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Investigation of Astringent and Antimicrobial Activity of Leaf and Stem of *Ageratum conyzoides*

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ABSTRACT

The chemical constituent of *Ageratum conyzoides*, a plant widely distributed in Nepal, India, West Africa, and South America, is well known for its potent bleeding control property and antibacterial effect. The study used an 80% ethanol extract of the leaf and stem to evaluate phytochemical screening, antibacterial properties (Standard: Tigecycline 15mcg; Test organisms: *Pseudomonas aeruginosa*, *E. coli*, *S. typhi*, *S. aureus*), and astringent properties (Standard: Tranexamic acid 650mg/2ml; Experimental organism: Albino mice).

The study revealed that the leaf and stem extract contain secondary metabolites (Alkaloids, Tannins, Flavonoids, Glycosides, and Phenolic Compounds). The ethanolic extract of both the leaf and stem of *Ageratum conyzoides* at different concentrations (Undiluted, 500mg/0.3ml, and 200mg/0.3ml) showed robust astringent activity. Both the leaf and stem extracts were found to control bleeding better than Tranexamic acid when applied topically to open wounds on the tail of mice. The study also revealed that the leaf extract exhibited better astringent activity compared to the stem extract of *Ageratum conyzoides*.

The ethanol extract of both leaf and stem exhibited an antibacterial effect against *Pseudomonas aeruginosa* at different concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.1mg/ml, 1.6mg/ml, and 0.8mg/ml) but showed resistance against *E. coli, S. typhi, and S. aureus*, while Tigecycline 15mcg showed sensitivity towards all the test bacteria.

Keywords: *Ageratum conyzoides*, Astringent, Ethanol, Phytochemical, Tigecycline, Tranexamic acid.

1. INTRODUCTION

Injuries and wound healing are crucial aspects of healthcare, as open wounds are highly vulnerable to bacterial infections. These infections can hinder the healing process, leading to prolonged recovery times and increased healthcare costs. Traditional wound care often involves using astringents and antimicrobial agents to facilitate healing and prevent infection. In recent years, there has been a growing interest in natural remedies due to their potential effectiveness and lower risk of side effects compared to synthetic drugs. According to data from the World Health Organization, approximately 40% of pharmaceutical products today are derived from natural and traditional knowledge.

Ageratum conyzoides, commonly known as Gandhe Jhar in Nepal and Goat weed in English, is widely distributed in tropical and subtropical regions, often found in open fields, roadside areas, and cultivated lands. It is a small herbaceous plant that is very common in West Africa, as well as some parts of Asia and South America. It is a softly hairy, erect, and branched annual weed that can grow up to 80-90 cm in height. The flowers range in color from purple to white, the fruits are achenes that are easily dispersed, and the leaves are simple, ovate, and covered in fine hairs, giving them a slightly rough texture (1).

Despite being classified as an invasive species in several regions, *A. conyzoides* is of significant interest due to its potential pharmacological properties. It is used to treat pneumonia, but its most common use is in the treatment of wounds and burns. Traditional communities in India use this species as a bactericide and anti-dysenteric. In Asia, South America, and Africa, extracts of this plant are used as a bactericide. In Congo and Cameroon, it is used for curing fever, headache, and colic (2). The plant has shown promising astringent and antimicrobial activities, which are attributed to its rich phytochemical composition, including

alkaloids, flavonoids, glycosides, phenols, and tannins (1). These bioactive compounds are believed to contribute to the plant's effectiveness in inhibiting bacterial growth and reducing inflammation.

The current study focuses on analyzing the secondary phytochemical compounds in the leaves and stems of *A. conyzoides* and assessing their astringent and antimicrobial activites. The aim of this research is to investigate the potential of *A. conyzoides* as a natural remedy for controlling bleeding and its antimicrobial activity. This study could offer valuable insights into its therapeutics properties and contribute to the development of natural remedies for bleeding control and treatment for different bacterial infection.

Figure No. 1: *Ageratum conyzoides* by Umesh Joshi.

2. MATERIALS AND METHODS

Plant materials: The matured plant of *A.conyzoides* was collected from Godawari, Lalitpur, Nepal (Collection Period June 2024). The plant was authenticated by the National Herbarium and Botanical Laboratories (NHBL).

Chemicals: Ketamine hydrochloride was obtained from Sah Medical Hall Pvt. Ltd. Tigecycline 15mcg disc, blue litmus paper,Muller Hinton Agar, Nutrient Broth, and Nutrient Agar were obtained from the Microbiology Lab of Nobel College. Tranexamic acid was obtained from Deurali Janta Pharmaceutical Pvt. Ltd. Mayer's reagent, Lead acetate, Concentrated Sulphuric acid, Concentrated Hydrochloric acid, Ammonia solution, and 80% Ethanol. All other chemicals were of standard analytical grade.

Preparation of extract: The plant extraction process involved using modified procedures as outlined by (3). Fresh leaves and stems were cleaned with distilled water to remove any waste or dust particles, then dried in the shade for 4 weeks at a normal room temperature of about 15-20°C. Once completely dried, the leaves and stems were separately turned into fine powder using an electric mixer. 80% ethanol was used as a solvent to extract the powder material. 50 grams of each leaf and stem powder were extracted using the Soxhlet apparatus for 10 hours. Subsequently, a vacuum evaporator was utilized to evaporate the solvent from the extract, concentrate it, and allow it to dry at room temperature below 40°C. The resulting dried extract was stored in the refrigerator at 4°C for further investigations.

Ethical statement: This study was approved by the review committee of the Nobel College Pvt Ltd ,Sinamangal, Kathmandu, Nepal. Albino mice were taken from Department of Plant Resources for this study. They were handled with care under the supervision of supervisor, before experiment they all were anethesized by Ketamine Hydrochloride.

2.1. Phytochemical screening: Phytochemical screening was conducted to inspect secondary metabolites.

Alkaloids Test (4)**:** 1 ml of Mayer's reagent was added to the 2 ml of crude extract. If a pale yellow precipitate was seen, it indicated the presence of alkaloids.

Tannins Test (5)**:** 3-4 drops of 10% FeCl3 were added to the diluted crude extract. If a blue color appeared, it indicated the presence of Gallic tannins, while a green color indicated the presence of catechol tannins.

Flavonoids Test (6)**:** 4ml of crude extract solution was mixed with 1.5ml of 50% methanol solution containing a magnesium chunk, and then heated. 4-5 drops of hydrochloric acid were added. The appearance of a red color indicated the presence of flavonoids.

Glycoside Test (7)**:**Mix 0.5 ml of glacial acetic acid with 2-3 drops of ferric chloride, then add 1 ml of crude extract to it.

After that, add 1 ml of concentrated sulfuric acid. If a deep blue color appears at the junction of the liquids, it indicates the presence of glycosides.

Phenolic compound Test (8)**:** 2-3 drops of crude extract was added on moist blue litmus paper, if color changed into red that indicate the presence of phenolic compounds.

2.2. Astringent Test:

Thirty-two albino mice weighing between 20-25 grams were purchased from the Department of Plant Resources, Kathmandu, for use in this study. The mice were fed a standard pellet diet and had access to water ad libitum. They were kept under laboratory conditions with a 24-hour dark-light period. The mice were randomly divided into eight groups, with every four mice in each group: 1; Negative control, 2; Positive group, 3; Test Group I (Low dose L), 4;Test Group II (High dose L), 5; Test Group III (Undiluted L), 6; Test Group IV (Low dose S), 7; Test Group V (High dose S), 8; Test Group VI (Undiluted S).

All mice were anesthetized using Ketamine Hydrochloride (87mg/kg) (9). The tails of the mice were cleaned using povidone-iodine, and a 2cm proximal part of the tail was excised using a surgical blade. Once bleeding started, Group 1 was left untreated, Group 2 was treated with Tranexamic acid 650mg/2ml, Group 3 with 200mg/0.3ml of leaf extract, Group 4 with 500mg/0.3ml of leaf extract, Group 5 with Undiluted leaf extract, Group 6 with 200mg/0.3ml of stem extract, Group 7 with 500mg/0.3ml of stem extract, and Group 8 with Undiluted stem extract topically.

Small pieces of filter paper were used to blot the blood from each cut every 5 seconds until the paper no longer showed any red from the blood, indicating that the bleeding had stopped, according to the modified procedure described by (10).

2.3. Antimicrobial Test: Each test bacteria was inoculated into 5ml of sterile nutrient broth and then incubated for 20-24 hours at 37°C. The concentration of each bacteria was determined by comparing their turbidity with McFarland, resulting in a bacterial concentration of 1.5 X 10^8 CFU/ml. Each bacteria of the above concentration was then transferred into sterile nutrient agar at 45°C and aseptically cultured into sterile Petri dishes containing Muller Hinton Agar by lawn culture. Afterward, four bores of 6 mm were created in each plate using a sterile borer to incorporate the crude extract at various concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.1mg/ml, 1.6mg/ml, and 0.8mg/ml). Each extracted sample of volume 150µl was poured into the bore to carry out the antibacterial activity. All the Petri dishes were then incubated for 18-24 hours at 37°C, and the diameter of the zone of inhibition was measured using a ruler, according to the modified procedure described by (11).

3. Result

3.1 Phytochemical test: The 80% ethanol extract (leaf and stem) was subjected to phytochemical screening, revealing the presence of alkaloids, tannins, flavonoids, glycosides, and phenolic compounds. The visual observation of color change was used to confirm the presence or absence of phytochemicals.

Table No. 1: Result of phytochemical screening of leaf and stem extract of *A. conyzoides*

SN	Phytochemical test	Test performed	Result
1	Alkaloids	Mayer's test	+
2	Tannins	Ferric chloride test	+
3	Flavonoids	Shinoda test	+
4	Glycosides	Borntrager's test	+
5	Phenolic compounds	Lead acetate test	+

Table shows result of phytochemical screening in leaf and stem extract of *A.conyzoides.*

(+) represent presence of tested phytochemicals.

(-) represent absence of tested phytochemicals.

Figure No 2: Ethanolic extract of leaf and stem of *Ageratum conyzoides* shows presence of alkaloids, glycosides, tannins, flavanoids and phenolic compounds.

3.2 Astringent Test: The study investigated the astringent activity of *A. conyzoides* (leaves and stems) in albino mice. The bleeding time in the animals was reduced by applying the crude extract topically. The astringent effect of the *A. conyzoides* plant was found to be concentration-related. Analysis of data reveals that undiluted extract controls bleeding much faster than that of 500mg/0.3ml and 200mg/0.3ml of extract. The result indicate that both leaf extract and stem extract have better astringent activity than that of Tranexamic acid (650mg/2ml) the standard drug (12). And similarly leaf extract has slightly better astringent property in comparison to stem extract as shown in (table no 2).

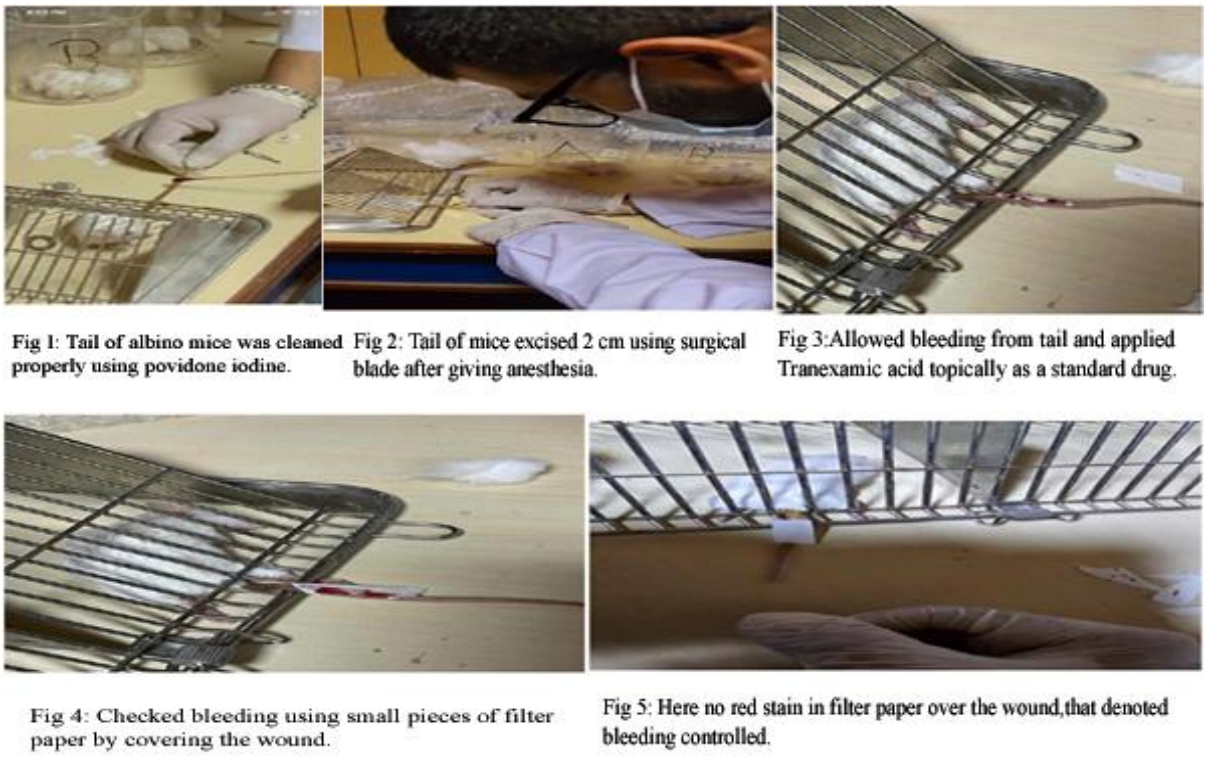

Figure No 3: Checking astringent activity of leaf, stem extract *Ageratum conyzoides* and tranexamic acid in albino mice.

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Table No. 2: Results of bleeding control time

SN	Groups (n=4)	Average time of bleeding control in minutes
1	Negative control	6.36±0.25
2	Positive group (Tranexamic acid 650mg/2ml)	4.12±1.20
3	Test Group I (Leaf extract 200mg/0.3ml)	3.1±0.53*
4	Test Group II (Leaf extract 500mg/0.3ml)	2.69±0.67*
5	Test Group III (Leaf extract Undiluted)	2.30±0.48*
6	Test Group IV (Stem extract 200mg/0.3ml)	3.45 ±0.07*
7	Test Group V (Stem extract 500mg/0.3ml)	3.27±0.25*
8	Test Group VI (Stem extract Undiluted)	2.58±0.30*

Results are expressed as mean±SD, n=4 *explaining significant difference in bleeding control time compared with the Negative control, Positive group.

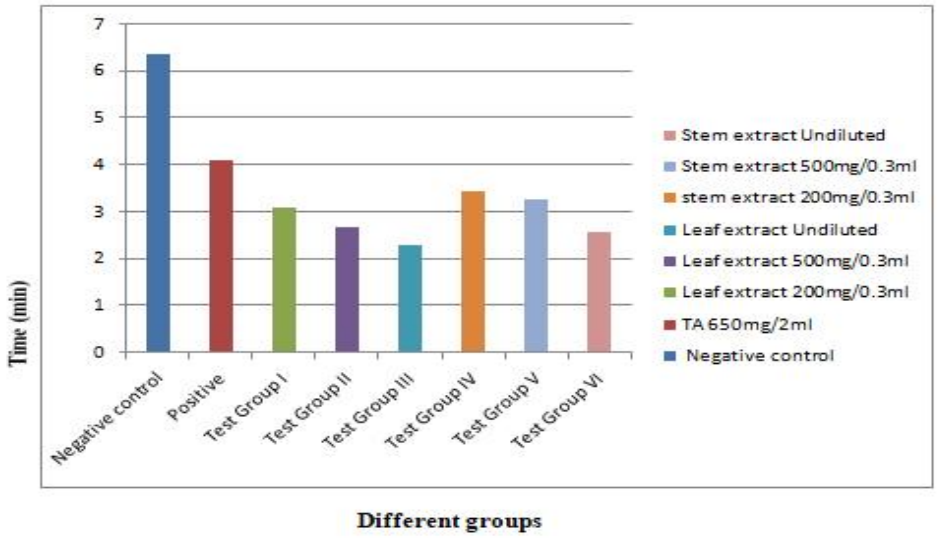

Figure No. 4: Showing the significant difference in bleeding control time between the Negative control, Positive, Test Group I, Test Group II, Test Group III, Test Group IV, Test Group V, and Test Group VI.

3.3. Antimicrobial test: The antibacterial activity of *A. conyzoides* was examined using the agar well diffusion technique to test the sensitivity or resistance of an 80% ethanol extract against both gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) and gram-positive (*Staphylococcus aureus*) bacterial strains. Tigecycline 15mcg was used as the standard antibiotic. The zone of inhibition by both the standard and test samples indicated the sensitivity or resistance against the bacteria.

The results of the antibacterial activity of the stem extract of *A. conyzoides* showed that the stem extract was sensitive to *Pseudomonas aeruginosa* and exhibited zones of inhibition measuring 15mm, 14mm, 14mm, 13mm, 12mm, 12mm, 10mm, and 9mm at different concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.1mg/ml, 1.6mg/ml, and 0.8mg/ml) respectively. However, the stem extract showed resistance against *Salmonella typhi, Escherichia coli, and Staphylococcus aureus* as shown in Table No.3.

The leaf extract also exhibited sensitivity to *Pseudomonas aeruginosa*, showing zones of inhibition measuring 15mm, 14mm, 14mm, 13mm, 13mm, 12mm, 10mm, and 7mm at concentrations of 100mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.1mg/ml, 1.6mg/ml, and 0.8mg/ml respectively. However, the leaf extract did not show sensitivity against *Salmonella typhi, Escherichia coli, and Staphylococcus* aureus as shown in Table No. 4.

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Tigecycline 15mcg exhibited sensitivity against all the test bacteria (*Pseudomonas, Salmonella typhi, Escherichia coli, and Staphylococcus aureus*) with zones of inhibition measuring 14mm, 13mm, 13mm, and 14mm respectively.

From this study, it was identified that both leaf and stem extracts were active against *Pseudomonas* only and had almost similar potency. Both leaf and stem extracts at 25mg/ml, 50mg/ml, and 100mg/ml showed similar potential to that of Tigecycline 15mcg against *Pseudomonas*.

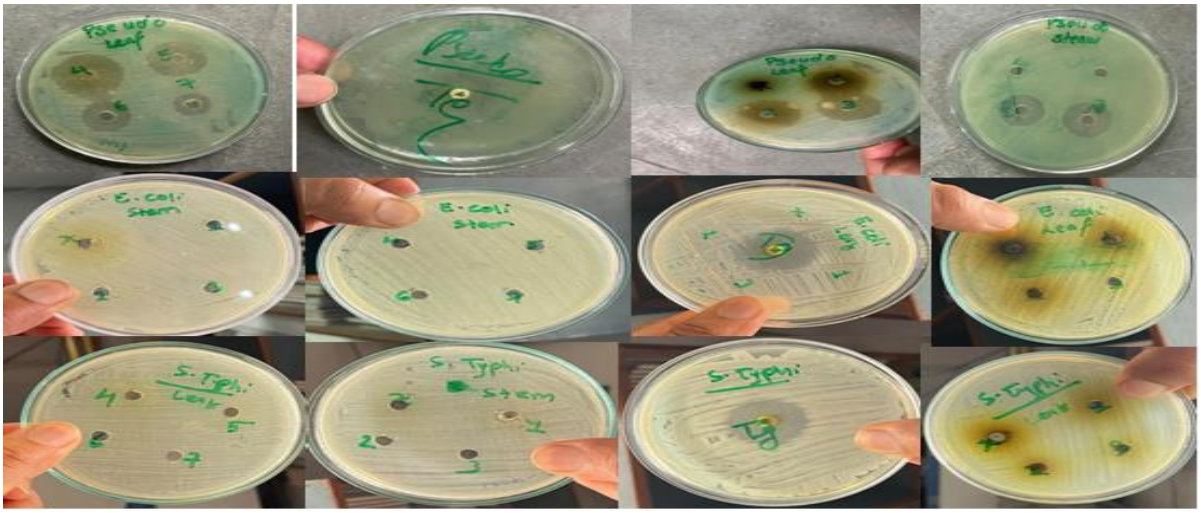

Figure No. 5: Sensitivity and resistivity of different concentration of leaf and stem extract of *Ageratum conyzoides* and Tigecycline 15mcg against various test bacteria.

Table No. 3: Zone of inhibition by ethanolic extract of *A. conyzoides* (stem) and Tigecycline on *P. aeruginosa*, *E. coli*, *S. typhi*, and *S. aureus*.

SN	Test organism	The concentration of stem extract (mg/ml) & zone of inhibition (mm)								Tigecycline (15mcg)
		100mg /ml	50mg /ml	25 mg/ml	12.5m g/ml	6.25mg /ml	3.1m g/ml	1.6 mg/ml	0.8 mg/ml	
1	*P. aeruginosa*	15 mm	14m m	14 mm	13mm	12 mm	12 mm	10 mm	9 mm	14mm
2	*S. typhi*	R	R	R	R	R	R	R	R	13mm
3	*E .coli*	R	R	R	R	R	R	R	R	13mm
4	*S. aureus*	R	R	R	R	R	R	R	R	14mm

Table No. 4: Zone of inhibition by ethanolic extract of*A. conyzoides*. (Leaf) & Tigecycline on *P.aeruginosa*, *E.coli*, *S.typhi* & *S.aureus.*

SN	Test organism	The concentration of leaf extract (mg/ml) & the zone of inhibition (mm).								Tigecycline (15mcg)
		100 mg/m l	50 mg/m l	25 mg/m l	12.5 mg/ml	6.25 mg/m l	3.1 mg/ml	1.6 mg/ml	0.8 mg/m l	
1	P. aeruginosa	15m m	14 mm	14 mm	13 mm	13 mm	12 mm	10 mm	7 mm	14mm
2	S. typhi	R	R	R	R	R	R	R	R	13mm
3	E. coli	R	R	R	R	R	R	R	R	13mm
4	S. aureus	R	R	R	R	R	R	R	R	14mm

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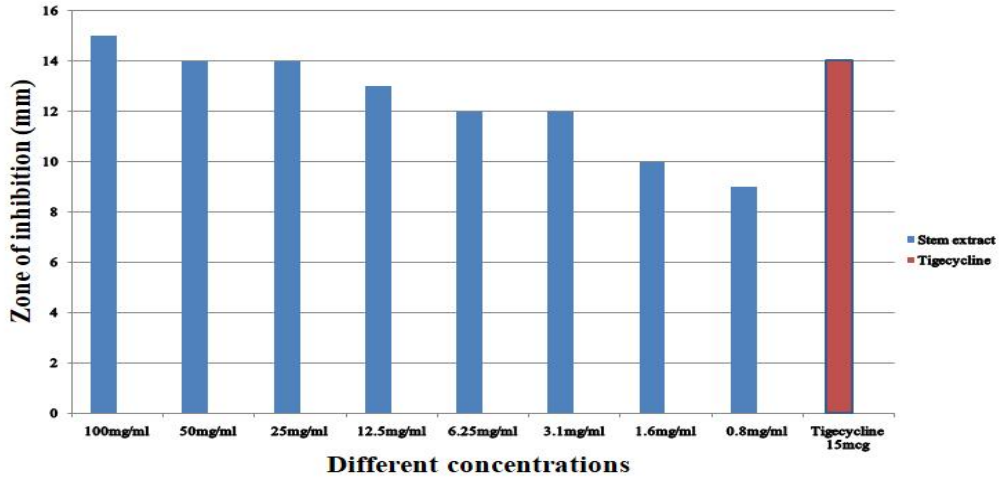

Figure No. 6: Antibacterial activity of ethanolic extract of *A. conyzoides* (stem)and Tigecycline against *Pseudomonas aeruginosa*.

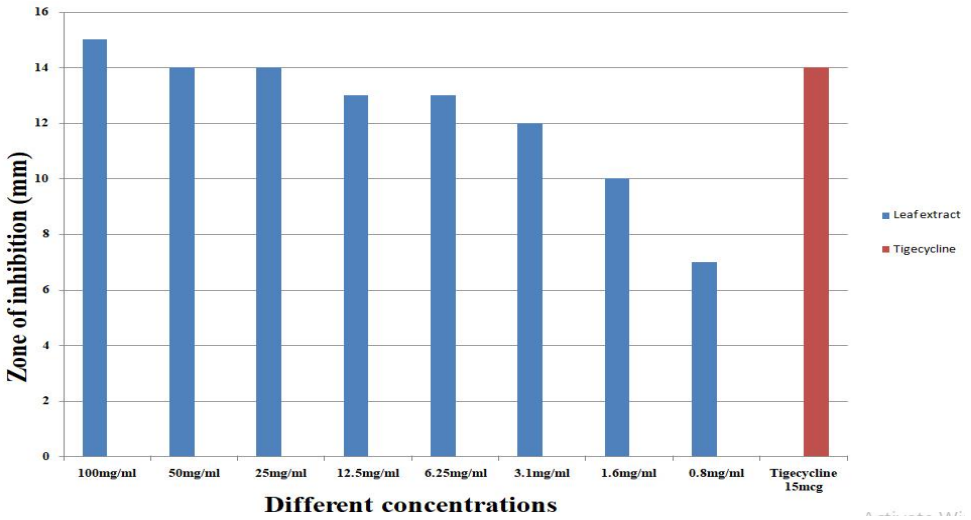

Figure No. 7: Antibacterial activity of ethanolic extract of *A. conyzoides* (leaf) and Tigecycline against *Pseudomonas aeruginosa.*

4. Discussion

In our research, we collected *A. conyzoides* from Godawari, Lalitpur, Nepal, and evaluated the 80% ethanol extract of leaves and stems for phytochemical screening, antibacterial, and astringent activities. According to (13), the moisture content in *A. conyzoides* is 9±4.24%, which meets the requirement of less than 10%. We choose 80% ethanol as the solvent to prevent increased chances of fungus growth due to higher moisture content. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, glycosides, and phenolic compounds in both leaf and stem extracts, consistent with previous works (14). Tannins provide astringent activity and wound-healing properties by causing vasoconstriction, forming artificial clots to control bleeding (15), reducing scar tissue formation, relieving pain, limiting secondary infection, preventing blood loss, and promoting keratinization (16).The study showed that the leaf extract has higher astringent activity than the stem extract due to its higher tannin concentration (17). Both extracts exhibited antibacterial activity against *Pseudomonas aeruginosa* and resistance against *S.typhi, E.coli, and S.aureus*. This antibacterial activity is attributed to the inhibitory effects on nucleic acid biosynthesis and other metabolic processes due to the richness of alkaloids, flavonoids, and phenolic compounds (18) . Phenolic compounds also contribute to antibacterial activity and are commonly used in disinfection (19). Flavonoids exhibit antibacterial, anti-inflammatory, anti-allergic, and antiviral activity (18). The extracts can be used to treat bacterial infections caused by Pseudomonas. This study supports the usage of *A. conyzoides* for treating bacterial infections and controlling bleeding.

5. Conclusion

The study results suggest that the ethanolic extract of the *A. conyzoides* plant has potential astringent and antibacterial activity. This plant could help control bleeding in open wounds, preventing excessive blood loss and aiding in healing. With healthcare costs on the rise, the availability of this plant makes it a cost-effective option for managing bleeding and certain bacterial infections. The antibacterial activity of the leaf and stem extract of A. conyzoides was found to be comparable to Tigecycline against *Pseudomonas*, while its astringent activity was slightly better than that of Tranexamic acid. While the use of this plant was already known in rural areas, scientific evidence now supports its beneficial effects in treating bacterial infections and managing bleeding. The plant's astringent properties make it an economically and environmentally friendly option with potential medical applications in the development of novel astringent materials.

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CONSENT FOR PUBLICATION

All the authors provided the consent for the publication in this journal.

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