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Original Research Article

Phytochemical Screening and Antibacterial Potential of Wild Leafy Vegetable-*Cayratia auriculata* (Roxb.) Gamble Against Selected Enteric Pathogens

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ABSTRACT

The emergence of antibiotic-resistant bacteria, combined with the negative effects of antibiotics on human health, necessitates the pursuit of plant-derived antimicrobial compounds. Wild leafy vegetables with ethnomedicinal uses can be investigated to reveal their efficacy for the discovery of novel nutraceuticals and herbal drugs. An underutilized leafy vegetable,*Cayratia auriculata*, is used against gastrointestinal disorders by the tribal communities inhabiting the Balasore district of Odisha. Therefore, the current study was designed to test the presence of phytochemicals in different leaf extracts of *Cayratia auriculata*, such as petroleum ether, methanol, and distilled water (aqueous), using the Soxhlet apparatus, and to screen its antibacterial activity using the agar well diffusion method as well as to determine the minimum inhibitory concentration (MIC) using the broth dilution method. The results of the phytochemical screening showed that the plant metabolites were present abundantly in the methanol extract. The plant extracts in different test solvents were observed to have broad-spectrum activity, thereby showing their efficacy against both gram positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli, Shigella flexneri, and Salmonella typhimurium*). The methanolic extract of *Cayratia auriculata* showed maximum antimicrobial potential against *Escherichia coli*, with a larger zone of inhibition (17.11 ± 0.09mm) and lower MIC value (6.25 mg/ml) as compared to the other two extracts against the four bacterial strains. Hence, further investigations could reveal that the leaf extracts of *C. auriculata* can be used as an effective natural antibacterial agent for the treatment of enteric diseases like diarrhoea and dysentery caused by those pathogenic bacteria.

***Keywords*:** *Cayratia auriculata*, Phytochemical, Antibacterial, MIC.

Introduction

With the overuse of antibiotics in the synthetic era, there has been an increase in the development of antibiotic-resistant bacteria, thereby decreasing their efficiency in the treatment of infectious diseases. In addition, they are responsible for damaging effects on the host; consequently, there is a worldwide increase in mortality and morbidity. This encourages the exploration of novel antimicrobial compounds from natural products.[1] Plant-derived antimicrobials appear to be one of the most promising alternatives for dealing with antibiotic-resistant human pathogens.[2] Besides, they are relatively safer and more cost-effective than synthetic drugs. Recently, the search for phytochemicals and the pharmacological investigation based upon folkloric claims of medicinal plants have gained momentum for establishing their scientific authenticity and efficacy for the discovery of new effective drugs. Thus, several ethnomedicinal plants have been screened for their possible pharmacologically active compounds for the benefit of humankind.Since time immemorial, green leafy vegetables have been consumed both as food and as medicine, providing dual benefits of nutrition and healthcare. They are nutritionally rich with antimicrobial and antioxidant properties, which can be explored for the development of therapeutic agents.[3]

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Cayratia auriculata (Roxb.) Gamble (Family: *Vitaceae*) is an underutilised leafy vegetable with potent ethnomedicinal properties (Figure 1).It is a climbing shrub found in dry evergreen to dry deciduous forests and is well distributed in Andhra Pradesh, Karnataka, Kerela, Tamilnadu, Odisha, and Maharashtra. The young shoots and leaves of this plant are consumed as a leafy vegetable and are reported to be used medicinally against several diseases like colds and coughs, fevers, diarrhoea, snake and dog bites, abscesses, rheumatism, ulcers, hydrocele, tumours, uterine disorders, and cardiac ailments.[4,5] This plant has also been used in veterinary medicine to treat blood dysentery and diarrhoea in animals.[6] The dried leaf powder of this plant is consumed for treating gastrointestinal problems such as dysentery and diarrhoea by the ethnic communities residing in the forest areas of Balasore district (Odisha). However, no scientific investigations regarding its biological evaluation have been reported.Several secondary metabolites like alkaloids, tannins, and flavonoids extracted from various medicinal plants have shown their antimicrobial efficacy.[7] The presence of flavonoids, phenols, coumarins, saponins, tannins, terpenoids, steroids, and cardiac glycosides is reported in a preliminary phytochemical screening utilising an ethanolic leaf extract of *Cayratia auriculata*.[8]Pharmacological investigations related to its anthelmintic, anticancer, cytotoxic, and acute toxic effects have been conducted.[8-10] However, phytochemical screening using different solvents to test their antimicrobial and antioxidant activity has not yet been undertaken. Hence, the present work was aimed at determining the antibacterial activity of various organic extracts of *Cayratia auriculata* leaves. The other objective of the present study was to screen the phytochemicals present in petroleum ether, methanol and aqueous extracts of *C. auriculata* leaves for the detection of any bioactive principles which could provide a scientific justification for the ethnomedicinal use of this plant against diarrhoea and dysentery.

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Materials and Methods

Plant Material Collection and Identification

Fresh leaves of *Cayratia auriculata* (Roxb.) Gamble were collected from the Nilagiri forest area of the Balasore district (Odisha) during the month of February, 2022. The plant was taxonomically identified by referring to the regional flora.[11-13] The voucher specimen [NN-335] was preserved at the Herbarium of Centurion University of Technology and Management's Department of Botany, School of Applied Sciences, Odisha, India.

Preparation of Solvent Extracts

The leaves were washed very well 2-3 times underneath running tap water, followed once by distilled water, and shade dried at room temperature for 7–10 days. By using a mortar and pestle, the dried plant material was made into a coarse powdered form, sieved and used for extraction. The dried plant material (50 g) was extracted in 3 distinct solvent systems (500 mL each) in the order of increasing polarity, consisting of petroleum ether, methanol, and distilled water (aqueous) separately in Soxhlet apparatus for seventy-two hours. The extracts obtained in each solvent were filtered respectively with Whatman No. 1 filter paper, concentrated with a rotary evaporator, and stored at 4^{o}C in airtight capped bottles in the freezer until further use. Preliminary phytochemical tests and an antibacterial activity assay were performed on all extracts.

Preliminary Phytochemical Screening

The petroleum ether, methanol and aqueous extracts of *C. auriculata* were subjected to qualitative phytochemical screening for active chemical constituents such as alkaloids, glycosides, flavonoids, saponins, steroids, phenols and tannins following the standard methods.[14,15]

Antibacterial Activity

Bacterial strains and culture media: One gram-positive human pathogenic bacteria, namely *Staphylococcus aureus* (MTCC-3160), and three gram-negative bacteria, such as *Escherichia coli* (MTCC-119), *Shigella flexneri* (MTCC-1457), and *Salmonella typhimurium* (MTCC 3231), were obtained from the Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India, for the study of antibacterial activity of plant extracts. All the bacterial strains were maintained by sub-culturing them on Nutrient Agar (Himedia) every fifteen days and then stored at 4°C. Ciprofloxacin, an antibiotic obtained from Himedia Laboratories, India, was used as a standard medicine or positive control in bacterial strain testing. As a negative control, Dimethyl Sulfoxide (Himedia) was utilised.

Antibacterial susceptibility assay: *Cayratia auriculata* leaf extracts were screened for antibacterial activity using the agar-well diffusion method with Nutrient Agar.[16] For each extract, three replicated trials were conducted against each organism. 30 mL of agar media (265 nm wavelength) was poured into Petri dishes and allowed to solidify for 15 minutes under ultraviolet light. Subsequently, the agar plate was inoculated by evenly streaking with a cotton swab that was dipped overnight in suspensions of test microorganisms, i.e., *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*, and *Salmonella typhimurium* (each adjusted to turbidity of 0.5 McFarland Standard) over the agar medium. Then, using a sterile cork borer, wells with a diameter of 6 mm were cut in the medium. Stock solutions of plant materials extracted in various solvents were diluted in DMSO to obtain concentrations of 6.25, 12.5, 25, and 50 mg/mL. The test samples as well as controls (50 µL) were loaded into the wells by using micropipettes and allowed to diffuse for 15 minutes. The plates were then incubated for 24 hours at 37°C. The antibacterial activities of various solvent extracts were assessed using an Antibiotic Zone Scale (Hi-Media) to measure the diameter of the zone of inhibition (ZI) against the test pathogens. The recorded value so obtained by each extract was rounded to the nearest millimeter. The efficient value for the average diameter of the zone of inhibition is recommended to be greater than 10 mm.

Minimum Inhibitory Concentration (MIC)

The broth dilution technique was used to determine the plant extract's minimum inhibitory concentration (MIC). The different plant extracts of petroleum ether, methanol and aqueous were taken (50, 25, 12.5 and 6.25 mg/mL) and serial dilution of the extract with nutrient broth for bacterial culture with respective inoculums was used. For bacteria, the tubes were incubated for 24 hours at 37 °C. Thereafter, each test tube was checked for turbidity. The minimum inhibitory concentration was calculated by measuring the least concentration of the extract for which no turbidity was detected.

Statistical analysis

The average value of inhibition obtained from each experimental result were statistically analyzed and standard deviation were calculated which is expressed as a mean ± standard deviation.

Results and Discussion

Phytochemical Constituents

The extraction process is vital for recovering and isolating phytochemicals from plant sources. The existence of 7 pharmaceutically active plant secondary metabolites was demonstrated in petroleum ether, methanolic, and aqueous extracts of *C. auriculata*, including alkaloids, flavonoids, tannins, saponins, steroids, glycosides, and phenols (Table 1). Flavonoids and steroids were found in all three extracts of *Cayratia auriculata* as secondary plant metabolites. Other secondary metabolites such as alkaloids and saponins were only found in methanolic extracts of the species, but not in aqueous or petroleum ether extracts. The polarity of the solute of interest determines the solvents used for phytochemical extraction. Methanol is known to be effective in the extraction of lower molecular weight phenols, whereas ethanol is an excellent phenol extraction solvent.[17] According to previous preliminary phytochemical analysis, high molecular weight chemicals were extracted with a highly polar solvent such as water, whereas low and medium molecular weight compounds were extracted with less polar solvents such as methanol and ethanol. Hence, the outcomes of the present experiment revealed that the majority of the phytochemicals were obtained from the methanolic extract of *C. auriculata*.

Table 1: Qualitative preliminary phytochemical analysis of leaf extracts of *C. auriculata*

Phytoconstituents	Tests	PE	MN	AQ
Alkaloids	Wagner's test	-	+	-
Flavonoids	Alkaline Reagent Test	+	+	+
Tannins	Lead acetate test	-	+	+
Saponins	Foam test	-	+	-
Steroids	Salkowski's test	+	+	-
Glycosides	Keller Killani test	-	+	+
Phenols	Ferric chloride test	-	+	+

+ Present, - Absent.PE- Petroleum ether, MN- Methanol, AQ- Aqueous

Antibacterial Activity

The antibacterial activity of *C. auriculata* was evaluated in terms of the inhibition zone (ZI) formed by the agar well diffusion test (Table 2). The findings of the antibacterial assay showed differential antibacterial activity of the leaf extract against the bacterial pathogens tested. All three extracts of *C. auriculata* showed strong antibacterial activity in the following inhibition sequence: *Escherichia coli* >*Salmonella flexneri* >*Salmonella typhimurium*>*Staphylococcus aureus*. The minimum antibacterial activity of the leaf extract was against the bacterial strain *Staphylococcus aureus*. The antibacterial competency of the plant extract was in the order of methanol > aqueous > petroleum ether extract.

The antibacterial activity of the leaf extract of *C. auriculata* was compared with the antibacterial activity of the standard drug

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ciprofloxacin (0.1 mg/mL). At 0.1 mg/mL, the antibiotic ciprofloxacin inhibited the test microbes in the following order: *Staphylococcus aureus* (26.3±0.63 mm) >*Shigella flexneri* (25.8±0.75mm) >*Salmonella typhimurium* (24.5±0.86mm) >*Escherichia coli* (22.1±0.89 mm).*C. auriculata's* antibacterial activity was evaluated in terms of minimum inhibitory concentration, as shown in Table 3. MIC is the minimum concentration of an antibacterial agent that retards the visible growth of microorganisms. The MIC concentration range of the various leaf extracts varied from 6.25 to 50 mg/mL. The methanol extract of *Cayratia auriculata* showed the minimum inhibitory concentration of *Escherichia coli* (6.25 mg/ml), while the aqueous extract showed the minimum inhibitory concentration of *Salmonella typhimurium* (6.25 mg/ml). This study demonstrated that methanol and aqueous extracts had lower MIC values for the tested bacterial pathogens than *C. auriculata's* petroleum ether extract (Figure 2). In this study, two gram-negative pathogens, such as *Escherichia coli* and *Shigella flexneri*, showed high sensitivity towards *C. auriculata* extract. Antimicrobial studies conducted in *Cayratia pedata* against two bacteria (*Staphylococcus aureus* and *Escherichia coli*) and two fungi (*Candida albicans* and *Aspergillus flavus*) in three different solvents like aqueous, methanol, and n-butanol reveal their antimicrobial potential against all the pathogens tested.[18]

Table 2: Antibacterial activity (Zone of inhibition ZI) of different leaf extracts of *C. auriculata*

Bacterial strains	Solvent	6.25mg/mL	12.5mg/mL	25mg/mL	50mg/mL	Negative Control DMSO	Positive Control Ciprofloxacin [0.1 mg/mL]
		[Different concentrations of plant extract] Zone of inhibition in millimetre					
Staphylococcus aureus	Petroleum ether	5.81 ± 0.31	5.99 ± 0.01	6.00 ± 0.00	6.03 ± 0.06	00 ± 0.00	26.3 ± 0.63
	Methanol	6.05 ± 0.04	6.06 ± 0.05	7.96 ± 0.06	8.07 ± 0.06	00 ± 0.00	
	Aqueous	9.07 ± 0.07	9.93 ± 0.04	10.06 ± 0.05	10.96 ± 0.04	00 ± 0.00	
Escherichia coli	Petroleum ether	10.09 ± 0.01	10.14 ± 0.03	11.28 ± 0.15	12.07 ± 0.06	00 ± 0.00	22.1 ± 0.89
	Methanol	12.09 ± 0.08	15.11 ± 0.01	16.13 ± 0.02	17.11 ± 0.09	00 ± 0.00	
	Aqueous	10.03 ± 0.03	10.09 ± 0.07	12.24 ± 0.03	13.23 ± 0.02	00 ± 0.00	
Shigella flexneri	Petroleum ether	10.09 ± 0.07	11.12 ± 0.02	12.09 ± 0.08	14.14 ± 0.02	00 ± 0.00	25.8 ± 0.75
	Methanol	10.15 ± 0.02	12.07 ± 0.06	13.07 ± 0.06	15.13 ± 0.03	00 ± 0.00	
	Aqueous	10.02 ± 0.04	10.07 ± 0.06	10.38 ± 0.17	11.10 ± 0.09	00 ± 0.00	
Salmonella typhimurium	Petroleum ether	10.00 ± 0.00	10.06 ± 0.06	10.08 ± 0.07	10.15 ± 0.02	00 ± 0.00	24.5 ± 0.86
	Methanol	9.60 ± 0.53	11.19 ± 0.03	13.30 ± 0.30	14.19 ± 0.02	00 ± 0.00	
	Aqueous	12 ± 0.08	13.03 ± 0.04	14.93 ± 0.04	15.03 ± 0.04	00 ± 0.00	

*Values in the table are mean ± SD of 3 replicates

Table 3: Minimum Inhibitory Concentration (MIC) of different leaf extracts of *C. auriculata* against pathogenic bacteria

Microorganisms	Petroleum ether	Methanol	Aqueous
	[Minimum Inhibitory Concentration in mg/mL]		
Staphylococcus aureus	50	50	25
Escherichia coli	25	6.25	25
Shigella flexneri	12.5	12.5	50
Salmonella typhimurium	50	12.5	6.25

Figure 1: *Cayratiaauriculata* (Roxb.) Gamble

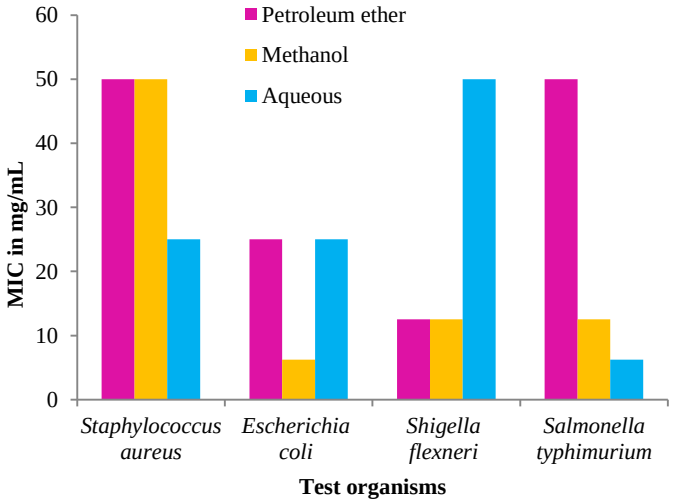

Figure 2: Comparison between Minimum Inhibitory Concentration (MIC) of different leaf extracts of *C. auriculata* against pathogenic bacteria.

Further, the inhibition was found to be greater in methanol extracts, as in the case of the present study. Hence, it can be concluded that both

the species of *Cayratia*, i.e., *Cayratia auriculata* and *Cayratia pedata*, possess antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacterial strains. From this study, it seems that enteric pathogens can be inhibited by various extracts from the leaves of *C. auriculata*.The leaf extract has shown its efficacy by inhibiting almost all bacterial strains tested. The potency of leaf extracts may be attributed to the presence of certain bioactive compounds in them, which play a key role in retarding bacterial pathogens in the gastrointestinal tract. The antibiotic ciprofloxacin, on the other hand, has shown inhibitory activity against the tested bacterial pathogens. But long-term usage of antibiotics results in bacterial resistance, endangering the efficacy of antibiotics against those bacterial pathogens. Therefore, herbal drugs, being inexpensive with no detrimental effects, can be used as natural antibiotics for treating gastrointestinal disorders. Further, many underutilized leafy vegetables with hidden nutritional and medicinal properties exist in nature, which need immediate documentation and investigation to reveal their potential benefits.

Conclusion

The present work authenticates the antimicrobial aspect of *Cayratia auriculata*, which is an ethnomedicinally potent but underutilized leafy vegetable. In addition, the existence of several secondary plant metabolites in plant extracts may be the reason for their effectiveness. All the extracts were found to be more or less effective against all test organisms, revealing their broad-spectral activity. Further, the methanolic extract of *C. auriculata* showed the strongest antibacterial activity in terms of larger zones of inhibition and lower MIC values among the three extracts against the *E. coli* and *Shigella flexneri* bacterial strains. Hence, the leaf extracts of *C. auriculata* can be used as alternative herbal therapy for treating diarrhoea and dysentery caused by different bacterial strains. Additionally, it has the potential for the development of pharmaceuticals and new drugs in the future.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Fair RJ and Tor Y. Antibiotics and bacterial resistance in the 21st Century. Perspect. Medicinal Chem. 2014; 6:25–64.
2. Lingaraju DP, Sudarshana MS, Mahendra C, Rao KP. Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of *Eryngium foetidum* L. (Apiaceae).Indo Am J Pharm Res. 2016; 6(2): 4339-44.
3. Noor N and Satapathy KB. Floristic inventory of leafy vegetables with special reference to their ethnomedicinal uses in Balasore district of Odisha, India. J Indian Bot Soc. 2021; 101 (1&2):131-139.
4. Trias-Blasi A, Chayamarit K, Parnell J. *Cymphostemma auriculatum* (Roxb.) P. Singh & BV Shetty (Vitaceae):Typification and a new generic record confirmed for Thailand. Kew Bulletin. 2015; 70(2):1-7.
5. Tetali S and Karkamkar SP. *Cayratia* sps. of family Vitaceae for utilization in the grape improvement programme. J Plant Sci Res. 2016; 2(2):14-21.
6. Patil US, Deshmukh OS.Traditional ethno-veterinary practices in Betul district (M.P) India. Int J Curr Res Life Sci. 2015; 4(10): 423-428.
7. Fernandez SP, Karim N, Mewett KN, Chebib M, Johnson GAR, Hanrahan JR . Flavan-3-olesters: new agents for exploring modulatory sites on GABAA receptors. Br J Pharmacol.2012; 165(4):965–977.
8. Lalitha S, Anusha D, Murkunde Y, Devaanand V, Maheshkumar K. Phytochemical screening and evaluation of cytotoxicity and acute toxicity of ethanolic leaf extract of *Cayratia auriculata*. *Int J Curr Res* Rev. 2021; 13(13):101-107.
9. Kancherla N, Dakshinamoorthi A, Chitra K, Komaram RB. Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of *Cayratia auriculata* (*In Vitro*). Maedica J Clin Med. 2019; 14(4): 350-356.
10. Lalitha S, Anusha D, Murkunde Y, Devanand V, Maheshkumar K. Anti-Cancer activity of *Cayratia auriculata* ethanolic extracts against Cancer Cell Line A549 - An *in vitro* analysis. Pharmacog J. 2021; 13(2):495-499.
11. Haines HH. The Botany of Bihar and Orissa. 6 parts London. Botanical Survey of India, Calcutta. 1921-1925.
12. Saxena HO and Brahmam M. The Flora of Orissa. Vol. 1-4. Bhubaneswar (Orissa): Regional Research Laboratory and Forest Development Corporation of Orissa,1994-1996.
13. Mooney HF. Supplement to the Botany of Bihar and Orissa.Catholic Press, Ranchi.1950.
14. Harborne JB. Phytochemical Methods ; A Guide to Modern Techniques of Plants Analysis. 3rd Edition.Chapman & Hall, England. 1998.
15. Edeoga HO, Okwu DE, Mbaebre BO. Phytochemical constituent of some Nigerian Medicinal Plants. Afr J Biotechnol. 2005; 4(7):685-688.
16. Mounyr B, Moulay S, Saad KI. Methods for *in-vitro* evaluating antimicrobial activity: A review. J Pharm Anal. 2016; 6(2):71-79.
17. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15(10):7313-7352.
18. Nayak BK, Lazar J. Antimicrobial efficacy of Leaf Extract of *Cayratia pedata* Lam.Vitaceae. Int.J. ChemTech Res.2014; 6(14):5721-5725.