



Evaluation of phytochemical analysis and antibacterial activity of *Andrographis echiioides* (L) nees (Green chiretta)

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Abstract

Andrographis echiioides (L) is a valuable medicinal plant belongs to Acanthaceae family. The whole plant is having highly medicinal value such as the leaf juice of this plant can be used to cure fever. It is used to treat a very long list of illnesses and conditions. The plant extract is used to treat liver diseases, fever, fertility problems, bacterial, malarial, helminthic, fungal infections and diarrhoea and it also possesses larvicidal property. Leaf juice boiled with coconut oil is used to control falling and graying of hair. Qualitative phytochemical analysis revealed the presence of alkaloids, terpenoids, steroids, flavonoids, phenols, tannins, glycosides, carbohydrates, amino acids and saponins. The antibacterial activity of different solvent extracts by the micro-dilution bio assay method revealed that *Andrographis echiioides* root, stem and leaves extract possess bioactive compounds, which inhibits the growth of microorganisms. Among the six pathogens tested the Gram negative bacteria *Klebsiella pneumoniae* and *Vibrio cholerae* was most susceptible to the extracts and the rest of the pathogens showed almost similar kind of MIC values.

Keywords: phytochemical analysis, antibacterial activity, secondary metabolites, zone of inhibition, *Andrographis echiioides* and green chiretta

Introduction

People are dependent upon plants for their foods, shelter and medicine since prehistoric time. They used the plants for their daily life although chemical constituents of those plants were unknown at that time. The tribal people have much more knowledge than others. Now a days tribal medicines developed to a great extent (Roy *et al.*, 2020) [14]. Plants drugs are traditionally utilized in customary therapeutic frameworks; ethnomedicine, folk remedy, and herbalism give a rational and obvious source of contenders for focused identification of lead substances with unique structures, combinations, and mechanisms of action (Das *et al.*, 2019) [1]. The genus *Andrographis* is a native of India contains 28 species of small annual shrubs essentially distributed in tropical Asia (Jeevanantham and Zahir, 2018) [5]. The plants from genus *Andrographis* are used in goiter, liver diseases (Nadkarni and Nadkarni, 1976) [9] fever, fertility problems, bacterial (Qadrie *et al.*, 2009) [12], malarial, helminthic, fungal, diarrhea and larvicidal disorders (Jeevanantham and Zahir, 2018) [5]; (Nazar *et al.*, 2009) [10]. *Andrographis echiioides* leaf juice boiled with coconut oil is used to decrease the falling and graying of hair (Kanchana and Rubalakshmi, 2014) [6]. Leaf, stem, and root extract of *Andrographis* spp. were proved to possess anti-bacterial, anti-fungal, and antihelminthic activities (Qadrie *et al.*, 2009) [12].

Scientific classification

Class: Dicotyledonae

Subclass: Gamopetalae

Series: Bicarpellatae

Order: Personales

Family: Acanthaceae

Genus: *Andrographis*

Species: *echiioides*

Synonyms: *Justicia echiioides*, *Indoneesiella echiioides*

Materials and Methods

Plant material

Andrographis echiioides (L) was collected from Annamalai University, Annamalai Nagar, Chidambaram during the month of January 2021. The aerial parts of the plant samples were separated, and first washed under running water to remove the soil particles, then washed with distilled water, cut into small pieces, and shade dried. The dried parts were ground to a fine powder using a mixture grinder.

Preparation of Extracts

Dried finally powdered samples (5g) were extracted for 24 h in 50 ml of hexane, chloroform, ethyl acetate and methanol separately at room temperature under dark condition. The extraction was twice repeated and filtered using Whatman No. 1 filter paper. Each filtrate was concentrated to dryness by evaporation stored at refrigerator until analysed.

Qualitative Analysis of Phytochemicals

The different extracts of *Andrographis echiioides* were used for qualitative phytochemical studies like alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, glycosides and tannins, carbohydrates and amino acids (Harborne, 1973; Trease and Evans, 1989) [73, 15].

Test for alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's test

A fraction of each of the extracts was treated with Mayer's reagent (1.36 g mercuric chloride and 5g of potassium iodide in 100 mL of distilled water). Development of yellow coloured precipitate indicates the presence of alkaloids.

Test for Terpenoids**Copper acetate test**

A fraction of each of the extracts was dissolved in water and treated with 3-4 drops of copper acetate solution. Development of emerald green colour indicates the presence of terpenoids.

Test for Steroids

Salkowski's test: A fraction of each of the extracts was treated with chloroform and filtered. The filtrates were treated with 3 to 4 drops of Con. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of steroids.

Test for Flavonoids

Alkaline reagents test: A fraction of each of the extracts was treated with 3 to 4 drops of sodium hydroxide solution. Development of dense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test for Phenols

Ferric chloride test: A fraction of the extracts was treated with a few drops of ferric chloride solution. Development of green colour indicates the presence of phenols.

Test for Tannins

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Development of white precipitate indicates the presence of tannins.

Test for glycosides: Extracts were hydrolysed with dilute HCL, and then subjected to test for glycosides.

Modified borntreger's test: A fraction of the extract was treated with ferric chloride solution and immersed in water bath for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The layer of benzene was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonial layer indicates the presence of glycosides.

Test for Carbohydrates

A fraction of each of the extracts were dissolved separately in 5 ml distilled water and filtered. The filtrates were used to test for the detection of carbohydrates.

Molisch's Test

Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Development of the violet ring at the junction indicates the presence of carbohydrates.

Test for Amino Acids

Xanthoprotein test: The plant extracts were treated with a few drops of Conc. Nitric acid. Development of yellow colour shows the presence of proteins.

Test for Saponins

Froth test: A fraction of the extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Development of 1 cm thick layer of foam indicates the presence of saponins.

Antibacterial Activity

Minimum inhibitory concentrations (MIC) of compounds for antibacterial activity were determined using the microdilution bioassay as described by Eloff (1998) [3]. Overnight cultures (incubated at 37°C in a water bath with an orbital shaker) of Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*,) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Vibrio cholerae*) bacterial strains were diluted with sterile Mueller-Hinton (MH) broth to give final inocula of approximately 10⁶ CFU/ml (colony forming units). The different extracts were re-suspended in respective solvents to known concentrations (50mg/ml). One hundred microliters of each extract were serially diluted two-fold with sterile distilled water in a 96-well microliter plate for each of the six bacterial strains. A similar two-fold serial dilution of Streptomycin (Sigma Aldrich) (0.1mg/ml) was used as a positive control against each bacterium. One hundred microliters of each bacterial culture were added to each well. Water and different extracts used in the present study were included as negative and solvent controls respectively. The plates were covered with parafilm and incubated at 37°C for 24 h. Bacterial growth was indicated by adding 50ul of 0.2 mg/ml p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich) with further incubation at 37°C for 2 h. Since the colourless tetrazolium salt is biologically reduced to a red product due to the presence of active organisms, the MIC values were recorded as the concentrations in the last well in which no colour change was observed after adding the INT indicator. Bacterial growth in the wells was indicated by a reddish-pink colour. The assay was repeated twice with two replicates per assay.

Result and Discussion

In the present study methanolic root extract of *A. echioides* revealed the presence of phytochemicals such as alkaloids, steroids, phenols, glycosides, carbohydrates and saponins. The ethyl acetate extract showed presence of terpenoids, phenols, glycosides and carbohydrates. In the chloroform extract alkaloids, steroids, glycosides and amino acids were present. In the hexane extract flavonoids, phenols and saponins were present (Table 1). The methanolic stem extract of *A. echioides* revealed the presence of phytochemical such as alkaloids, terpenoids, flavonoids, phenols, glycosides, carbohydrates and saponins. In ethyl acetate extract terpenoids, steroids, phenols and carbohydrates were present. In chloroform extract alkaloids, terpenoids, steroids, glycosides carbohydrates and amino acids were present. In hexane extracts alkaloids flavonoids phenols and saponins were present (Table 2). The methanolic leaf extract of *A. echioides* showed more phytochemical such as alkaloids, terpenoids, steroids, flavonoids phenols, tannins glycosides carbohydrates amino acids and saponins when compared to other solvent extracts. The ethyl acetate extract revealed the presence of terpenoids, steroids, phenols, glycosides and carbohydrates. In chloroform extracts alkaloids, terpenoids, steroids glycosides and amino acids were present. In hexane extract flavonoids, phenols and carbohydrates were present (Table 3). The methanolic leaf extract possessed strong phytochemicals when compared to root and stem. Jeevanantham and Zahir (2018) [5] and Rashmi *et al.* (2017) reported similar results on *Andrographis echioides* plant. The results revealed the presence of alkaloids, flavonoids,

steroids, phenols, saponins, amino acids and tannins. Pandey *et al.*, (2019) ^[11] reported similar trend with different results the results showed alkaloids, saponins, terpenoids, flavonoids, protein, glycosides, phytosterol, tannin and oils and fats. Similarly Maheshwari and Vijayarengan, (2020) ^[7], (2021) ^[8] reported that *Tylophora indica* leaf extracts the results revealed the presence of alkaloids, flavonoids, terpenoids, glycosides, saponins, steroids, phenols, carbohydrates and amino acids. In the present investigation, hexane, chloroform, ethyl acetate and methanol extracts of leaves of *Andrographis echioides* were used to study the antibacterial activities against gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Vibrio cholera*) and the results are presented in Tables 4, 5 & 6. In general all the extracts process antibacterial activities. Minimum Inhibitory Concentration (MIC) was estimated for all the compounds over the test pathogens. The results of antibacterial activity of root of *A. echioides* are presented in Table 4. The range of MIC to inhibit bacterial strains are *Staphylococcus aureus* (6.25-1.56 µg/ml), *Escherichia coli* (12.50-3.125 µg/ml), *Pseudomonas aeruginosa* (6.25 -1.56 µg/ml), *Bacillus subtilis*(12.50-3.125 µg/ml), *Klebsiella pneumoniae* (12.50-3.125 µg/ml) and *Vibrio cholerae* (6.25-3.125µg/ml) and the results of antibacterial activity of stem of *A. echioides* are presented in Table 5. The range of MIC to inhibit bacterial strains are *Staphylococcus aureus* (12.50-1.56 µg/ml), *Escherichia coli* (6.25-3.125 µg/ml), *Pseudomonas aeruginosa* (6.25-3.125 µg/ml), *Bacillus subtilis*(12.50-3.125 µg/ml), *Klebsiella pneumoniae* (3.125 -1.56 µg/ml) and *Vibrio cholerae* (6.25-3.125 µg/ml) and the results of antibacterial activity of leaves of *A. echioides* are presented in Table 6. The range of MIC to inhibit bacterial strains are *Staphylococcus aureus* (6.25-3.125 µg/ml), *Escherichia coli* (6.25-3.125 µg/ml), *Pseudomonas*

aeruginosa (3.125 -1.56 µg/ml), *Bacillus subtilis*(3.125-1.56 µg/ml), *Klebsiella pneumoniae* (3.125 -0.78 µg/ml) and *Vibrio cholerae* (3.125-0.78 µg/ml). Among the six pathogens tested the bacteria *Klebsiella pneumoniae* and *Vibrio cholerae* (0.78µg/ml) was most susceptible to the extracts and the rest of the pathogens showed almost similar kind of MIC values. Among the different solvents tested, methanol was most active followed by hexane, chloroform and ethyl acetate extract showed moderate inhibitory effect to the test pathogens. The same plant exhibited antimicrobial properties but the results are differed. Our results are different with the results of Elayaraja *et al.*, (2015) who reported that the highest antibacterial activity was recorded in the crude and methanol extracts of *Indoneesiella echioides* against *Escherichia coli* at diameter of zone inhibition by 10 mm at 150 µL, for *Staphylococcus aureus* bacteria the highest zone of inhibition was observed by 12 mm at 150 µL for methanolic extract, and *Pseudomonas aeruginosa* bacteria the highest zone of inhibition was observed by 18 mm at 150 µL for ethanolic extract, and *Proteus vulgaris* bacteria the highest zone of inhibition was demonstrated by 15 mm at 150 µL for ethanolic extract, when compared with chloramphenicol as standard. The acetone and methanol extracts showed the best zone of inhibition against the severe pathogens such as *S. aureus* (A.E-33 mm, M.E-28mm) and *S. typhi* (A.E-26.67 mm, M.E-28.33 mm) (Roy *et al.*, 2020) ^[14]. It is in contrast with the current experiment. In the present study the antibacterial activity of *A. echioides* showed similar observations as previous studies. Methanol extracts have been shown to result in high extraction yield with strong antibacterial activities (Maheshwari *et al.*, 2021) ^[8]. Maheshwari and Vijayarengan, (2020) ^[7] reported that antibacterial activities of ethanolic extracts of leaves of *Tylophora indica* was more active against *Bacillus subtilis* in microdilution bioassay methods.

Table 1: Phytochemical analysis of root extracts of *A. echioides*

S. No	Phytochemical compounds	Hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	+	-	+
2	Terpenoids	-	-	+	-
3	Steroids	-	+	-	+
4	Flavonoids	+	-	-	-
5	Phenol	+	-	+	+
6	Tannin	-	-	-	-
7	Glycosides	-	+	+	+
8	Carbohydrates	-	-	+	+
9	Amino acids	-	+	-	-
10	Saponins	+	-	-	+

(+) =Positive (Present), (-) = Negative (Absent)

Table 2: Phytochemical analysis of stem extracts of *A. echioides*

S. No	Phytochemical compounds	Hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	+	+	-	+
2	Terpenoids	-	+	+	+
3	Steroids	-	+	+	-
4	Flavonoids	+	-	-	+
5	Phenol	+	-	+	+
6	Tannin	-	-	-	-
7	Glycosides	-	+	-	+
8	Carbohydrates	-	+	+	+
9	Amino acids	-	+	-	-
10	Saponins	+	-	-	+

(+) =Positive (Present), (-) = Negative (Absent)

Table 3: Phytochemical analysis of leaf extracts of *A. echioides*

S. No	Phytochemical compounds	Hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	+	-	+
2	Terpenoids	-	+	+	+
3	Steroids	-	+	+	+
4	Flavonoids	+	-	-	+
5	Phenol	+	-	+	+
6	Tannin	-	-	-	+
7	Glycosides	-	+	+	+
8	Carbohydrates	-	-	+	+
9	Amino acids	-	+	-	+
10	Saponins	+	-	-	+

(+) =Positive (Present), (-) = Negative (Absent)

Table 4: Minimum inhibitory concentrations (MIC- $\mu\text{g/ml}$) of root extract of *A. echioides* against bacterial strains

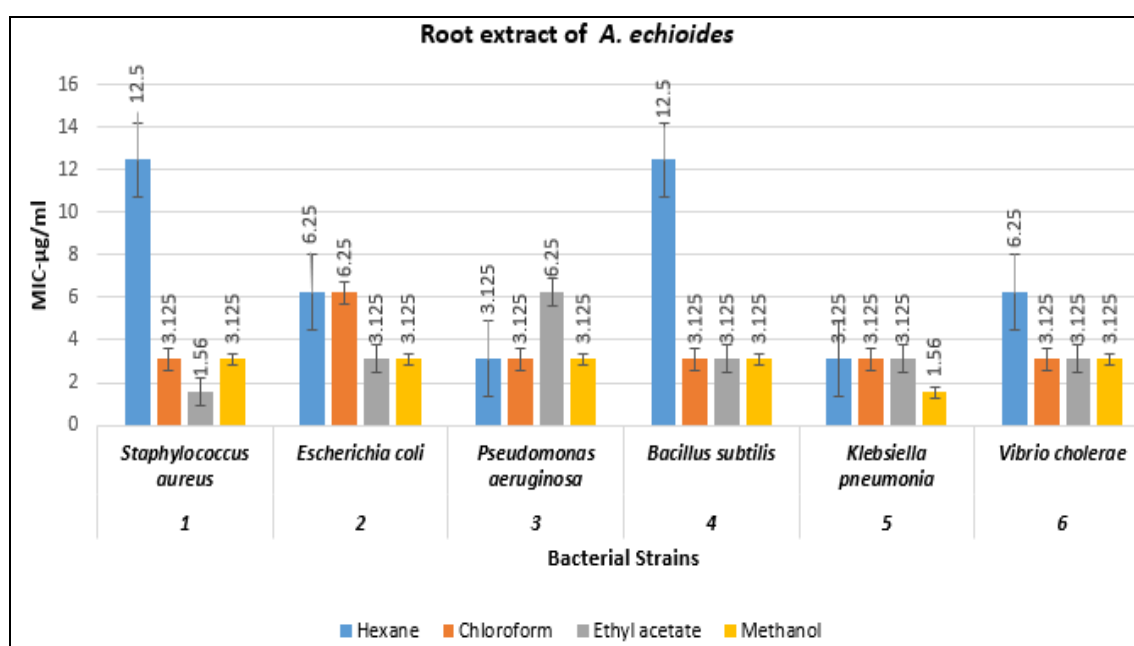
S. No	Pathogens/solvents	Hexane	Chloroform	Ethyl acetate	Methanol
1	<i>Staphylococcus aureus</i>	6.25	3.125	3.125	1.56
2	<i>Escherichia coli</i>	12.50	6.25	3.125	3.125
3	<i>Pseudomonas aeruginosa</i>	6.25	3.125	1.56	1.56
4	<i>Bacillus subtilis</i>	3.125	12.50	3.125	6.25
5	<i>Klebsiella pneumonia</i>	12.50	3.125	3.125	3.125
6	<i>Vibrio cholerae</i>	6.25	3.125	3.125	3.125

Table 5: Minimum inhibitory concentrations (MIC- $\mu\text{g/ml}$) of stem extract of *A. echioides* against bacterial strains

S. No	Pathogens	Hexane	Chloroform	Ethyl acetate	Methanol
1	<i>Staphylococcus aureus</i>	12.50	3.125	1.56	3.125
2	<i>Escherichia coli</i>	6.25	6.25	3.125	3.125
3	<i>Pseudomonas aeruginosa</i>	3.125	3.125	6.25	3.125
4	<i>Bacillus subtilis</i>	12.50	3.125	3.125	3.125
5	<i>Klebsiella pneumonia</i>	3.125	3.125	3.125	1.56
6	<i>Vibrio cholerae</i>	6.25	3.125	3.125	3.125

Table 6: Minimum inhibitory concentrations (MIC- $\mu\text{g/ml}$) of leaf extract of *A. echioides* against bacterial strains

S. No	Pathogens/solvents	Hexane	Chloroform	Ethyl acetate	Methanol
1	<i>Staphylococcus aureus</i>	6.25	3.125	3.125	3.125
2	<i>Escherichia coli</i>	3.125	6.25	3.125	3.125
3	<i>Pseudomonas aeruginosa</i>	3.125	3.125	1.56	1.56
4	<i>Bacillus subtilis</i>	3.125	3.125	3.125	1.56
5	<i>Klebsiella pneumonia</i>	3.125	3.125	3.125	0.78
6	<i>Vibrio cholerae</i>	3.125	3.125	3.125	0.78

**Fig 1:** Minimum inhibitory concentrations (MIC- $\mu\text{g/ml}$) of root extract of *A. echioides* against bacterial strains

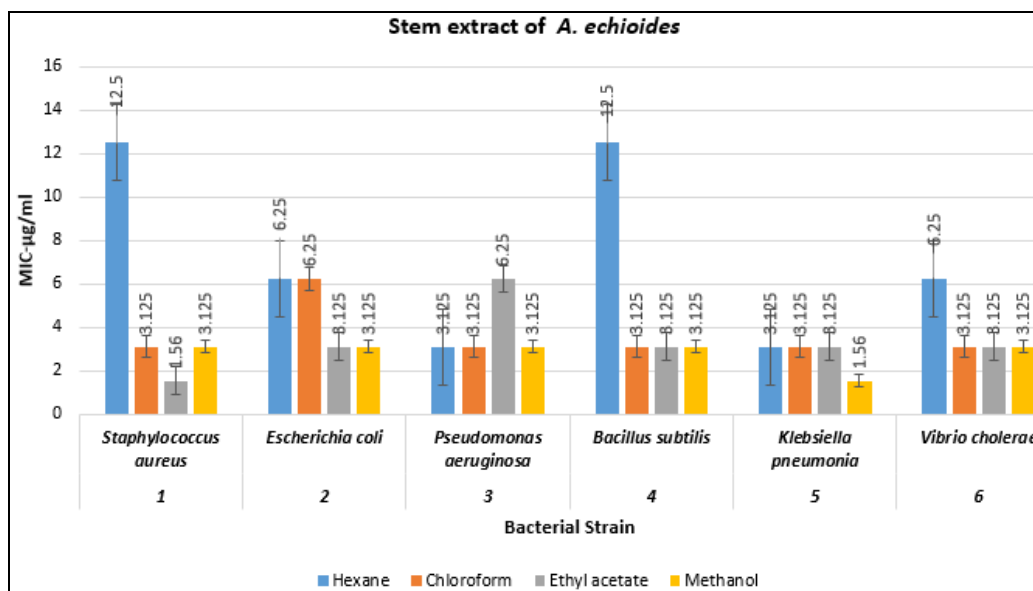


Fig 2: Minimum inhibitory concentrations (MIC-µg/ml) of stem extract of *A. echioides* against bacterial strains

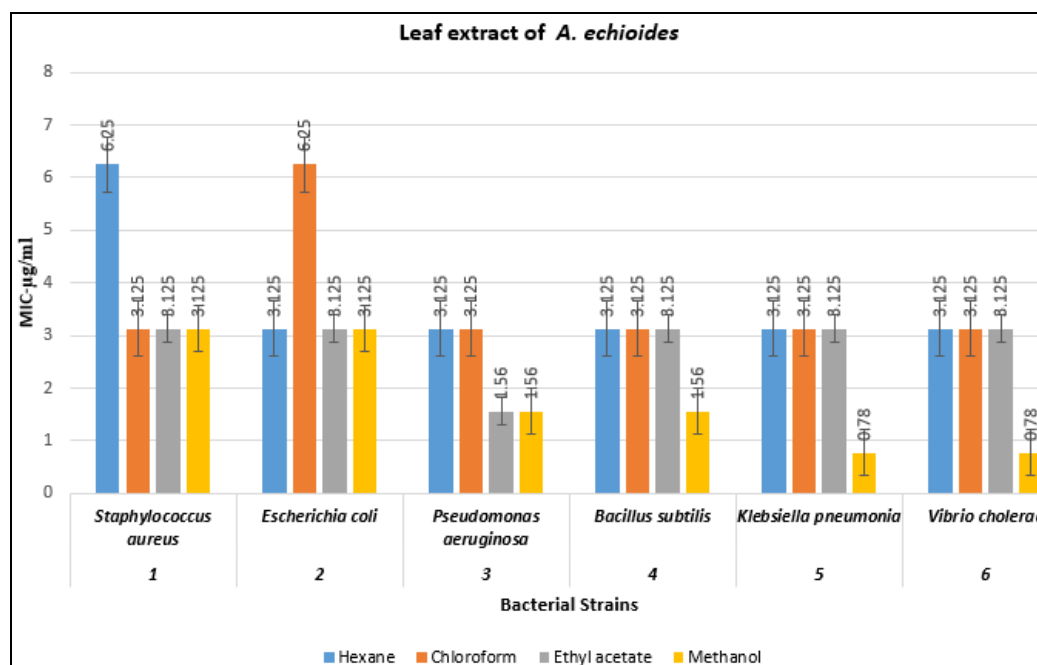


Fig 3: Minimum inhibitory concentrations (MIC- µg/ml) of leaf extract of *A. echioides* against bacterial strains

Conclusion

Andrographis echioides is an important medicinal plant. Phytochemical evaluation revealed the presence of alkaloids, terpenoids, steroids, flavonoids, phenols, tannins, glycosides, carbohydrates, amino acids and saponins in these plants have been claimed to be responsible for their antimicrobial activity. Hence leading to the conclusion that, these plants would serve as sources of novel antimicrobial agents.

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