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Phytochemical analysis and antibacterial activity of *Andrographis lineata* Nees (Acanthaceae)

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ABSTRACT

Andrographis lineata Nees is an erect herb and has been used as a traditional medicine against many diseases. The present study was to investigate the phytochemical analysis and antibacterial activity of *A. lineata* plant extracts. The methanolic extracts of *A. lineata* showed a variety of phytochemicals such as alkaloids, flavonoids, phenols, tannins, terpenoids etc. The quantitative estimation of major phytochemical constituents revealed maximum amount of phenolic content in the roots and alkaloids in both the stem and leaf. Correspondingly, the highest amount of tannins and terpenoids were recorded in the leaf and flavonoids in the root samples. Furthermore, the plant extracts of *A. lineata* showed antibacterial activity against both Gram-positive and Gram-negative bacterial strains. Comparatively, stem extract was effective against *Streptococcus mutans* (18.19±0.04 mm), leaf extract against *Enterobacter faecalis* (24.13±0.14 mm) and root extract against *Bacillus subtilis* (24.09±0.14 mm) at 30 µg/µL concentration. However, the leaf extract was more effective against *Escherichia coli* (25.13±0.16 mm), *Pseudomonas aeruginosa* (20.20±0.13 mm) and *Klebsiella pneumoniae* (18.18±0.17 mm) at 30 µg/µL concentration. The studies infer that the phytochemical constituents of *A. lineata* have antibacterial properties and these herbs may be considered as the medicinal plant for treating bacterial diseases.

KEYWORDS: *Andrographis lineata*, Phytochemical analysis, Flavonoids, Phenols, Antibacterial activity

INTRODUCTION

The oxidative stress caused by free radicals may induce several metabolic and chronic disorders in the human body (Forman & Zhang, 2021; Chaudhary *et al.*, 2023). Generally, synthetic drugs cause various side effects such as gastrointestinal disturbances, hypoglycemia, and liver dysfunction (Li *et al.*, 2022; Rang *et al.*, 2003). Furthermore, the increase in the prevalence of multiple drug resistance demands the search for new antimicrobials from alternative sources (León-Buitimea *et al.*, 2020). The use of herbal medicinal products and supplements has increased tremendously over the past three decades worldwide in curing various diseases of human beings (Abu-Rabia, 2005; Rashrash *et al.*, 2017; Welz *et al.*, 2018). According to an estimate, about 80% of the population across the world depends on herbal treatment (Parsaeimehr *et al.*, 2017). The rich source of secondary metabolites such as alkaloids, together with terpenoids and flavonoids, etc., made medicinal plants a major source of therapeutic remedies (Bishnu *et al.*, 2009). Secondary metabolites have medicinal properties and are known to possess antioxidant, anti-inflammatory and antimicrobial properties (Umadevi *et al.*, 2013).

Among the genera, *Andrographis* (Acanthaceae), *A. paniculata* is one of the potential sources of traditional medicines for treating various diseases due to its wide spectrum of biological activities (Akbar, 2011; Sareer *et al.*, 2014). Similarly, *A. lineata* Nees is one of the most potential herbs distributed widely in the Deccan, Carnatic, and Nilgiri hills of South India (Gamble, 1956). It is also used in traditional medicine as a substitute for *A. paniculata* (Polash *et al.*, 2017). *A. lineata* is found to be effective in the treatment of various diseases of human beings (Ayyanar *et al.*, 2008; Sangameswaran *et al.*, 2008). Furthermore, it is known to possess antihelminthic, anti-inflammatory, and antipyretic properties (Renugadevi *et al.*, 2013). Pharmacological studies of leaf extracts of *A. lineata* showed antibacterial, diuretic, hepatoprotective, and antidiabetic properties (Sangameswaran *et al.*, 2008). Screening of such active compounds from plants leads to the discovery of new medicinal drugs that have efficient protection against various diseases, including cancer (Sheeja & Kuttan, 2007) and Alzheimer's disease (Mukherjee *et al.*, 2007). Thousands of medicinal plants were identified as valuable resources of antibacterial compounds and are effective in curing bacterial infections (Manandhar *et al.*, 2019). The present study was aimed to screen phytochemical constituents of *A. lineata*,

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and also to evaluate their antibacterial efficiency against a few selected disease causing Gram-positive and Gram-negative bacterial strains.

MATERIALS AND METHODS

Collection of Plant Samples and Preparation of Plant Extracts

The fresh and healthy plants of *Andrographis lineata* were collected from the forest patches of the Environmental Management and Policy Research Institute (EMPRI), Department of Forest, Ecology and Environment, Government of Karnataka, Doresanipalya, Bengaluru, India. The voucher specimen (RBMAL-1) documenting its collection is on deposit at the Department of Botany, Bangalore University, Bengaluru, India. The collected samples were double-washed with distilled water. Further, the root, stem, and leaves of the plants were separated, shade dried, and finely powdered. About 10 g of each powdered plant sample were soaked in 25 mL of test solvents (aqueous, ethanol, methanol, and chloroform) separately for two days (Saxena *et al.*, 2013). Later, these extracts were filtered using Whatman no. 1 filter paper and these filtrates were lyophilized and stored at 4°C until further use. The protocol was repeated for two cycles to complete the extraction of biochemical compounds.

Qualitative Analysis of Phytochemicals

The phytochemicals of the plant extracts such as alkaloids, flavonoids, phenols, coumarins, glycosides, phytosterols, saponins, resins, tannins, terpenoids, and steroids were analyzed using the standard protocol. The phytochemicals found in root, stem, and leaf extracts of *A. lineata* were extracted using different solvents viz. aqueous, ethanol, butanol, methanol and chloroform, separately. The following standard procedures were followed to determine the presence of alkaloids (Mayer's and Wagner's test), flavonoids (Ferric Chloride and Alkaline reagent test), phenols (Ferric chloride test), coumarins, glycosides (Keller Killani's test), phytosterols, saponins (Froth test), resins, tannins and terpenoids (Salkowski test) and steroids (Liebermann-Burchards's test). The qualitative assessment was based on the color development of the resultant solution and was classified as present (+) and absent (-) for each phytochemical analysis (Harborne, 1984).

Quantitative Estimation of Phytochemicals

The amount of phytochemicals found in the methanolic extracts of the leaf, stem, and roots of *A. lineata* was determined. 10 g of the dried powder of each plant material was subjected to Soxhlet extraction. The Soxhlet thimble was fitted with a 250 mL round bottom flask containing 200 mL of 90% methanol. The flask was heated for 18-20 hrs and the extraction temperature was maintained at 50 °C. After extraction, the contents were filtered and dried. A known amount of dried plant extract was collected in an airtight vial and stored at 4 °C until further use (Azwanida, 2015). About 100 mg of the plant extract was

dissolved in 100 mL of methanol, vortexed, and filtered using Whatman no.1 filter paper. The clear filtrates were collected and stored in an airtight vial for phytochemical estimation. All the experiments were conducted in three replications.

The total flavonoid content of the plant extract was determined by the aluminium chloride method (Rajendran *et al.*, 2014). About 2.5 mL of ethanol and 0.2 mL each of aluminium chloride and potassium acetate were added to the 0.5 mL of plant extract and made up the final volume of 9 mL with distilled water. The test solution was incubated for 30 min at room temperature. The absorbance of each test solution was read at 415 nm using a UV-spectrophotometer compared with a standard curve. The total flavonoid content was measured in µg of quercetin equivalent (QE) per mg of extract. Similarly, the total phenolic content of plant extract was estimated by the Folin-Ciocalteu reagent (FC) method (Singleton *et al.*, 1999). In brief, 0.2 mL of FC reagent was mixed with 0.5 mL of extract and the mixture was allowed to stand for 5 min at room temperature. 2 mL of sodium carbonate was added to the above mixture and was made up to 7 mL with distilled water. The test solution was incubated in the dark at room temperature for 90 min. The absorbance of the blue color mixture was measured at 725 nm against the blank and was compared with a standard. The total phenolics content of plant extracts was expressed in µg of Gallic acid equivalent per mg of extract.

The total tannins in plant extracts were estimated by the Folin-Denis (FD) method (Polshettiwar & Ganjiwale, 2007). In this method, 0.5 mL of the plant extract was added with 0.5 mL of FD reagent followed by 1 mL of sodium carbonate and the reaction mixture was made up to 10 mL with distilled water. The absorbance of the blue colour developed was measured at 700 nm. The total tannins were expressed in µg/mL of extract. The total alkaloid content in plant extracts was determined by the 1, 10-phenanthroline method (Singh *et al.*, 2004). To 0.5 mL of plant extract, 1 mL of ferric chloride in hydrochloric acid and 1 mL of alcoholic 1,10-phenanthroline was added. The mixture was incubated at 70°C in the water bath for 30 min. The absorbance of the red color complex was measured at 510 nm and the total alkaloid content was expressed in µg of colchicine equivalent/mg of extracts.

Similarly, the terpenoids were detected by the method described by Pandey *et al.* (2019). In this method, 2 mL of chloroform and 5 g of each extract were mixed, and then a layer of concentrated H₂SO₄ (3 mL) was carefully added. To demonstrate that terpenoids were present, a reddish brown coloration of the interface was created.

Antibacterial Assay

The bacterial types used for antibacterial assay were Gram +ve and Gram -ve strains. The Gram +ve strains were *Streptococcus mutans* (MTCC 497), *Enterobacter faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC 6633), Similarly, the Gram -ve strains utilized in the study were *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) and *Klebsiella pneumoniae* (MTCC 3384). The bacterial strains were obtained

from the Department of Microbiology, Bangalore University, Bengaluru, India.

Determination of Antibacterial Activity

The *in vitro* antibacterial activity of methanolic extracts of root, stem, and leaf of *A. lineata* against Gram-positive and Gram-negative bacterial strains was carried out using disc diffusion method (Hassan & Ullah, 2019). The Sterile Mueller Hinton agar plates were prepared to grow bacterial strains and inoculated by spread plate method under aseptic condition. Plant extracts of known concentrations (10 µg/µL, 20 µg/µL, and 30 µg/µL) was added to sterile filter paper discs (6 mm diameter) of holding capacity of 10 microlitres. These sterile impregnated discs with plant extracts were placed on the agar surface. The standard antibiotic Ciprofloxacin and DMSO were used as +ve and -ve control respectively. The plates were incubated at 37 °C for 24 hrs for optimum growth of the bacterial strains. After incubation, the antibacterial activity of the extracts was determined by measuring the diameter of zone of inhibition in millimetres with a transparent scale. The tests were repeated three times to ensure reliability. The results obtained in the present study were analyzed and presented in the form of mean ±SD.

RESULTS

Qualitative Analysis

The phytochemical analysis of the leaf, stems and roots of *A. lineata* extracted with different solvents such as aqueous, ethanol, butanol, methanol and chloroform showed the presence of alkaloids, flavonoids, phenols, coumarins, glycosides, phytosterols, saponins, resins, tannins, terpenoids and steroids (Table 1). Notwithstanding, the maximum number of phytochemical constituents was found in methanolic extract compared to other test solvent extracts due to variation in their polarity. Nonetheless, saponins were found only in the aqueous extracts of the root and stem. Interestingly, glycosides were present only in the methanolic and chloroform extracts of roots.

Quantitative Analysis

Secondary metabolites are bioactive compounds that play an important role in the protection of plants against pathogens. The quantification of selected phytochemical constituents viz. the total phenolics, alkaloids, tannins, flavonoids, and terpenoids were performed in methanolic extract of root, stem, and leaf and results are presented in Figure 1. The roots showed higher total phenolic content (64.62 ± 1.23 µg/mL) followed by the stem (35.00 ± 1.23 µg/mL) and leaf (20.65 ± 1.40 µg/mL). Similarly, the significant amount of total alkaloid content was found in the stem (162.51 ± 2.26 µg/mL) followed by the leaf (158.33 ± 3.65 µg/mL). Nonetheless, lower alkaloid content was recorded in the root (112.50 ± 2.45 µg/mL).

The total tannins present in the plant extracts of *A. lineata* were determined using tannic acid as standard. The results showed higher tannin content in the leaf (72.75 ± 2.89 µg/mL) followed by the root (52.53 ± 1.80 µg/mL). However, the minimum amount of tannins was recorded in the stem (30.26 ± 1.51 µg/mL). Furthermore, the methanolic extract of roots showed a high content of total flavonoids (27.54 ± 1.34 µg/mL) followed by stem (25.73 ± 1.23 µg/mL) and leaf (19.52 ± 1.10 µg/mL). Similarly, the maximum amount of terpenoids was recorded in leaves (119.53 ± 1.00 µg/mL) followed by root (64.65 ± 2.01 µg/mL) and stem (29.29 ± 1.62 µg/mL).

Antibacterial Activity

The secondary metabolites synthesized from medicinal plants are good sources of antibiotics against many pathogens (Hassan & Ullah, 2019). The antibacterial activity of the methanolic extract of *A. lineata* i.e. root, stem, and leaf were tested against three Gram +ve bacterial strains viz. *Streptococcus mutans*, *Enterobacter faecalis* and *Bacillus subtilis* by exposing to three different concentrations (10, 20 and 30 µg/µL) of plant extracts (Table 2). The observations revealed a better zone of inhibition of the leaf extracts against *S. mutans* (25.13 ± 0.07 mm), *E. faecalis* (24.13 ± 0.14 mm) and *B. subtilis* (20.12 ± 0.23 mm) at 30 µg/µL. Similarly, the stem extract showed moderate levels

Table 1: Phytochemical constituents of *Andrographis lineata* screened with polar and non-polar solvents

Chemical constituents	Aqueous			Methanol			Ethanol			Chloroform			Butane		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Alkaloids	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+
Flavonoids															
a. Fe Cl ₃	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
b. Alkaline	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Phenols	-	+	+	+	+	+	+	-	-	-	-	-	+	-	-
Coumarins	-	-	-	+	+	-	+	+	-	+	-	-	+	-	-
Glycosides	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Phytosterols	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Saponins	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-
Tannins															
a. Lead Acetate	-	+	+	+	+	+	-	-	+	+	+	-	-	-	+
b. Gelatine	-	-	-	+	+	+	-	-	-	+	+	+	+	-	+
Terpenoids	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	-	-	+	-	+	+	+	-	-	+	+

+: Present, -: Absent

of inhibition against *S. mutans* (25.15±0.06 mm), *E. faecalis* (22.10±0.21 mm) and *B. subtilis* (24.08±0.12 mm) at 30 µg/µL. Furthermore, the diameter of the zone of inhibition caused by methanolic extract of *A. lineata* against the bacterial strains such as *S. mutans*, *E. faecalis* and *B. subtilis* were 18.09±0.05, 20.24±0.15 and 24.09±0.14 mm respectively at 30 µg/µL.

The antibacterial activity of *A. lineata* leaf, stem and roots was studied against three Gram –ve bacterial strains viz. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* with three different concentrations (10, 20 and 30 µg/µL) of plant extracts (Table 3). The results showed that the leaf extracts of *A. lineata* exhibited greater zone of inhibition ability against *E. coli*, (25.13±0.16 mm) followed by *P. aeruginosa* (16.11±0.08 mm), and *K. pneumoniae*

(18.18±0.17 mm) at 30 µg/µL. Furthermore, the stem extract also showed a moderate zone of inhibition and was *E. coli* (22.15±0.18 mm), *P. aeruginosa* (19.11±0.09 mm), and *K. pneumoniae* (9.09±0.11 mm) at 30 µg/µL. Similarly, the diameter of the zone of inhibition showed by root was *E.coli* (22.18±0.12 mm), *P. aeruginosa* (17.15±0.10 mm) and *K. pneumoniae* (17.13±0.13 mm) at 30 µg/µL.

DISCUSSION

The present study determined the phytochemical composition of plant extracts of *A. lineata* and their antibacterial activity on selected Gram-positive and Gram-negative bacteria. The preliminary screening of phytochemicals in the methanolic extract of *A. lineata* showed various phytochemicals viz. alkaloids, flavonoids, phenols, glycosides, tannins, terpenoids, etc. The results of the present study are confirmatory with the findings of Alagesaboopathi (2011) and Gupta *et al.* (2010) who found almost similar phytochemical constituents in *A. lineata*. Similarly, Fardiyah *et al.* (2020) reported flavonoids, alkaloids, tannins, triterpenoids and polyphenol in the plant extracts of *Andrographis paniculata*, an important medicinal plant. Comparatively, the methanolic extract of plant parts showed a wide variety of phytochemicals than other solvents due to their high polarity nature.

The amount of secondary metabolites such as phenols, tannins, alkaloids, flavonoids and terpenoids were estimated in the methanolic extracts of leaf, stem, and root. Comparatively, greater amounts of metabolites were found in the leaf followed by roots and stem. Phenols are generally found in all parts of the plants and their concentration depends upon the season and stages of the growth and development of the plant

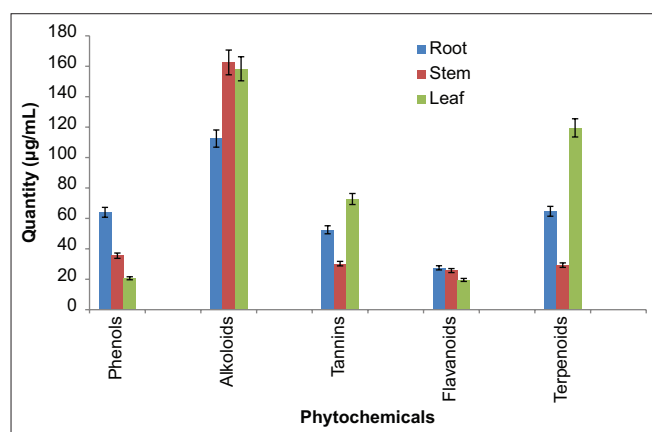


Figure 1: Quantity of phytochemicals found in methanolic extract of root, stem and leaf of *Andrographis lineata*. Data are Mean±SD of three replications in each experiment

Table 2: Antibacterial activity of the methanolic extract of *Andrographis lineata* leaf, stem and roots to Gram +ve bacterial strains viz. *Streptococcus mutans*, *Enterobacter pneumoniae* and *Bacillus subtilis* exposed to three different concentrations (10 to 30 µg/µL) of plant extracts

Plant extract	<i>Streptococcus mutans</i>			<i>Enterobacter faecalis</i>			<i>Bacillus subtilis</i>		
	Concentration (µg/µL)								
	10	20	30	10	20	30	10	20	30
	Diameter of zone of inhibition (mm)								
Leaves	12.13±0.04	13.14±0.05	25.13±0.07	18.15±0.03	22.10±0.21	24.13±0.14	12.09±0.07	16.09±0.45	20.12±0.23
Stem	6.14±0.02	14.16±0.07	25.15±0.06	18.19±0.04	20.12±0.25	22.10±0.21	14.10±0.08	20.10±0.50	24.08±0.12
Root	13.19±0.05	15.18±0.09	18.09±0.05	15.16±0.05	18.13±0.30	20.24±0.15	14.08±0.06	20.26±0.60	24.09±0.14

Values represent mean±SD of three different replications

Table 3: Antibacterial activity of the methanolic extract of *Andrographis lineata* leaf, stem and roots to Gram -ve bacterial strains viz. *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* exposed to three different concentrations (10 to 30 µg/µL) of plant extracts

Plant extract	<i>Escherichia coli</i>			<i>Pseudomonas aeruginosa</i>			<i>Klebsiella pneumoniae</i>		
	Concentration (µg/µL)								
	10	20	30	10	20	30	10	20	30
	Diameter of zone of inhibition (mm)								
Leaves	20.12±0.34	22.13±0.08	25.13±0.16	15.10±0.17	16.11±0.08	20.20±0.13	15.08±0.07	16.16±0.12	18.18±0.17
Stem	12.13±0.12	20.09±0.10	22.15±0.18	15.18±0.09	16.19±0.14	19.11±0.09	5.06±0.05	8.07±0.09	9.09±0.11
Root	12.22±0.09	20.13±0.07	22.18±0.12	10.08±0.10	13.12±0.09	17.15±0.10	10.08±0.08	13.12±0.15	17.13±0.13

Values represent mean±SD of three different replications

(Bhattacharjee *et al.*, 2010). However, in the present study, elevated levels of phenols were found in the root followed by the stem and leaf. Similarly, the level of alkaloids was higher in the stem and roots of *A. lineata*. A higher content of nitrogen in the root led to a greater amount of alkaloids in plants.

The level of tannins was high in the methanolic extracts of the leaf. Results of the study showed higher amounts of flavonoids in the root and stem of *A. lineata*. Flavonoids are generally abundant in leaves and are precursors of biosynthetic pathways in the photosynthesis of leaves. Our observations are consistent with the findings of Chaithada *et al.* (2018), who found high amounts of flavonoids in the leaves of *Mesua ferrea*. Flavonoids defend plants from abiotic and biotic stress and play an important role in protecting plants from drought and freezing (Panche *et al.*, 2016). Terpenoids are the largest and structurally diverse group of secondary metabolites derived from natural sources (Isah *et al.*, 2018) and were found in greater amounts in the leaf of *A. lineata*.

The methanolic extract of the root, stem, and leaf of *A. lineata* showed higher antibacterial activity against both Gram-positive and Gram-negative bacteria. The antibacterial activity of plants was primarily due to the presence of different secondary metabolic constituents like alkaloids and polyphenols (Duraipandiyani *et al.*, 2006). Plants that are rich in phenolic compounds exhibit therapeutic activities like anti-inflammatory, antimicrobial, antioxidant, and anticancer (Tungmunthum *et al.*, 2018). Similarly, alkaloids also exhibit antimicrobial, anti-inflammatory, and anti-cancer properties (Kurek, 2019).

Results of the study showed a greater influence of phytochemicals on Gram-positive bacterial strains than on Gram-negative bacterial strains. Similarly, the Gram-positive bacteria were more sensitive to plant extracts compared to that of the Gram-negative bacteria (De Zoysa *et al.*, 2019). Gram-negative bacteria possess an outer membrane and a unique periplasmic space which is not found in Gram-positive bacteria and therefore, they showed different antimicrobial activity against tested sample extracts (Duffy & Power, 2001). The significant antibacterial effect of leaf extracts of *A. lineata* was possibly due to the availability of greater amounts of flavonoids and phenols in the leaf parts. Safari *et al.* (2019) reported that flavonoids act as antimicrobial agents by inhibiting nucleic acid synthesis. Similarly, the antibacterial property of *A. lineata* was due to the presence of three flavonoids found in these plants (Kishore *et al.*, 2003).

CONCLUSION

The plant extracts of the *A. lineata* showed therapeutically significant secondary metabolites which could inhibit pathogenic Gram-positive and Gram-negative bacterial strains. Accordingly, *A. lineata* can be considered a potential source of biologically important phytochemical constituents and can be used as medicines against bacterial diseases. However, further studies on these lines are needed to confirm their potentiality in *in vivo* conditions.

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