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Phytochemical screening and antimicrobial activity of the plant extracts Andrographis paniculata against selected microbes

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ABSTRACT

Background: Andrographis paniculata is a medicinal plant with a long history of traditional use in various herbal medicine systems. **Objective:** This study aims to conduct aphytochemical screening and antibacterial activity from leaf, stem and root parts of *Andrographis paniculata*. **Methods:** The preliminary screening was done by cold maceration technique using chloroform, butanol, methanol, ethanol and aqueous solvents. This phytochemical screening showed the presence of alkaloids, glycosides, flavonoids, terpenoids, phenols, tannins and saponins. Antibacterial activity was performed by using well diffusion method from leaf, stem and root butanolic extracts of *A. paniculata*. **Results:** Glycosides are abundantly present in leaf, stem and root explant extracts. Whereas, alkaloids and flavonoids are moderately present in leaf, stem and root explant extracts and followed by less presence of tannins, phenols and saponins. The maximum zone of bacterial inhibition was observed in leaf butanolic extract against of the *E. coli*, for stem butanolic extract against of *B. subtilis* and for root butanolic extract against *B. sphaericus*. **Conclusion:** This study revealed the potential photochemical constituents and antibacterial activity in *A. paniculata*. The presence of these bioactive substances they use in treatment.

Keywords: Phytochemicals, Glycosides, Alkaloids, Flavonoids, Terpenoids, Phenols

INTRODUCTION

Medicinal plants can offer valuable therapeutic effects; they are not a replacement for modern medical treatments when it comes to serious health conditions. The use of medicinal plants should be approached with caution and ideally under the guidance of a qualified health care professional, especially when taken alongside prescription medications. Most of the world's population depends on medicine derived from plants, which has drawn the interest of researchers to invest new medicines ^[1]. According to WHO, plants are the best sources for discovering new drugs.

Andrographis paniculata belongs to the family acanthaceae. *A. paniculata* is a good source of Andrographiles. Andrographiles are active compounds present in the aerial parts of the plant and has treatment for cold, hypertension, diabetes, cancer, malaria and snake bite ^[2]. It acts as anticancer ^[3], antidiarrheal ^[4] anti HIV ^[5], anti hyperglycaemic ^[6], antihepatatis ^[7], anti-inflammatory ^[8], antimicrobial ^[9], antimalarial ^[10], antioxidant ^[11], cytotoxic ^[12], cardiovascular ^[13], Hepatoprotective ^[14], sexual dysfunctions ^[15], immunostimulatory ^[16].

Andrographis paniculata has various pharmacological effects and used as medicinal herb for treating upper gastrointestinal tract, upper respiratory infections, herpes and other chronic diseases ^[17].

Scientific research continues to explore the potential benefits, safety and mechanisms of action of various medicinal plants, integrating traditional knowledge with modern evidence-based medicine ^[18]. Phytochemicals provide many potential benefits to human health when introduced into our diet. Consuming a wide variety of plant-based foods such as fruits, vegetables, whole grains, legumes, nuts, and seeds can provide a broad spectrum of phytochemicals that help maintain a balanced diet.

Plants act as anti-microbial compounds due to the presence of secondary metabolites ^[19]. To overcome the microbial infection problems agents like anti-microbial and resistance towards the infection were used. Anti-microbial activity is a method that prevents the growth of the disease causing microbes. There are different types of anti-microbial agents which play important role in suppression of infections such as anti-fungal, anti-bacterial and anti-viral. Many medicinal plants have been identified as natural anti-microbial compounds for the treatment of bacterial infections ^[20].

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The main purpose of this study is phytochemical screening of leaf, stem and root parts of explant and antibacterial activity of leaf, stem and root butanolic extracts of *Andrographis paniculata*.

MATERIAL METHODS

Collection of Plant material

Fresh leaves, stems and roots of *A. paniculata* plants growing in the medicinal harbour of Department of Biotechnology, Kakatiya University, India were collected for experimental purpose. The plant material was thoroughly washed with running tap water to remove dirt and soil and cut the plant into three parts i.e., roots, stem and leaves.

Preparation of plant extracts

Preliminary phytochemical screening was performed by using cold maceration technique. The powders were extracted with 30ml of butanol, ethanol, methanol, chloroform and aqueous separately. Add 30ml of each solvent to each conical flask containing 3g of shade dried powder in it, next cover with aluminium foil and kept in orbital shaker for 48hrs at 120rpm of 22 ^oC for 48hrs. The extracts were filtered using whatman filter paper and stored at 4 ^oC for further biological application. The presence or absence of secondary metabolites in these extracted samples was determined by phytochemical screening protocols ^[21].

Microbial culture and culture conditions

The butanolic leaf, stem and root extract of *A. paniculata* were screened against gram-positive and gram-negative strains. The test organisms were *Escherichia coli*, *Proteus vulgaris*, *Bacillus sphaericus and Bacillus subtilis* strains were obtained from Microbiology lab, Department of Biotechnology, Kakatiya University, Warangal. The 24hrs old bacterial strains were prepared by inoculating a loopful of original cultures in nutrients broth and incubate overnight at 37^oC. These cultures were maintained at Muller Hinton Agar (MHA) plates at 4 °C for further use.

Agar well diffusion method was performed for screening of antibacterial activity and the antibiotic *streptomycin* (10 µg/ml) was used as standard. The media was prepare and poured at a rate of 15ml each in a petri dish and allowing for solidification for about 5min. A loopful of inoculums was swabbed uniformly onto the media and left them drying. The different concentration of leaf, stem, root extract of (20 µg/ml, 40 µg/ml, 60 µg/ml and 80 µg/ml) and standard (10 µg/ml) was loaded into each well and allowed diffusion of extract. Plates are incubated overnight at 37 °C zone appeared after incubation period and the percent of incubation was determined in millimetres using scale.

Preliminary phytochemical screening

The presence or absence of phytochemical compounds can be determined by performing certain qualitative tests.

Test for alkaloids

Mayers test

Few drops of reagent were added to each 1ml of plant extract (leaf, stem, and root). It is dissolved indifferent solvents and it results in the formation of pale or cream coloured precipitate which indicates the presence of alkaloids.



Figure 1: Represents parts of *Andrographis paniculata*. (a). Leaves (b). Leaves powder (c). Leaves extract (d) Stem (e). Stem powder (f). Stem extract (g). Root (h). Root powder (i). Root extract.

Hagers test

Few drops of Hagers reagent was added to 0.5ml of each plant extract (leaf, stem and root). It is dissolved in different solvents and it results in the formation of yellow colour precipitate indicates the presence of alkaloids.

Tannic acid test

Few drops of 10% tannic acid was added to 0.5ml of each plant extract (leaf, stem and root). It is dissolved in different solvents and it results in the formation of buff colour precipitate indicates the presence of alkaloids.

Test for flavonoids

Alkaline reagent test

1ml of each plant extract (leaf, stem and root) which is dissolved in different solvents and also treated with few drops of NaOH solution, and it results in the formation of yellow colour precipitate and it disappears on addition of dilute acid indicates the presence of flavonoids.

Ferric chloride (FeCl₃ test)

Few drops of Fecl₃wasadded to the plant extract (leaf, stem, root). It shows black colour precipitate indicates the presence of flavonoids.

Test for glycosides

Molisch test

1ml of each plant extract (leaf, stem, root) dissolved in different solvents and treated with a few drops of α - napthol alcohol and 2ml of concentrated H₂SO₄ along the walls of the tube. If brown ring appears it indicates the presence of carbohydrates.

Conc. H₂SO₄ test

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1ml of con. H_2SO_4 is added to 1ml of test solution and allows it to stand for 2minutes. Red colour precipitate indicates the presence of glycosides.

Test for saponins

Foam test

Few drops of each plant extract (leaf, stem, and root) dissolved in different solvents and diluted with distilled water to up to 25 ml and mixed thoroughly for 10 minutes after that it shows a layer of foam which indicates the presence of saponins in the extract.

Test for phenols

FeCl₃ test

In 2 ml of each plant extract add a few drops of FeCl3 and its shows blue colour which indicates the presence of phenols.

Ellagic acid test

In 3 ml of each plant extract (leaf, stem and root) add few drops of 5% (W/V) of sodium nitrate solution and it shows niger brown precipitate which indicates the presence of phenols in the plant extract.

Test for tannins

FeCl₃ test

In 2 ml of plant extract (leaf, stem, root) add few drops of Fecl₃ which shows a blue or green colour precipitate indicates the presence of tannin.

Gelatine test

Table 1: Preliminary screening of Phytochemicals from leaf, stem and root powder extract of A. Paniculata

In 1 ml of each plant extract (leaf, stem, root) add few drops of 1% gelatine solution in 10% NaCl were added. It forms a white precipitate which indicates the presence of tannins.

Alkaline reagent test

In 1 ml of each plant extract (leaf, stem, root) add few drops of NaOH solution, which forms yellow or red colour precipitate indicates the presence of tannins.

Test for steroids/tri-terpenoids

Salkowski test

In 1 ml of each plant extract (leaf, stem, root) add few drops of conc. H2SO4 which forms lower red colour indicates the presence of steroids whereas, yellow colour indicates the presence of triterpenoids.

Test for quinones

Alcoholic KOH test

In 1ml of each plant extract (leaf, stem, root) add few drops of KOH solution. If colour changes from red to blue indicates the presence of quinones.

RESULTS

The preliminary screening of phytochemicals in leaf, stem and roots extracts of *A. Paniculata* showed the abundant presence of glycosides, moderate amount of tannins andalkaloids and followed by flavonoids, quinones, phenols, terpenoids and saponins (Table 1).

Part of the plant	Phytochemical component	Methanol extract	Butanol extract	Chloroform extract	Ethanol extract	Aqueous extract
Leaf	Alkaloids	+	++	++	-	+
	Flavonoids	+	++	+	-	+
	Glycosides	++	++	++	++	++
	Phenols	+	+	-	+	-
	Tannins	+	++	+	++	++
	Steroids/triterpenoids	+	-	-	+	-
	Quinones	-	-	++	-	++
	saponins					+
Stem	Alkaloids	+	++	++	++	+
	Flavonoids	+	+	+	+	++
	Glycosides	++	++	++	++	++
	Phenols	-	+	-	-	-
	Tannins	+	++	++	++	++
	Steroids/triterpenoids	++	++	-	+	+
	Quinones	+	-	+	-	-
	saponins					+
Root	Alkaloids	+	++	++	+	+
	Flavonoids	+	+	+	+	++
	Glycosides	++	++	+	+	++

-	Phenols	-	+	-	+	-
	Tannins	++	++	++	++	++
	Steroids/triterpenoids	++	++	-	+	+
	Quinones	+	-	+	+	-
	saponins					-

Antibacterial activity

The anti-bacterial activity of leaf, stem and root butanolic extract of *A. paniculata* evaluated against gram-positive (*Bacillus subtilis and Bacillus sphaericus*) and gram-negative (*Escherichia coli and Proteus vulgaris*) bacteria by measuring the zone of inhibition (fig 2, 3, 4 and 5).

Anti-bacterial activity for leaf butanolic extract

The anti-bacterial activity of leaf butanolic extract $(20 \ \mu g/ml)$, $40 \ \mu g/ml$, $60 \ \mu g/ml$ and $80 \ \mu g/ml$) and the control streptomycin $(10 \ \mu g/ml)$ was evaluated (fig 2 and graph 1). The butanolic leaf extract showed maximum zone of inhibition at $80 \ \mu g/ml$ when compare to other concentrations. As the plant concentration increase, zone of inhibition also increase. The maximum anti-bacterial activity has showed against of E. *coli* at $(80 \ \mu g/ml)$ concentration (fig 2 and graph 1).

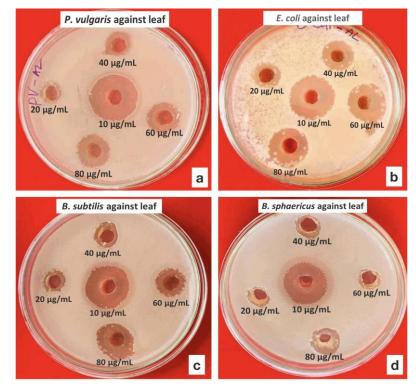
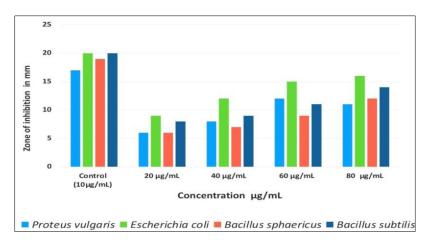


Figure 2: Antibacterial activity of butanolic leaf extracts against different bacterial strains (a). Zone of inhibition against *P. valgaris* (b). Zone of inhibition against *E. coli* (c). Zone of inhibition *against B. subtillis* (d). Zone of inhibition against *B. spheaericus*



Graph 1: Zone of inhibition for butanolic leaf extract of A. paniculata.

Anti-bacterial activity for butanolic stem extract

Anti-bacterial activity of stem butanolic extract ($20 \ \mu g/ml$, $40 \ \mu g/ml$, $60 \ \mu g/ml$ and $80 \ \mu g/ml$) and control streptomycin ($10 \ \mu g/ml$) was evaluated. It is similar to leaf extract were maximum zone of inhibition showed at $80 \ \mu g/ml$ comparing to other concentration. Stem butanolic extract has showed maximum antimicrobial activity by *B. subtilis* ($80 \ \mu g/ml$) concentration (fig 3 and graph 2).

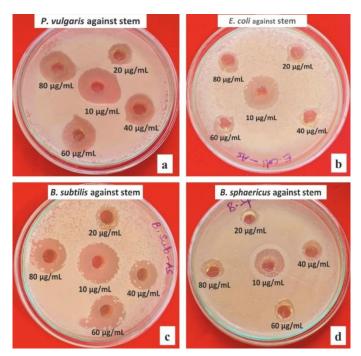
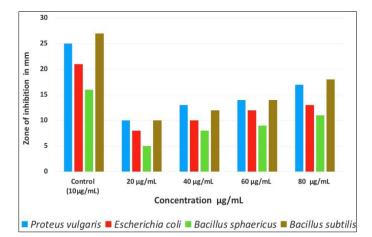


Figure 3: Antibacterial activity of butanolic stem extracts against different bacterial strains (a). Zone of inhibition against *P. valgaris* (b). Zone of inhibition against *E. coli* (c). Zone of inhibition *against B. subtillis* (d). Zone of inhibition against *B. spheaericus*



Graph 2: Zone of inhibition for butanolic stem extracts of A. paniculata

Anti-bacterial activity for butanolic root extract

Anti-bacterial activity of root butanolic extract ($20 \mu g/ml$, $40 \mu g/ml$, $60 \mu g/ml$ and $80 \mu g/ml$) and control streptomycin ($10 \mu g/ml$) was evaluated. It is similar to leaf and stem extract were maximum zone of inhibition showed at $80 \mu g/ml$ comparing to other concentration. Root butanolic extract showed maximum antimicrobial activity by *B. sphaericus* ($80 \mu g/ml$) concentration (fig 4 and graph 3).

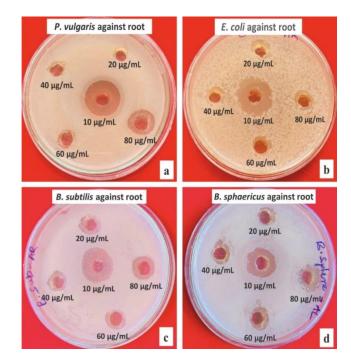
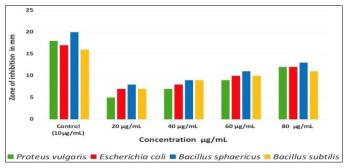


Figure 4: Antibacterial activity of butanolic root extracts against different bacterial strains (a). Zone of inhibition against *P. valgaris* (b). Zone of inhibition against *E. coli* (c). Zone of inhibition *against B. subtillis* (d). Zone of inhibition against *B. spheaericus*



Graph 3: Zone of inhibition for butanolic root extracts of A. paniculata

DISCUSSION

Phytochemical analysis of A. paniculata leaf, stem and root extract were conducted using different solvents such as butanol, methanol, ethanol, chloroform and aqueous extract. This phytochemical analysis resulted in the presence of alkaloids, flavonoids, terpenoids, glycosides, phenols, saponins, tannins, quinones. The analysis shows that glycosides are abundant in butanolic extract (Table 1). The highest anti-bacterial for leaf butanolic extract showed against E. coli at 80 µg/ml concentration, followed by B. subtilis, B. sphaericus and P. vulgaris (fig 2 and graph 1) and similar findings were reported in leaf extracts of Baeckea frutescens [22]. Our results coincided with results reported by shakil ahmed^[23] in leaf extracts of A.paniculata which showed more presence of glycosides and phenols in aqueous and ethanol extracts.

Analysis revealed that the butanolic extract of the stem contained more glycosides showing similarities with the results reported by Shakil Ahmed^[23] glycosides, flavonoids and saponins were abundant in aqueous and ethanol. Stem butanolic extract showed maximum antimicrobial activity against *B. subtilis* at 80µg/ml concentration, followed by *P. vulgaris, E. coli and B. sphaericus* and similar work

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reported in stem extracts of *Nericum indicum Solanum khasianum and Habiscus rosasinensis* ^[24, 25].

The analysis reveals that butanolic extract of root contains more tannins and less amount of phenols and saponins coordinated with results reported by Senguttavan ^[26], shows abundant amount of glycosides, phenols, steroids, tannins, terpenoids in root methanolic extracts of *Hypochaeris radicata*. The maximum zone of inhibition for root butanolic extract showed against B. *sphaericus* at 80 µg/ml concentration, followed by *P. vulgaris*, *E. coli and B. subtilis*and similar findings reported in root extract of *Berberis vulgaris* ^[27].

CONCLUSION

The preliminary phytochemical screening of different parts of the plant such as leaf, stem and root of *A. paniculata* showed the various phytochemical compounds present in it. In qualitative analysis we examined presence of various phytochemical compounds such as alkaloids, flavonoids, glycosides, phenols, terpenoids, quinones, saponins and tannins. The current study showed that various butanolic plant extracts of leaf, stem and root derived from *A. paniculata* had strong antibacterial activity against gram-positive and gram-negative bacteria. These discoveries were also useful in evaluating suggestions made on the use of these plants medicinal properties to treat a variety of illnesses and in leading to the development of new medications.

Consent for publication

All the authors read and agreed that the manuscript be published in its present form.

Availability of data and material

Data and materials related to the present work will be made available on request by the corresponding author

Competing interests

Authors declare that there is no conflict of interest of any kind.

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