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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *Ageratina adenophora* (Spreng.) R.M. King & H. Rob.

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ABSTRACT

Ageratina adenophora, a native flora of Central America and Mexico from the family Asteraceae is often considered a weed that has been reported for aggressive growth in many other parts of the world including India. The parallel reports of therapeutic potential of the plant is earlier reported. In India, the species is often observed or records have shown to be present in higher altitudes of Himalayas and with comparatively lower temperature. The present study aims to study the phytochemical analysis and antibacterial activity of *A. adenophora* collected from the non-Himalayan habitat i.e., Deomali hills of Koraput, Odisha. The present study revealed the presence of tannin, phenolic compounds and alkaloids from the three solvent extracts (aqueous, ethanol and methanol). The antibacterial activity of the leaves extracts of ethanol extract was found to show highest inhibition followed by methanol extract. Additional phytochemicals such as flavonoids and saponin present in aqueous and methanol extract may have other therapeutic potentials that could be studied in future assessment of the bioassays. This study determines the preliminary test of antibacterial activity and its corresponding phytochemicals detected. Such weeds having therapeutic potential could have positive uses towards the development of drugs for various ailments for human in future.

Keywords **:** Deomali hills, *E. coli*, phytochemical compounds, weeds.

Introduction

Ageratina adenophora (Fig. 1) commonly known as Crofton weed, is a perennial invasive shrub belonging to the family Asteraceae which is native to Central America and Mexico. The species has rapidly spread across tropical and subtropical regions of Asia, Africa, Europe and Oceania due to its high reproductive capacity, adaptability and allelopathic nature (Fried *et al.*, 2025; EPPO, 2023). The plant thrives in disturbed habitats, forest margins, roadsides, grasslands and agricultural fields, where it competes aggressively with native vegetation and causes serious ecological and economic impacts. In India, the species has become widely distributed in Himalayan regions and several temperate and subtropical ecosystems, posing a threat to biodiversity and natural habitats (Negi *et al.*, 2026). It is characterized by erect branched stems, opposite triangular leaves with serrated margins, and clusters of small white flowers. The plant produces abundant wind-dispersed seeds, facilitating rapid colonization in new environments (Guacchio, 2013). Due to its invasive behavior, extensive studies have focused on its ecology, distribution, habitat suitability and management strategies, including biological control approaches (Heystek *et al.*, 2011; Zhao *et al.*, 2012). Despite being recognized as a noxious invasive weed, it possesses significant ethnomedicinal and pharmacological importance. Traditional medicinal systems have utilized different parts of the plant for treating wounds, inflammation, fever, skin infections, diabetes and microbial diseases (Tripathi *et al.*, 2018; Gupta and Gupta, 2021).

Fig. 1: *Ageratina adenophora* in its habitat

Phytochemical investigations have revealed the presence of flavonoids, terpenoids, alkaloids, coumarins, phenolic compounds, tannins and essential oils that contribute to its diverse biological activities (Sun *et al.*, 2023). Several studies have reported antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, larvicidal and mosquito repellent properties of the plant extracts (Rajeswary and Govindarajan, 2013a & 2013b; Chanu *et al.*, 2023; Gupta and Gupta, 2024). These pharmacological attributes indicate its potential as a source of bioactive compounds for pharmaceutical and agricultural applications. Despite its remarkable therapeutic potential, *A. adenophora* is also recognized for its toxic effects on livestock and other grazing animals. Ingestion of the plant has been reported to cause respiratory complications, liver damage, oxidative stress and other pathological disorders. This is primarily due to the presence of toxic sesquiterpenes and related secondary metabolites (Ren *et al.*, 2021). Considering its widespread distribution, invasive nature and rich phytochemical profile, it has attracted considerable scientific attention in recent years. The present study explores the phytochemical analysis and antibacterial activity against *E. coli*.

Materials and Methods

Collection and Identification of Plant Material

Fresh plant samples of *Ageratina adenophora* were collected from Deomali hills of Koraput, Odisha during field surveys. Healthy leaves were carefully harvested and transported to the laboratory for further investigation. Photographic documentation of the plant was carried out, and taxonomic identification was performed using standard floristic literature and authenticated references (Jain and Rao, 1976).

Preparation of Plant Extracts for qualitative phytochemical analysis

The collected plant materials were thoroughly washed with water to remove adhering debris and shade dried at room temperature. The dried samples were then pulverized into coarse powder using a mechanical grinder. Approximately 5 g of powdered material was soaked separately in 50 ml of ethanol and distilled water for 24 hours for extraction of phytoconstituents. The mixtures were filtered using Whatman filter paper, and the obtained filtrates were preserved for phytochemical screening and antibacterial assays. Extraction of phytochemicals was carried out following standard Soxhlet and solvent extraction procedures using solvents of different polarities (Sadasivam and Manickam, 2009).

Qualitative Phytochemical Screening was done by the methods suggested by Kumar *et al.*, 2013; Marndi *et al.*, 2024.

Preliminary phytochemical evaluation of the plant extracts was conducted to determine the presence of important secondary metabolites using standard qualitative methods.

Detection of Tannins

About 1 ml of plant extract was treated with a few drops of 10% lead acetate solution. Formation of a gelatinous precipitate indicated the occurrence of tannins.

Detection of Saponins

1 ml of extract was mixed with equal volume of distilled water and vigorously shaken. Persistent froth formation confirmed the presence of saponins.

Detection of Flavonoids

To 1 ml of extract, 2 ml of 2% sodium hydroxide solution was added followed by a few drops of dilute hydrochloric acid. Development of a yellow coloration that disappeared upon acidification indicated the presence of flavonoids.

Detection of Terpenoids

1 ml of the plant extract was mixed with chloroform and gently heated in a water bath. Concentrated sulphuric acid was then added carefully. Appearance of a reddish-brown interface confirmed terpenoids.

Detection of Phenolic Compounds

A small quantity of extract was treated with 5% ferric chloride solution. Formation of a dark bluish-black coloration indicated the presence of phenolic compounds.

Detection of Reducing Sugars

The extract was mixed with Fehling's solutions A and B and heated in a water bath. Formation of a brick red or orange precipitate confirmed reducing sugars.

Detection of Steroids

1 ml of extract was treated with chloroform and concentrated sulphuric acid. Development of a reddish upper layer and yellow lower layer with green fluorescence indicated steroids.

Detection of Alkaloids

Plant extract was treated with Dragendorff's reagent. Formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Antibacterial Activity against *Escherichia coli*

Agar Well Diffusion Method

Antibacterial activity of the aqueous, ethanol and methanol extracts of *A. adenophora* was evaluated against Escherichia coli using the agar well diffusion technique following NCCLS guidelines. Sterile nutrient agar plates were inoculated with bacterial culture using the pour plate method and allowed to solidify. Wells measuring 6 mm diameter were made aseptically using a sterile cork borer. Different extract concentrations (25, 50, and 100 mg/ml) were prepared and 100 µl of each concentration was introduced into the respective wells. Kanamycin (1 mg/ml) was used as the positive control. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured. All experiments were conducted in triplicate (Owusu *et al.,* 2021).

Minimum Inhibitory Concentration (MIC) Assay

The MIC of the plant extracts against *E. coli* was determined using the broth dilution method. Serial dilutions of the extract were prepared to obtain concentrations ranging from 10 to 100 mg/ml. To each sterile nutrient broth tube, 50 µl of bacterial inoculum and 100 µl of plant extract were added. Positive control tubes contained bacterial inoculum in nutrient broth, while negative controls contained only sterile broth. All tubes were incubated at 37°C for 24 hours. The lowest concentration showing no visible turbidity was considered the MIC value (Owusu *et al.,* 2021).

Results and Discussion

Phytochemical analysis

The qualitative phytochemical screening of different solvent extracts of *A. adenophora* revealed the presence of several bioactive secondary metabolites (Table 1 and Fig. 2). Among the three extracts analyzed, the aqueous extract exhibited the highest diversity of phytochemicals, containing tannins, saponins, flavonoids, phenolic compounds, reducing sugars and alkaloids. The methanol extract showed the presence of tannins, phenolic compounds, reducing sugars, and alkaloids, whereas the ethanol extract contained tannins, phenolic compounds and alkaloids only. This indicate that the extraction solvent plays a significant role in isolating phytoconstituents from plant materials. Polar solvents such as water and methanol were more effective in extracting a broader range of compounds compared to ethanol. The detection of tannins, phenolic compounds, flavonoids and alkaloids in the present investigation correlates with the observations of earlier studies (Tripathi *et al.,* 2018; Gupta and Gupta, 2021; Bisht and Sharma, 2024). The higher occurrence of phenolic compounds and tannins is often related to occurrence in antioxidant-related metabolites. Phenolic compounds and flavonoids are known for their antimicrobial, antioxidant and anti-inflammatory properties, while alkaloids are associated with antimicrobial and pharmacological activities. The presence of saponins exclusively in the aqueous extract may contribute to enhanced biological activity due to their membrane-permeabilizing effects on microorganisms. Similar findings have been reported in several medicinal plants where aqueous and methanolic solvents demonstrated superior extraction efficiency for polar bioactive compounds.

Table 1: Detection of qualitative phytochemical analysis of solvent extracts of *A. adenophora*

Solvent Extract(s)	Phytochemicals detected
Ethanol Extract	Tannin, Phenolic compounds, Alkaloids
Methanol Extract	Tannin, Phenolic compounds, Reducing sugar, Alkaloids
Aqueous Extract	Tannin, Saponin, Flavonoids, Phenolic compounds, Reducing sugar, Alkaloids

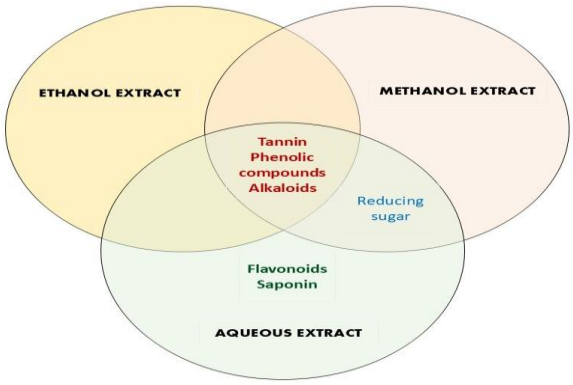

Fig. 2: Diagrammatic representation of the phytochemical compounds present in different solvent extracts of *A. adenophora*

Antibacterial Activity

The antibacterial activity of different extracts of *A. adenophora* was evaluated by measuring the zone of inhibition against the test organism (*E. coli*) (Table 2). Among the extracts tested, the ethanol extract exhibited the highest antibacterial activity at all concentrations, with the maximum zone of inhibition recorded at 100 mg/ml (2.2 ± 0.1 mm), followed by 50 mg/ml (2.0 ± 0.1 mm) and 25 mg/ml (1.8 ± 0.0 mm). The methanol extract showed moderate antibacterial activity with inhibition zones ranging from 0.8 ± 0.1 mm to 1.5 ± 0.0 mm, while the aqueous extract demonstrated the least activity. The standard antibiotic, kanamycin (1 mg/ml), produced a zone of inhibition of 2.5 ± 0.0 mm, which was comparatively higher than all plant extracts. However, the ethanol extract showed activity close to the standard, indicating its promising antibacterial potential (Fig. 3). The stronger antibacterial activity of the ethanol extract may be attributed to the presence of tannins, phenolic compounds and alkaloids, which are known to interfere with microbial cell wall synthesis, membrane permeability and enzymatic activity. Although the aqueous extract contained a greater number of phytochemicals, its lower antibacterial activity suggests that the bioactive compounds responsible for antibacterial action may be more efficiently extracted in ethanol.

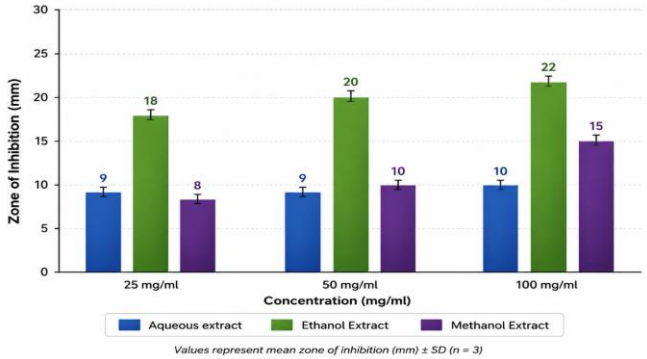

Fig. 3: Antibacterial activity of different solvent extracts of *A. adenophora* against *E. coli*

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the ethanol and methanol extract against *Escherichia coli* was determined by the broth dilution method and was found to be 80 mg/ml and 100mg/ ml respectively. Different concentrations of the plant extract ranging from 10 to 100 mg/ml were prepared through serial dilution. Each sterile nutrient broth tube received 50 µl of bacterial inoculum along with 100 µl of the plant extract. Positive control tubes containing bacterial inoculum in nutrient broth and negative control tubes containing only sterile broth were maintained for comparison. After incubation at 37°C for 24 hours, the tubes were examined for visible turbidity. The lowest concentration of the extract that showed complete absence of turbidity was considered as the MIC value. The antibacterial activity may be attributed to the synergistic action of one or more phytoconstituents present that can interfere with bacterial cell wall synthesis, membrane permeability, protein synthesis or enzymatic activity.

Conclusion

The study demonstrated the presence of various phytochemical compounds such as alkaloids, flavonoids, tannins, saponins and phenolic compounds which may contribute to its therapeutic properties. The extracts also exhibited appreciable antibacterial activities, particularly against *E. coli*, showing its potential as a natural source of antibacterial agents. The present findings of *A. adenophora* could serve as a promising candidate for the development of plant-based pharmaceutical formulations. Further studies involving other bioassays and isolation of active compounds, toxicity evaluation and *in vivo* investigations are recommended to validate its medicinal applications and ensure safe utilization.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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