaves contain 1-deoxynojirimycin (DNJ), a potent anti-diabetic alkaloid (Asano et al., 2001; Kimura et al., 2007), along with flavonoids and polyphenols that offer anti-inflammatory, antioxidant, and hypoglycaemic benefits (Kadam, 2019; Ge et al., 2018; Van Dam et al., 2013). These constituents not only improve human health but also affect silkworm nutrition and silk productivity (Krishnaswami et al., 1971; Das & Vijayaraghavan, 1990;).

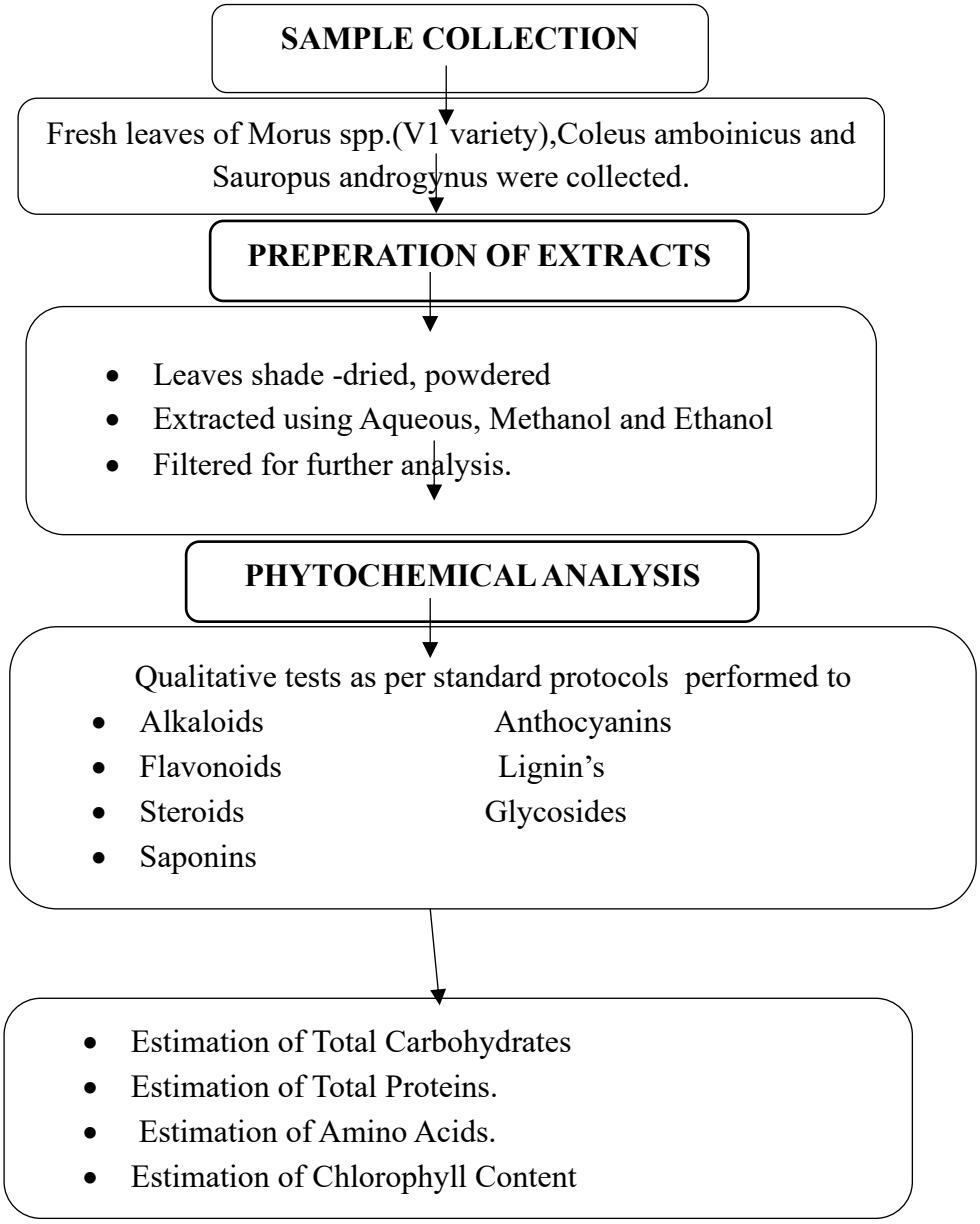
Coleus amboinicus is a widely used aromatic herb with traditional applications in treating respiratory and digestive disorders. The essential oils and phenolic compounds present in Coleus contribute to its antimicrobial activity (Chaitali et al., 2014; Hammer et al., 1999). Sauropus androgynus, commonly known as the multivitamin plant, is rich in protein, iron, and bioactive compounds, and is consumed both as food and medicine across South and Southeast Asia (Edeoga et al., 2005). The leaf extracts of this shrub contain ascorbic acid, eugenol, gallic acid, caffeic acid, syringic acid, p-coumaric acid, sinapic acid, ferulic acid, and different types of flavonoids (Dhaneswar Swain (2024).

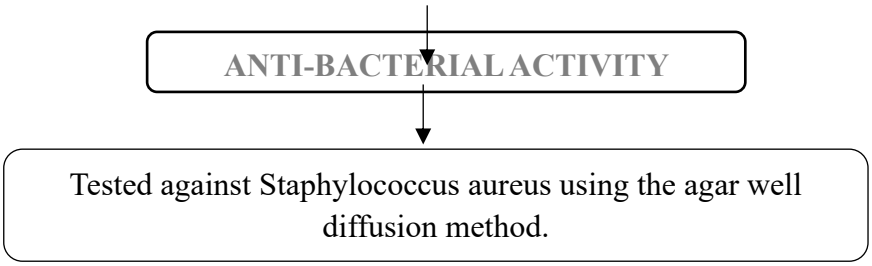
Due to considerable global rise in antibiotic-resistant infections, particularly from pathogens like Staphylococcus aureus, the exploration of plant-based antimicrobials has become increasingly important (Hammer et al., 1999; Hafiza et al., 2002). Therefore, this study aims to evaluate the phytochemical composition, biochemical constituents, and antimicrobial potential of these three medicinally significant plant species.

Methodology:

This study was carried out during 2024 in the Dept. Of Biosciences and Sericulture, Sri Padmavati Mahila Visvavidyalayam, Tirupati.

Flow chart of Preliminary Phytochemical Screening, Biochemical Analysis, and Antibacterial Activity of Mulberry (*Morus* spp.), *Coleus amboinicus*, *and Sauropus androgynus* (L.)





1. Sample Collection

Fresh leaves of Morus spp. (V_1 variety), Coleus amboinicus, and Sauropus androgynus were collected from the Medicinal Garden located in Biosciences and Sericulture Department, Sri Padmavati Mahila Visvavidyalayam, Tirupati

2. Preparation of Extracts

Leaves were shade-dried, powdered, and extracts are prepared using three different solvents aqueous, methanol, and ethanol by taking 100 mg of each sample. Leaf Samples were dissolved in 100 ml of the different solvents, filtered using Whatman No. 1 filter paper (Elvino Nortjie et.al (2022), and used for further analysis.

3. Phytochemical Screening.

Standard qualitative tests were performed to identify the following constituents:

- **Alkaloids**: Mayer's and Wagner's tests
- **Flavonoids**: Shinoda and Ferric chloride tests
- **Phenolics**: Ferric chloride and Ellagic acid tests
- **Steroids**: Salkowski and Liebermann–Burchard tests
- **Saponins**: Froth formation test
- **Tannins**: Gelatin and Ferric chloride tests
- **Anthocyanins**: Methanolic HCl test
- **Lignin's**: Furfuraldehyde and Labat tests
- **Glycosides**: Acetic acid-ferric chloride–H_2SO_4 ring test

Biochemical Analysis: The biochemical constituents were estimated using standard methods.

1. Estimation of Total Carbohydrates (Anthrone Method)

Samples were hydrolysed with 2.5N HCl, neutralized with Na_2CO_3, and reacted with Anthrone reagent. The absorbance was measured at 630 nm.

2. Estimation of Total Proteins (Lowry's Method)

The extracts were treated with alkaline copper reagent and Folin–Ciocalteu's phenol reagent. Absorbance was recorded at 660 nm.

3. Estimation of Amino Acids (Ninhydrin Assay)

Extracts were reacted with ninhydrin reagent and heated in a boiling water bath. The absorbance was measured at 570 nm.

4. Estimation of Chlorophyll Content

Chlorophyll was extracted with 80% acetone. Absorbance was recorded at 645 nm and 663 nm to determine chlorophyll a, b, and total content using standard equations.

5. Anti-bacterial Activity

1. Antibacterial efficacy was tested against Staphylococcus aureus using the agar well diffusion method. Wells were filled with 20 µL of each extract (methanol, ethanol, and aqueous), and zones of inhibition were measured after incubation at 37°C for 24 hours.

RESULTS AND DISCUSSION

1. Phytochemical Profile (Table -1)

- **Mulberry leaf extract** (Methanol, Ethanol and aqueous): The extracts were screened for the presence of various Phyto-constituents. The phytochemical screening of mulberry leaf extract was found positive for Alkaloids, Flavonoid, Phenolics, Steroids, Saponins, Tannins,Anthocyanins,lignins,Glyosides ."Mulberry leaves contain amino sugar Alkaloids (1-deoxynojirimycin (DNJ))which exhibit an inhibitory effect on mammalian glycosidase enzymes" (Asano et al.,2000).

- **Coleus amboinicus leaf extracts (Methanol, Ethanol, Aqueous):** Phytochemical screening was carried out on the leaf extracts to determine the presence of different classes of phytoconstituents. The study revealed that Alkaloid, flavonoid, Tannins, Glyosides are present in the Coleus amboinicus leaves extract.
- **Sauropus androgynus leaf extracts** (Methanol, Ethanol and aqueous) The leaf extracts were analysed for the presence of various phytochemicals to detect and characterize the major groups of phytoconstituents. This investigation revealed that Alkaloid,flavonoid,steroids,sapoins,Tannins,Glyosides are present in the Sauropus androgynus leaf extract.
- "Flavonoids possess a number of medicinal benefits including anti-cancer,anti-inflammatory, anti-viral properties anti-oxidant activities. Enhance cerebrovascular and coronary blood flows, decrease sugar and fat" (Chaital Nikar ker et al.,).
- Alkaloids have the importance in medicine, they are helpful in pain relief, control the excess activity of gastrointestinal tract and adjuvant to the anaesthesia (J.Joanna kurek,2019).
- Saponins reduce the uptake of certain nutrients including glucose and cholesterol at the gut through intra-luminal physiochemical interaction (Price et al.,1987).
- The therapeutic values of the secondary metabolites are due to the occurrence of chemical substances. That Secondary metabolites produced by plant extracts against particular organism produce a definite physiological action on the body. Toxic affects/pests are produced by the compound like steroids, phenols, anthocyanins, flavonoids, tannins, alkaloids and glycosides (Smita G.Bhat,2021).

2. Biochemical Composition(Table -2)

Carbohydrates:

The total amount of carbohydrate present the mulberry leaf extract was 40mg/gm. Total carbohydrates in Sauropus androgynus leaves extract was estimated as 20.33 mg/g of plant material. Total carbohydrates in Coleus amboinicus leaves extract was 41 mg/g of plant material.

Proteins

The Total protein in Morus Sauropus androgynus, Coleus amboinicus leaves extract is estimated by lowry's method. Total protein in the aqueous was done by spectrophotometer measuring at 660nm.The total amount of protein obtained was 94mg/g, 61mg/g , 21.3 mg/g of plant material respectively.

Amino acid

Quantification of total amino acids in Morus, Sauropus androgynus, Coleus amboinicus leaves extract using 80% ethanol extract was done by spectrophotometer. The total amount of amino acids obtained was 47mg/g, 21.43mg/g, 26 mg/g of plant material respectively.

Chlorophyll

Quantification of total chlorophyll in Morus, Sauropus androgynus, Coleus amboinicus leaves extract by using 80% acetone extract was done by spectrophotometer. The total amount of chlorophyll obtained was 3.16 mg/gm , 1.492mg/g , 2.43 mg/g of plant material respectively.

3. Antibacterial Activity (Table -3)

Anti-bacterial activity was tested against Staphylococcus aureus with three different extracts (Aqueous. Ethanol and methanol) of Morus, Coleus amboinicus and Sauropus androgynus (L.) Inhibition of Staphylococcus aureus growth was observed on ethanol extracts of all the three selected plant extracts. The maximum inhibition was observed as 18.16 mm on Sauropus followed by Coleus with 15 mm and 12.3mm in Morus The inhibition Staphylococcus aureus growth was maximum on Coleus extract with 19.8 mm followed by Sauropus 12.13 mm and Morus 10.1 mm respectively. Aqueous extract shown inhibition zone of 8.73mm,7.6mm, and 7.06mm on Coleus, Morus and Sauropus respectively.

Phytochemical compounds such as alkaloids, saponins, tannins, flavonoids and steroids have been known to be biologically active and thus responsible for the antimicrobial activity plans. Due to the presence of these phytochemicals Morus plant , Sauropus androgynus (L.) and Coleus amboinicus might be showing anti-bacterial activity on Staphylococcus aureus gram positive bacteria.

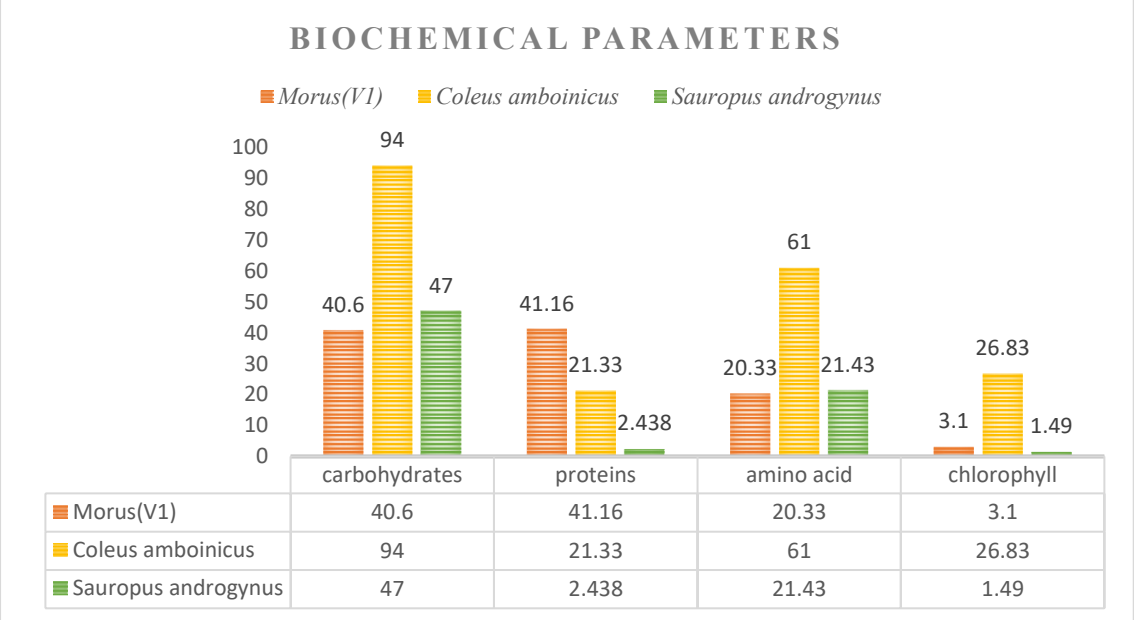
Table-1.Phytochemical screening of Morus ,Coleus and Sauropus

S.no	Name of the test	Morus (V1 mulberry variety)			Coleus amboinicus			Sauropus androgynus(l.)		
		D.H2O	Ethanol	Methanol	D.H2O	Ethanol	Methanol	D.H2O	Ethanol	Methanol
1	**Test for alkaloids** (a)Mayers test	+	__	+ +	+ +	+ +	+ +	+	+ +	+ +
	(b)Wagner test	+	-	+ +	+ +	-	-	+	+ +	+ +
2	**Test for flavonoids** (a) Shinoda's test	+ +	++	+ +	+ +	+ +	+ +	-	+ +	+
	(b) Ferric chloride test	+ +	++	+ +	+ +	+ +	+ +	-	+ +	+
3	**Test for phenols** (a) Phenols test	_ _	+ +	+	-	-	-	-	++	-
	(b) Ellagic acidic test	+	_ _	_	-	-	-	-	-	-
4	**Test for steroids** (a) Salkowski test	_ _	_ _	_ _	+	-	-	-	-	-
	(b) Libermann's burchard test	+	+ +	+ +	+	+ +	+ +	-	-	-
5	**Test for saponins**	+ +	+ +	+ +	+ +	-	+ +	-	-	-
6	**Test for tannins** (a) Gelatin test	+ +	_ _	_ _	-	-	-	++	+	-
	(b) Ferric chloride test	+ +	+ +	+ +	+	+	-	++	++	-
7	**Test for anthocyanins**	_ _	_ _	_ _	-	-	-	-	-	
8	**Test for lignin's** (a) Lignin's test	_ _	+	+	-	-	-	-	-	-
	(b) Labat test	+ +	+ +	+ +	+ +	+	-	-	-	-
9	**Test for glycosides**	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+ +

Table-2. Biochemical Composition of Morus, Coleus and Sauropus leaf extracts.

Biochemical	Mulberry (V1)	Coleus amboinicus	Sauropus androgynus
Carbohydrates	40.6 mg/g	41.16 mg/g	20.33 mg/g
Proteins	94 mg/g	21.33 mg/g	61.00 mg/g

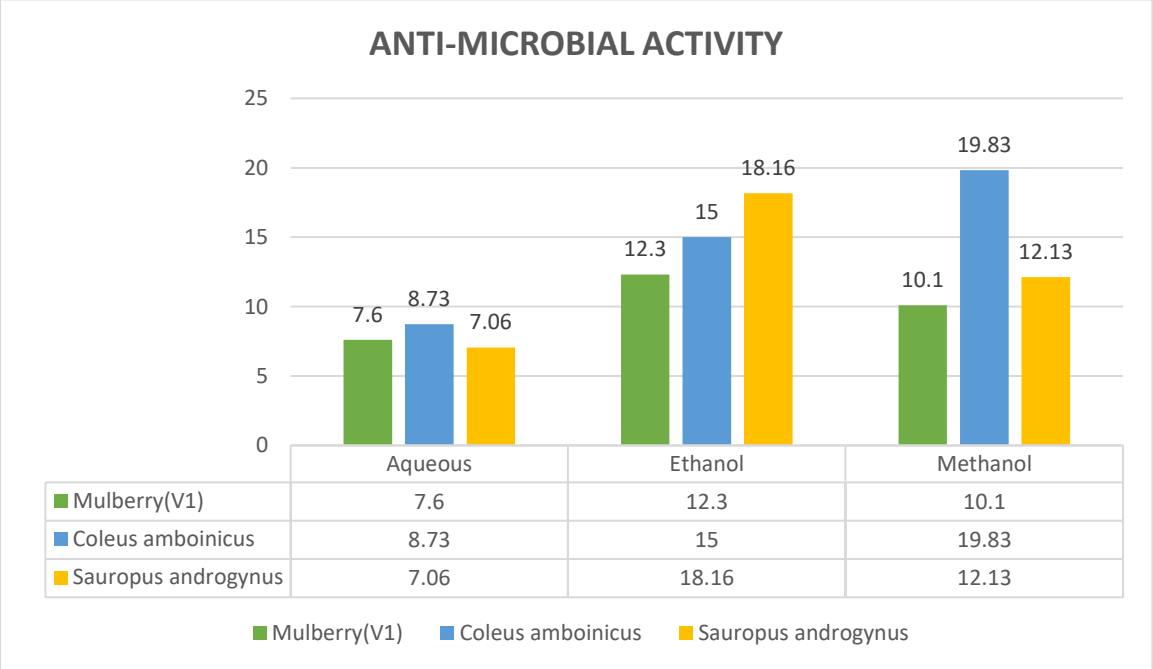
Amino Acids	47 mg/g	2.438 mg/g	21.43 mg/g
Chlorophyll	3.10 mg/g	26.83 mg/g	1.49 mg/g



Mulberry exhibited the highest protein and amino acid content, while Coleus showed the highest chlorophyll concentration, indicating potential for both nutritional and industrial applications.

Table -3. Antibacterial Activity of Plant extracts on Staphylococcus aureus

Extract Type	Mulberry (V_1)	Coleus amboinicus	Sauropus androgynus
Aqueous	7.6 mm	8.73 mm	7.06 mm
Ethanol	12.3 mm	15 mm	18.16 mm
Methanol	10.1 mm	19.8 mm	12.13 mm



The ethanol extract of Sauropus androgynus showed the highest antibacterial activity, followed by methanol extract of Coleus amboinicus. These effects are attributed to the presence of bioactive secondary metabolites known to disrupt bacterial growth.

"Antibiotics or antibacterial substances like glycosides ,flavonoids and alkaloids etc are found to be distributed in plants"(Hamed Zandavar et al.2023).

CONCLUSION

The present study confirms that the leaves of Morus spp., Coleus amboinicus, and Sauropus androgynus are rich in phytochemicals and essential biomolecules. Their significant antibacterial activity, particularly in ethanol and methanol extracts, suggests their potentiality in herbal drug development and natural health supplements. Further investigation into isolation and characterization of active compounds is recommended to harness their full therapeutic potential. Anti-bacterial activity result of the present research revealed that among the different solvents methanol extract showed high zone of inhibition of the growth of Staphylococcus aureus . Biochemical analysis of the three selected revealed that, their leaves serves as rich source of nutrients including carbohydrates, protein, amino acids and chlorophyll.

The present study indicates that natural plant extracts possess significant anti-microbial potential, effectively inhibiting the growth of microbial populations.Hence,these plants can be utilized in the pharmaceutical industry for the development of cost-effective medicines aimed at safeguarding human health.

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