

Antimicrobial and Phytochemical Studies of *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees

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Abstract: The genus *Andrographis* which belongs to the family Acanthaceae comprises about 40 species. Only a few are famous for their use in folk medicine for different health concerns. Carbohydrate, coumarins, glycosides, phenol saponin, steroids, and tannin are present in the methanol extract of *Andrographis paniculata* and *Andrographis echioides*. The efficiency of antimicrobial activity from the aqueous and various solvents extract of ethnomedicines were screened against gram-negative bacteria of *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Vibrio cholera* and gram-positive bacteria of *Bacillus subtilis*, *Lactococcus lactis*, and *Staphylococcus aureus*. The antimicrobial activity of *A. paniculata* showed the maximum activity in ethyl acetate extract among the tested crude extracts such as methanol, aqueous cold and boiled against the tested human pathogen. The *A. echioides* showed maximum activity in ethyl acetate extracts is 30 mm against *B. subtilis* and *E. cloacae*, 28 mm against *S. aureus*, followed by 28 mm against *P. aeruginosa*, 25 mm against *V. cholera* and *S. aureus* in methanol extract, 24 mm against *E. coli* in ethyl acetate extract and 18 mm against *L. lactis* both ethyl acetate and methanol extracts and 19 mm against *P. aeruginosa* in methanol extract. The difference of the antimicrobial activity has dependent on the concentration and types of active principle present in the extracts.

Keywords: *Andrographis paniculata*, *Andrographis echioides*, human pathogenic bacteria, phytochemicals.

I. INTRODUCTION

The World Health Organization (WHO) opines that about 80% of the World's population is dependent on the traditional herbal medicines for their primary health care needs [1]. The practice of transmitting healing knowledge from generation to generation has enabled the consistency of application of given herbs for specific ailments.

The genus *Andrographis* which belongs to the family Acanthaceae comprises about 40 species. Only a few are famous for their use in folk medicine for different health concerns. One of the critical species is *Andrographis paniculata*, commonly known as King of Bitters. It is native to peninsular India and Sri Lanka and also distributed in different regions of Southeast Asia, America, China, Christmas Island and West Indies. It is cultivated because of its well-known medicinal value, and it grows well in most soil types thus it is widely distributed [2]. The aerial parts and roots of *Andrographis* are have been widely used as traditional medicine to treat many diseases in China, India, Thailand and other Southeast Asian countries.

The aerial parts, roots and whole plant of *A. paniculata* have been used for centuries in Asia as a traditional drug for the treatment of various diseases. It has been used by the traditional medical practitioners for inflammation, and intermittent fevers, pyrexia, and stomachaches [3], [4], [5], [6]. The entire plant extract has been used for several applications such as antidote for snake-bite and poisonous stings of some insects, treat dyspepsia, influenza, dysentery, malaria and respiratory infections [3],[4]. The leaf extract is used for the treatment of colic pain, diarrhea, fevers, loss of appetite, infectious disease and irregular stools [7].

In Malaysia, a hot water extraction of the aerial parts is used to treat the common cold, hypertension, diabetes, cancer, malaria, and snakebite [8]. In traditional Chinese medicine, it is seen as the cold-property herb used to rid the body of heat and fever and to dispel toxins from the body [9]. In Ayurvedic medicinal system, tribals of Tamil Nadu, India use this herb for a variety of ailments like dysmenorrhea, leucorrhoea, pre-natal and post-natal care, complicated diseases such as malaria, jaundice, gonorrhoea and general ailments like wounds, cuts, boils and skin diseases [10], [11], [12], [13].

The whole plant of *A. echioides* crushed oil and paste applied on snake bite; also the entire plant dried and powdered, mixed with water and drunk; whole plant decoction is the cure for fever, dysentery, stomachache and liver disorders. Leaves juice used as a febrifuge, cathartic, anthelmintic, laxative, alterative, stomachic, antimalarial; leaf paste applied to cure a headache; leaves decoction given against intestinal worms, constipation, fever, and jaundice. Physical and chemical properties and medicinal uses of this plant are considered similar to those of *A. paniculata*. The demand for the raw materials and derivatives of the *Andrographis* for the green drug industries is satisfied mainly from the wild source. The present study focuses on the comparison of phytochemical and antimicrobial efficiency and to find the potential of Ethnomedicines.

II. MATERIALS AND METHODS

2.1 Plant Materials:

Andrographis paniculata (Burm.f.) Wall. ex Nees in Wall. Pl. Asiat. Rar.3:116, 1832; Wight, Ic.t. 518, 1842; Clarke in Hook.f. Fl. Brit. India 4: 501. 1884; Gamble, Fl. Pres. Madras 2:734.1957 (repr.ed.). *Justicia paniculata* Burm.f. Fl. Ind. 9. 1768. Family: Acanthaceae, Siryaa nangai/ Nil vembu/ Milagai nangai in Tamil, Green chirayta, King of bitters in English.

Andrographis echioides (L.) Nees in Wall., Pl. Asiat. Rar. 3: 117. 1832; Hook. f., Fl. Brit. India 4: 505. 1884; Gamble, Fl. Pres. Madras 1051(736). 1924; Vajr., Fl. Palghat Dist. 341. 1990. *Justicia echioides* L., Sp. Pl. 16. 1753. *Neesiella echioides* (L.) Sreem., Phyt. 15: 271. 1967. *Indoneesiella echioides* (L.) Sreem., Phytologia 16: 466. 1968; Manilal & Sivar., Fl. Calicut 215. 1982; Mohanan, Fl. Quilon Dist. 304. 1984; Antony, Syst. Stud. Fl. Kottayam Dist. 303. 1989; Anil Kumar *et al.*, Fl. Pathanamthitta 380. 2005; M. Mohanan & Henry, Fl. Thiruvananthapuram 348. 1994; Sivar. & Mathew, Fl. Nilambur 504. 1997; Mohanan & Sivad., Fl. Agasthyamala 508. 2002; Sunil & Sivadasan, Fl. Alappuzha Dist. 540. 2009. Family - Acanthaceae, locally known as Gopuram tangi in Tamil, False Water Willow in English.

The plant materials are collected from the Government Arts College for Men Campus, Krishnagiri and allowed to dry in the shade. The shade-dried plant materials were used in this study.

2.2 Extraction:

The plant materials (*Andrographis paniculata* and *Andrographis echioides*) were cut into pieces, shade-dried, coarsely powdered and extracted with ethyl acetate and followed by methanol using a Soxhlet apparatus. The extracts so collected and distilled off on a water bath at atmospheric pressure, then the final traces of the solvents were removed *in vacuo* [14].

2.3 Qualitative Analysis of Phytochemical Constituents:

The solvent (ethyl acetate and methanol) extracts of plant materials were tested for preliminary phytochemical screening [15], [16], [17] and the observations were recorded.

2.4 Antimicrobials study:

Seven human pathogenic bacterial strains *Bacillus subtilis*, *Escherichia coli*, *Enterobacter cloacae*, *Lactococcus lactis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Vibrio cholerae* were obtained from the Department of Microbiology, Government Arts College for Men, Krishnagiri, Tamil Nadu and used for antimicrobial studies. The strains were kept at 4°C on agar slant and subculture at 37°C for 24 hr. in nutrient agar (Himedia, Mumbai) before susceptibility tests.

2.5 Determination of the antimicrobial activity of aqueous extracts of the plant:

The 20 gm of plant materials were weighed individually; chopped and divided into two portions, each portion was crushed by grinding in a mortar and transferred to a suitable glass bottle, and a 50 ml of distilled water was added. One glass bottle with the extract was boiled (100°C) for 20 min and the second was mechanically shaken (200 rpm) in a cold

condition for two hours. The extracts were filtered off using cheesecloth, followed by 0.45 μ filter paper and transferred into a sterile closed container. The crude extract was considered as 100 % extract. By adding sterile distilled water, 50 % of the extract was prepared [18].

2.6 Screening of antimicrobial activity:

Agar well diffusion method [19], [20] was followed to determine the antimicrobial activity. Two wells (6 mm diameter) were made in each of these plates using sterile cork-borer. Nutrient agar plates were swabbed (sterile cotton swabs) with 8 hours old broth culture of respective bacteria. About 0.3 ml of 100 % and 50 % aqueous extract and different concentrations of plant solvent extracts (20, 10, 5 and 2.5 mg/ml of ethyl acetate and methanol extract) were added using sterile dropping pipettes into the well and allowed to diffuse at room temperature for 2 hours. The Petri plates were incubated at 37°C for 18 - 24 hr. and the diameter of the inhibition zones was recorded. Triplicates were maintained, and the experiments were repeated thrice, and the average value of the zone of inhibition was recorded for antimicrobial activity.

III. RESULTS AND DISCUSSION

3.1. The solubility of phytochemicals in solvents:

The solvent extractive values revealed the presence of secondary metabolites such as steroids, alkaloids, terpenoids, fat-soluble hydrocarbons, flavonoids, phenols, etc. These values can be used for fixing pharmacopoeial standards along with other pharmacognostic and microbial parameters.

The preliminary phytochemical characters can be used as diagnostic tools in the correct identification of plants. The adulterants, if any in plant material can also be identified easily by these studies. The quantity of the methanol extract (9.7%) is higher than that of the Ethyl acetate (5.9 %) extract of *Andrographis paniculata*. Similarly, the amount of the methanol extract (8.2%) of *Andrographis echioides* is higher than the ethyl acetate extract (5.2 %). The color, appearance, and solubility of extracts indicate the quantity and nature of constituents in the extract [21].

3.2. Preliminary Phytochemical Analysis:

All the crude extracts (ethyl acetate and methanol) of selected ethnomedicines showed the alkaloid, anthraquinone, and terpenoid (Table – 1).

Table 1: Preliminary Phytochemical data of the selected Ethnomedicines

S.No.	Test	<i>A. paniculata</i>		<i>A. echioides</i>	
		EtOAc	MeOH	EtOAc	MeOH
1.	Alkaloid	+	+	+	+
2.	Anthraquinone	+	+	+	+
3.	Carbohydrate	-	+	-	+
4.	Coumarins	-	+	-	+
5.	Flavonoid	+	+	+	+
6.	Glycosides	-	+	-	+
7.	Phenol	-	+	-	+
8.	Quinone	+	-	+	-
9.	Saponin	-	+	-	+
10.	Steroids	-	+	-	+
11.	Tannin	-	+	-	+
12.	Terpenoid	+	+	+	+

+ present; - absent; EtOAc - Ethyl acetate; MeOH – Methanol

Carbohydrate, coumarins, glycosides, phenol, saponin, steroids, and tannin are present in the methanol extract of ethnomedicines. Quinone is present only in ethyl acetate extract of *A. paniculata*, *A. echioides*, these are mainly colour reaction, and the results may be used as standard parameters in quality control.

3.3 Antimicrobial activity:

The aqueous and various solvents (ethyl acetate and methanol) bio-crude extracts from selected Ethnomedicines (*A. paniculata*, *A. echioides*) were screened for their efficiency of antimicrobial activity. The crudes were tested against gram-negative bacteria of *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Vibrio cholera* and gram-

positive bacteria of *Bacillus subtilis*, *Lactococcus lactis*, and *Staphylococcus aureus*. Among the different solvent extracts, the antimicrobial activity was high in the ethyl acetate and followed by the methanol. The antimicrobial activity was much less in the aqueous extract. The efficiency of antimicrobial activity depends on the types and concentration of bioactive principles present in the tested extracts.

3.3.1 *Andrographis paniculata*:

The aqueous extract showed activity in both gram-positive and gram-negative bacteria except *B. subtilis* and *P. aeruginosa* (Table – 2). The ethyl acetate and methanol extract were inhibited both gram-negative and gram-positive bacteria; Graph - 1).

The cold aqueous extract showed maximum inhibition zone of 10 mm in 100% and 8 mm in 50 % concentrations against *E. cloacae*. Minimum inhibition zone of 8 mm in 100 % and 7 mm in 50 % concentration were observed in *V. cholerae* (Table – 2).

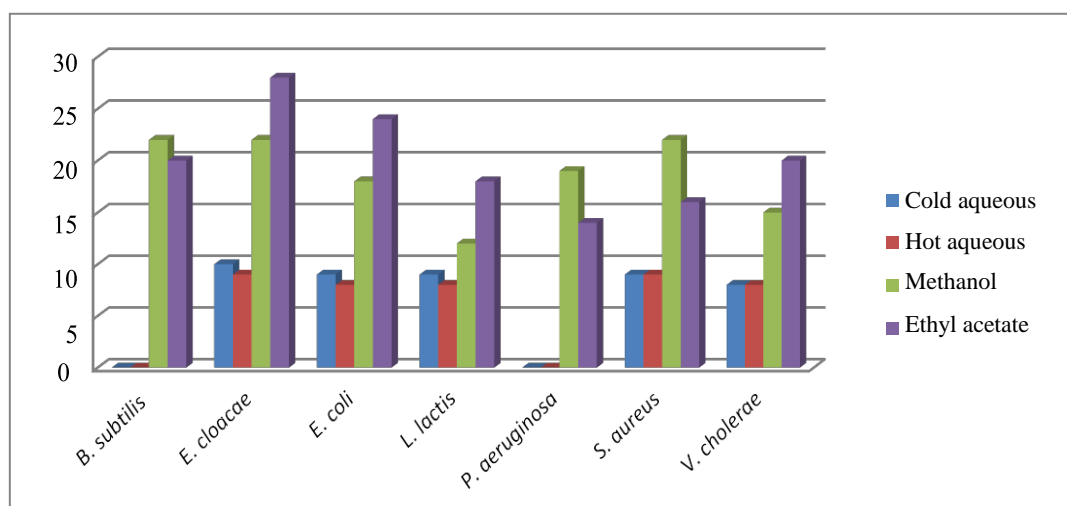
The boiled aqueous extract showed maximum inhibition zone of 9 mm in 100% and 8 mm in 50 % concentrations against *E. cloacae*. Minimum inhibition zone of 8 mm in 100 % and 7 mm in 50 % concentration was observed in *E. coli*, *L. lactis* *V. cholerae*. The antibacterial activity of methanol extract (Table - 2) showed maximum inhibition zone of 22 mm in 20mg/ml against *B. subtilis*, *E. cloacae* and *S. aureus*; 20 mm in 10 mg/ml against *B. subtilis* and *E. cloacae*; 18 mm in 5 mg/ml against *E. cloacae* and 15 mm in 2.5 mg/ml against *S. aureus* (Table – 2; Graph - 1).

The ethyl acetate extract showed the maximum inhibitory activity was 28 mm in 20 mg/ml against *E. cloacae* followed by 24 mm in 20mg/ml against *E. coli*. Minimum inhibitory zone of 14 mm in 20 mg/ml against *P. aeruginosa* was recorded. The Maximum inhibitory zone (22mm) was seven in 10 mg/ml concentrations against *B. subtilis* and *E. cloacae*, and minimum inhibitory zone (10 mm) was recorded in 2.5 mg/ml concentration against *P. aeruginosa* and *S. aureus* (Table – 2; Graph - 1).

Table 2: Antimicrobial activity aqueous, ethyl acetate and methanol extracts of *A. paniculata*

Test organism	Cold Aqueous		Boiled Aqueous		Methanol extract (mg/ml)				Ethyl acetate extract (mg/ml)			
	100	50	100	50	20	10	5	2.5	20	10	5	2.5*
<i>B. subtilis</i>	-	-	-	-	22	20	15	12	20	22	16	12
<i>E. cloacae</i>	10	8	9	8	22	20	18	14	28	22	20	18
<i>E. coli</i>	9	8	8	7	18	17	14	10	24	20	16	12
<i>L. lactis</i>	9	8	8	7	12	12	10	10	18	12	10	10
<i>P. aeruginosa</i>	-	-	-	-	19	12	10	8	14	10	8	8
<i>S. aureus</i>	9	8	9	8	22	19	15	15	16	14	12	10
<i>V. cholerae</i>	8	7	8	7	15	18	9	9	20	18	15	11

* Zone of inhibition in mm



Graph 1: Antimicrobial activity of *Andrographis paniculata* against human pathogens

The ethnomedicines *A. paniculata* showed maximum activity in ethyl acetate extract among the tested crude extracts such as methanol, aqueous cold and boiled against the tested human pathogen. The better zone of inhibition was observed in the ethyl acetate extracts is 28 mm against *E. cloacae*, 24 mm against *E. coli*, followed by 22 mm against *S. aureus* in methanol extract, 22 mm against *B. subtilis*, 20 mm against *V. cholera* in ethyl acetate extract and 19 mm against *P. aeruginosa* in methanol extract. The aqueous extracts show only minimum antimicrobial activity compare to methanol and ethyl acetate extracts.

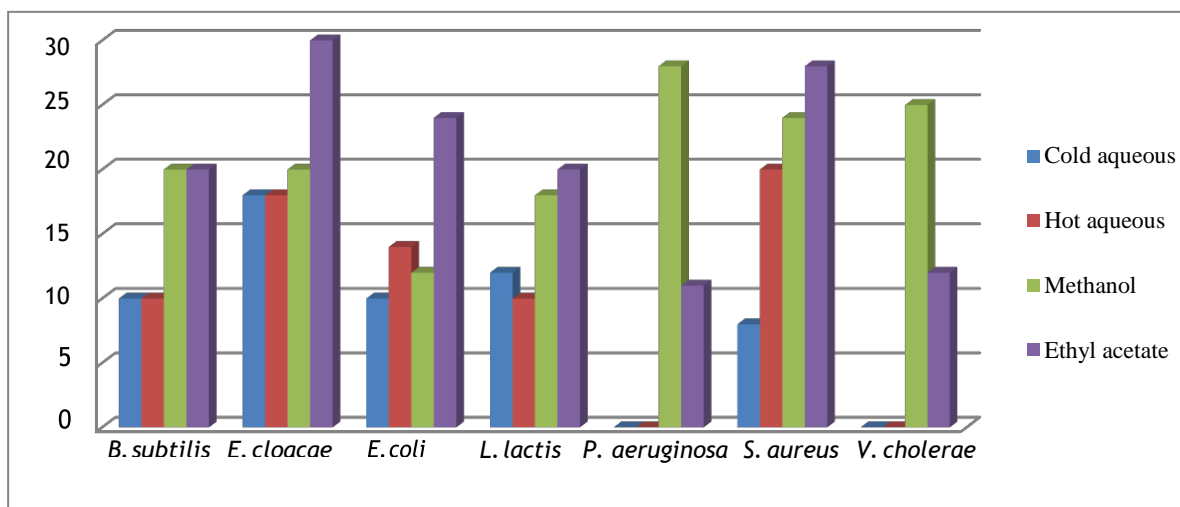
3.3.2 *Andrographis echioides*:

The cold and boiled aqueous extract showed activity in both gram-positive and gram-negative bacteria except *P. aeruginosa* and *V. cholera* (Table - 3; Graph - 2). The ethyl acetate and methanol extract inhibited both gram-negative and gram- positive bacteria.

Table 3: Antimicrobial activity aqueous, ethyl acetate and Methanol extracts of *A. echioides*

Test organism	Cold Aqueous		Boiled Aqueous		Methanol extract (mg/ml)				Ethyl acetate extract (mg/ml)			
	100	50	100	50	20	10	5	2.5	20	10	5	2.5*
<i>B. subtilis</i>	8	10	8	10	20	26	18	15	30	26	15	14
<i>E. cloacae</i>	18	12	18	12	20	18	15	12	28	30	14	12
<i>E. coli</i>	10	10	14	12	12	12	12	10	24	22	20	18
<i>L. lactis</i>	12	10	10	10	18	14	12	10	18	18	20	18
<i>P. aeruginosa</i>	-	-	-	-	28	28	20	18	11	10	9	8
<i>S. aureus</i>	8	8	20	18	24	18	10	10	28	22	20	18
<i>V. cholerae</i>	-	-	-	-	25	22	20	18	12	10	8	-

* Zone of inhibition in mm



Graph 2: Antimicrobial activity of *Andrographis echioides* against human pathogens

The cold aqueous extract showed maximum inhibition zone of 18 mm in 100% and 12 mm in 50 % concentrations against *E. cloacae*. Minimum inhibition zone of 8 mm in 100 % and 50 % concentrations were observed against *S. aureus*. The boiled aqueous extract showed maximum inhibition zone of 20 mm in 100% and 18 mm in 50 % concentrations against *S. aureus*. Minimum inhibition zone of 10 mm in 100 % and 8 mm in 50 % concentration were observed in *B. subtilis*.

The antibacterial activity of methanol extract (Table - 3) showed maximum inhibition zone of 28 mm in 20mg/ ml, and 10 mg/ ml against *P. aeruginosa* followed by 26 mm in 10 mg/ml against *B. subtilis*. The minimum inhibitory zone of 12 mm in 20 mg/ml against *E. coli*. The low concentration of 2.5 mg/ml showed a maximum zone of inhibition (18 mm) was recorded against *V. cholera* (Table – 3; Graph - 2).

The ethyl acetate extract showed the maximum inhibitory activity is 30 mm in 20 mg/ml against *B. subtilis* followed by 28 mm in 20mg/ml against *E. cloacae* and *S. aureus*. Minimum inhibitory zone of 11 mm in 20 mg/ml against *P. aeruginosa* was observed. The Maximum inhibitory zones of 30 mm in 10 mg/ml against *E. cloacae* followed by 26 mm in 10mg/ml against *B. subtilis* were recorded. The low concentration of ethyl acetate extract shows a

predominant zone of inhibition (20 mm) in 5 mg/ml and 18 mm in 2.5 mg/ml against *E. coli*, *L. lactis* and *S. aureus*. There was no antimicrobial activity recorded in 2.5 mg/ml concentration of ethyl acetate extract against *V. cholera* (Table – 3; Graph - 2).

The plant *A. echioides* showed maximum activity in ethyl acetate extract among the tested crude extracts such as methanol, aqueous cold and boiled against the tested human pathogen. The better zone of inhibition was observed in ethyl acetate extracts is 30 mm against *B. subtilis* and *E. cloacae*, 28 mm against *S. aureus*, followed by 28 mm against *P. aeruginosa*, 25 mm against *V. cholerae* *S. aureus* in methanol extract, 24 mm against *E. coli*, in ethyl acetate extract and 18 mm against *L. lactis* both ethyl acetate and methanol extracts and 19 mm against *P. aeruginosa* in methanol extract. The aqueous extracts show only minimum antimicrobial activity compare to methanol and ethyl acetate extracts except for *E. cloacae*. The difference of the antimicrobial activity has dependent on the concentration and types of active principle present in the extracts.

Medicinal plants are the primary sources of new drug and may initiate an alternative to the usual medicines. The therapeutic and aromatic plants are used on a vast, full scale in medicine against drug-resistant bacteria [22]. Singha [23] reported that aqueous extract, andrographolides and arabinogalactan proteins from *A. paniculata* were evaluated against the human pathogenic organism. The antibacterial activities of 75% methanol extract from *A. paniculata* leaves were observed only against the *S. aureus* ATCC 25923. The extract was not found active against *Proteus vulgaris* and *E. coli* ATCC 25922 [24]. This study is positively supported by our research of *S. aureus* against methanol extract. But, controversy observation was observed against *E. coli*. Our study, maximum zone of growth inhibition by ethyl acetate and methanol extract against *S. aureus*, *E. cloacae* is following the previous studies reporting that 75% methanol is a better solvent for extraction of antimicrobial substances from medicinal plants than other concentration of methanol as well as water and hexane. Geetha and Catherine [25] suggested the higher dose (200 mg) of methanol extract inhibited the growth of *Aeromonas hydrophila*, *E. coli*, *P. vulgaris*, *Salmonella typhi* and *S. aureus* with the zone of inhibition ranging from 10 - 16 mm. None of the extracts have inhibited the growth of *Pseudomonas aeruginosa*. In our study, the low concentration of ethyl acetate and methanol extract showed a maximum zone of inhibition against the tested pathogen. This result is similar to earlier observation of many researchers [26], [27], [28], [29].

Similarly, the *A. paniculata* extracts exhibited a varying degree of inhibitory activity against the growth of all the microorganisms tested except *Pseudomonas aeruginosa* [30]. This result was positively correlated with many of the researchers who already reported that *A. paniculata* as a potent antimicrobial activator. Mishra *et al.* [24] said that 75% methanol extract of *A. paniculata* leaves was found to be active against *S. aureus*, *E. faecalis*, and *M. tuberculosis*. Zaidan *et al.* [31] have reported that the water extracts of *A. paniculata* possess a potential antibacterial activity towards both gram positive and gram-negative bacteria. Humnabadkar and Kareppa [32] reported the aqueous extracts of *A. paniculata* showed maximum antibacterial activity against *S. aureus* and *P. aeruginosa*. Hosamani *et al.* [33] have reported that the acetone and alcohol extracts of *A. paniculata* with higher inhibitory activity against *B. subtilis* and *S. aureus* and also conducted on other plants showed a positive result on antimicrobial activity. Mahesh and Satish [34], showed a study on antimicrobial activity of methanol extracts of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritiana* and reported the useful antibacterial activity against *E. coli*, *P. fluorescens*, *B. subtilis*, *Xanthomonas axonopodis* and *S. aureus*.

The methanol extract of *A. echioides* has shown to have excellent antibacterial activity against gram-negative bacteria in high concentration. In comparison to the positive control drug Ampicillin, the antibacterial activity of this plant extract is not very significant against gram-positive bacteria [35] except *S. aureus*. The presence of Flavanoids in the ethanol extract of our preparation can be responsible for the antibacterial action against gram-negative organisms. A lot of factors like the binding capacity, chelation of iron and proteins of the bacterial cell membranes are suggested antibacterial mechanisms of the phytochemicals like tannins [36].

IV. CONCLUSION

The present study is revealed that the solvent extracts of *A. paniculata* and *A. echioides*, expressed high antimicrobial activity than aqueous extract and also confirmed that the number of active principles soluble mainly in a low polar solvent. This investigation is strongly recommended that the ethnomedicines to entire consumed material in the form of powder / isolated the active principles for controlling disease rather than the water extracts. Further study is essential, because of the solvent extracts of ethnomedicines containing a lot of useful active principles to be isolated and to discover new drugs for curing of emerging diseases.

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