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To evaluate the anti-ulcer activity of leaf extract of Conyza canadensis using experimental models and to assess its potential as a natural therapeutic agent for gastric ulcers.

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ABSTRACT

Conyza canadensis (L.) Cronquist, commonly known as Horseweed or Canadian fleabane, is an annual herb belonging to the family Asteraceae. Native to North America, it is widely distributed in Canada and other temperate regions. Traditionally, the plant has been valued for its astringent, diuretic, and anti-inflammatory properties quercetin anti-ulcer activity and invitro study murine macrophage cell line. Indigenous peoples used it for the treatment of diarrhoea, dysentery, internal bleeding, fever, and urinary tract disorders. The aerial parts contain flavonoids, tannins, and essential oils (such as limonene and linalool), which contribute to its medicinal actions. Modern phytochemical investigations indicate potential antimicrobial, antioxidant, and anti-inflammatory effects, supporting some of its traditional uses. Although generally considered safe in small therapeutic doses, its efficacy and safety require further pharmacological and toxicological studies. Overall, Conyza canadensis represents a promising herbal resource with diverse traditional applications and emerging scientific relevance.[1][2]

Keywords: Conyza canadensis Limonene Flavonoids Polyphenols quercetin anti-ulcer activity and invitro study murine macrophage cell line .

1. Introduction -

Conyza canadensis (L.) Cronquist, commonly known as Horseweed, Horsewood, or Canadian fleabane, is a medicinal herb belonging to the family Asteraceae. It is native to North America, particularly Canada and the United States, but has now become widely naturalized in many parts of the world, including Asia, Europe, and Australia. The plant grows abundantly in open fields, roadsides, and waste areas, and is often regarded as a weed due to its rapid growth and adaptability to different soils and climates. Despite its weedy nature, Conyza canadensis has a long history of use in traditional and folk medicine. Various Indigenous tribes in North America used it as a remedy for digestive disorders, coughs, fevers, bleeding, and urinary complaints.[3] The plant is known for its astringent, diuretic, styptic, and anti-inflammatory properties. Modern phytochemical studies have revealed the presence of flavonoids, tannins, and essential oils, which are believed to contribute to its medicinal actions. In recent years, scientific research has focused on the biological activities of Conyza canadensis, exploring its potential antioxidant, antimicrobial, and anti-inflammatory effects[4]. Because of its widespread availability and traditional reputation, it is being reconsidered as a valuable herbal resource for natural health care and pharmacological development. The increase in horseweed populations in cropping systems was the result of several factors. Most important was the shift to conservation tillage land management programs and the evolution and rapid spread of herbicide resistant biotypes [3] [4]. Horseweed produces thousands of seeds with single plant estimates greater than 200,000 plant−1

[5]. The seeds, botanically defined as achenes, do not require after-ripening and germination may exceed 80% [6]. The pericarp of the achene has a modified calyx, called the pappus, consisting of awns approximately 2 mm in length which enables wind dissemination up to 500 m from source populations [7]. Furthermore, horseweed may behave as a biennial germinating in the fall and growing to an overwintering rosette stage which bolts in the spring, or as a summer annual, germinating in spring, and completing its life cycle in a single season [2]. The biennial growth format affords early growth when favorable fall conditions persist positioning horseweed to resume growth from the more difficult to control rosette stage in the spring. Fortunately, from a management perspective, rosette survival in the following spring is highly variable with estimates between 3% and 91% depending on fall growth conditions [8] [9]. Rosette survival was highly correlated with rosette size [9].

2. Pharmacopieal Herbal Monograph Introduction

Pharmacopoeial herbal monograph is an officially recognized scientific document that provides comprehensive standards and specifications for an individual medicinal plant or herbal preparation. The purpose of such monographs is to establish uniform guidelines for the identification, purity, quality, safety, and efficacy of herbal raw materials and finished formulations. Unlike conventional synthetic drugs, herbal substances exhibit significant variability due to differences in geographical origin, cultivation practices, harvesting seasons, processing techniques, and storage conditions. Therefore, developing standardized monographs is essential to maintain consistency and authenticity in herbal medicine. A typical herbal pharmacopoeial monograph includes the botanical name (Latin binomial), common names, plant parts used, macroscopic and microscopic characteristics, chromatographic profiles, active phytochemical markers, quantitative assays, limits for contaminants, and permissible levels of heavy metals, pesticides, and microbial load. [10] These components allow manufacturers, researchers, and regulatory bodies to authenticate the plant material and ensure adherence to pharmacopoeial standards. Globally, several national and international pharmacopoeiassuch as the Indian Pharmacopoeia (IP), Ayurvedic Pharmacopoeia of India (API), Chinese Pharmacopoeia (ChP), European Pharmacopoeia (Ph. Eur.), United States Pharmacopoeia (USP), British Pharmacopoeia (BP), and Japanese Pharmacopoeia (JP)include herbal monographs relevant to traditional and modern medical systems. These monographs support regulatory approval, facilitate international trade, and provide scientific credibility to herbal medicines. [11][12]

With the growing acceptance of complementary and alternative medicine, the World Health Organization (WHO) has emphasized the need for harmonized herbal monographs to improve global standards. They act as reference documents for quality control laboratories, pharmacognosy research, standardization of formulations, clinical studies, and intellectual property protection. Furthermore, pharmacopoeial monographs promote the development of pharmaceutically acceptable herbal products by defining validated analytical methods such as Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and spectrophotometric assays.

In research and industry, these monographs serve as foundational resources for discovering new therapeutic agents, developing herbal formulations, and ensuring legal compliance. Ultimately, pharmacopoeial herbal monographs play a crucial role in integrating traditional knowledge with modern scientific standards, thereby promoting the safe and effective use of herbal medicines in global healthcare systems

3. History of Herbal Drug Standards in Indian Pharmacopoeia

The history of herbal drug standards in India is deeply rooted in traditional systems of medicine such as Ayurveda, Siddha, and Unani, but their formal incorporation into pharmacopeial standards began during the colonial period. The journey started with the publication of the Bengal Pharmacopoeia and the General Conspectus of Medicinal Plants (1844), which included information on indigenous medicinal plants used across India. Although focused mainly on local herbs, this early work laid the foundation for standardized documentation.

The first official Pharmacopoeia of India was published in 1868, and it included several drugs of European as well as indigenous origin. Later, the British Pharmacopoeia of 1867 also acknowledged certain Indian herbal substances. During the pre-independence period, the Indian Pharmacopoeia List (1946) was prepared to guide the use of both modern and indigenous drugs. After India gained independence, the Indian Pharmacopoeia Committee (IPC) was formed in 1948 to develop a national pharmacopoeia. Its efforts led to the first edition of the Pharmacopoeia of India (1955), which primarily covered allopathic drugs but gradually began including herbal medicines through supplements. The 1960 supplement included both western and traditional system drugs. This approach continued in later editions:

Indian Pharmacopoeia 1966 – included standardization efforts for plant-based drugs. Supplements in 1975 and 1985 – added more herbal formulations Indian Pharmacopoeia 1985, 1989, and 1991 – continued incorporating herbal standards selectively. A major shift occurred in 1996, when a separate publication called the “Indian Herbal Pharmacopoeia” was introduced in collaboration with the Indian Drug Manufacturers’ Association (IDMA) and the Regional Research Laboratory (RRL). This provided scientifically validated monographs of commonly used medicinal plants. Further developments include: Ayurvedic Pharmacopoeia of India (API) – First published in 1989, specifically to standardize Ayurvedic drugs.

Unani Pharmacopoeia of India and Siddha Pharmacopoeia – Introduced to regulate traditional systems separately.

Subsequent volumes and supplements – added identity, purity, safety, and quality parameters like TLC profiles, foreign matter limits, ash values, extractive values, and phytochemical markers. In recent decades,

regulatory standards for herbal drugs have been strengthened through:

Fig.no.01 Conyza Canadensis leave Fig.no.02dry Leave Fig.no.03 Dry Powder

Enlist of Extraction Methods Used in Conyza Canadensis

1. Maceration (Most Common)
2. Soxhlet Extraction
3. Ultrasound-Assisted Extraction (UAE)
4. Hot Extraction / Reflux Method
5. Sequential Solvent Extraction
6. Essential Oil Extraction (Hydrodistillation)

Soxhlet Extraction Method (Herbal Monograph Format :-

1. **Plant Material:-**
   Dried leaves of Conyza canadensis are collected, authenticated, washed with distilled water, and shade-dried at room temperature. The dried material is pulverized into a coarse powder and stored in airtight containers.

2. **Soxhlet Apparatus**
   Heating mantle.
   Whatman no. 1 Filter paper.
   Rotary evaporator (or Water bath)

3. **Solvent**
   Methanol or ethanol (commonly used) Other solvents (optional): hexane, chloroform depending on study .

4. **Procedure**

Accurately weigh 30–50 g of dried powdered leaves.
Place the powder in a cellulose extraction thimble.
Insert the thimble into the Soxhlet extractor.
Add 250–300 mL solvent into the round-bottom flask.
Assemble the Soxhlet apparatus and connect to a condenser.
Heat the system using a heating mantle.
Allow extraction to proceed for 6–8 hours (approximately 10–15 siphon cycles).
Continue until the solvent in the siphon tube becomes colorless.
After completion, allow the apparatus to cool.
Collect the extract from the flask.[30][31]

Fig.no.04 Soxhlet Apparatus

5. **Concentration of Extract**

The extract is concentrated under reduced pressure using a rotary evaporator.
Alternatively, evaporate solvent on a water bath below 50°C.
A semi-solid crude extract is obtained.

Fig.no.05 Filter of Extract **Fig.no.06 Final extract**

6. **Drying and Storage**
Dry the extract to constant weight.
Store in amber-colored, airtight containers.
Keep at 4°C until further use.

7. **Percentage Yield**
8. **Notes (Monograph Style)**
Methanol/ethanol extracts are rich in flavonoids, phenolics, and glycosides.
Non-polar solvents (hexane) extract lipids and terpenoids.
Avoid overheating to prevent degradation of phytoconstituents.
Ensure continuous water flow in condenser for efficient extraction.

9. **Phytochemical Analysis:**
Qualitative Tests for Phytochemical Screening:[33][34][35]

Test	Procedure	Observations (Indicating Positive Test)	References
Detection of alkaloids			
1. Dragendroff's/ Kraut's test	Few mL filtratea + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate	
2. Mayer's/Bertrand's/ Valser's test	Few mL filtratea + 1-2 drops of Mayer's reagent (Along the sides of test tube)	A creamy white/yellow precipitate	
3. Wagner's test	Few mL filtratea + 1-2 drops of Wagner's reagent (Along the sides of test tube)	A brown/reddish precipitate	
4. Hager's test	Few mL filtratea + 1-2 mL Hager's reagents	A creamy white precipitate	
5. Picric acid test	Few mL filtratea + 3-4 drops of 2% picric acid solution	An orange coloure	
6.Iodine Test	3mL extract solution + few drops of iodine solution	A blue colour, which disappears on boiling and reappears on cooling	
Detection of Carbohydrates			
1 .Molish's test	2mL filtrateb + 2 drops of alcoholic α–naphthol + 1mL conc. H2SO4 (along the sides of test tube)	A violet ring	
2 .Barfoed's test	1mL filtrateb + 1mL Barfoed's reagent + Heated for 2 min.	A red precipitate {monosaccharides}	
3 .Test for starch	Aqueous extract + 5mL 5% KOH solution	A cinary colouration	
Detection of Reducing sugars			
4 .Benedict's test	0.5mL filtrateb + 0.5mL Benedict's reagent + Boiled for 2 min.	Green/yellow/red colour	
5 .Fehling's test	1mL each of Fehling's solution A & B + 1mL filtrateb + boiled in water bath	A red precipitate	
6 .Aqueous NaOH test	Alcoholic extract + dissolved in 1mL of water + few drops of aqueous NaOH solution	A yellow colour	
Detection of Cardiac Glycosides			
1 .Keller-Killani test	1mL filtrate + 1.5mL glacial acetic acid	A blue coloured solution	

	+ 1 drop of 5% ferric chloride + conc. H2SO4 (along the side of test tube)		
2 .Baljet test	2mL extract + a drop of Baljet's reagent	A yellow-orange colour	
3 .Bromine water test	Plant extract + few mL of bromine water	A yellow precipitate	
Detection of Flavonoids			
1 .Alkaline reagent test	1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid	
2 .Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate	
3 .Shinoda's test/ Mghydrochloride reduction test	Plant extract is dissolved in 5mL alcohol + Fragments of magnesium ribbon + few drops of conc. HCl	A pink to crimson coloured solution {flavonal glycosides}	
4 .Ferric chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	A green precipitate	
5 .Conc. H2SO4 test	Plant extract + conc. H2SO4	An orange colour	
6 .Zinc-hydrochloride reduction test	Plant extract + pinch of zinc dust + conc. HCl along the side of test tube	Magenta colour	
Detection of Tannins			
1 .Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	
2 .Bromine water test	10 ml of bromine water + 0.5gm plant extract	Decoloration of bromine	
3 .10% NaOH test	0.4mL plant extract + 4mL 10% NaOH	shaken well Formation of emulsion {Hydrolysable tannins}	

Observation Table Anti-ulcer Activity Assay:- [36,37]
In-vitro Evaluation of Antiulcer Activity: Acid Neutralizing Capacity:
The Eethanolic extract of acid-neutralizing capacity value are 100mg, 500mg, 1000mg, 1500mg. The aluminium hydroxide and magnesium hydroxide (500mg) have compared for the standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1.0 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed. With 0.5N Sodium hydroxide, the excess HCl was- immediately titrated until the pink colour is attained . The moles of acid neutralized is calculated by,
mEq acid neutralized=V.HCl× N.HCl−V.NaOH ×N.NaOH (milliequivalents)
(here V.HCl = 30 mL, N.HCl = 1.0, N.NaOH = 0.5)
moles HCl neutralized=mEq/1000 **ANC (mol/g)**=moles HCl neutralized/sample weig ht (g) (sample weight in g = concentration (mg) / 1000) **ANC (mmol/g)**=ANC (mol/g)×1000

H+ /K+ -ATPase Inhibition Assay
Fresh goat stomach was collected from a local slaughterhouse. The fundus region was cut open and the inner mucosal layer was gently scraped to collect the parietal cells (which contain the H+/K+-ATPase enzyme). The collected cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 to release the enzyme. The homogenate was centrifuged at 6000 rpm for 10 minutes, and the clear supernatant was collected as the enzyme extract. The protein content of this extract was determined by the Bradford method using BSA as a standard.

1. **Evaluation test: [36,37]**

The anti-ulcer assay is based on the ability of the plant extract of Conyza canadensis to protect the gastric mucosa from ulcer formation. Ulcers are induced using agents such as ethanol or pylorus ligation, which damage the stomach lining by increasing acid secretion and oxidative stress. The plant extract is administered to experimental animals before ulcer induction. Bioactive compounds like flavonoids and phenolics present in the extract may reduce gastric acidity and enhance mucus production. After treatment, the stomach is examined for ulcer lesions and ulcer index. A decrease in ulcer severity compared to the control group indicates anti-ulcer activity of the extract.

Detection of Flavonoids[36,37]

1.Alkline reagent test	1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid +ve

2 .Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate +ve
3.Shinoda's test/ Mghydrochloride reduction test	Plant extract is dissolved in 5mL alcohol + Fragments of magnesium ribbon + few drops of conc. HCl	A pink to crimson coloured solution {flavonal glycosides} +ve
4 .Ferric chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	A green precipitate
5 .Conc. H2SO4 test	Plant extract + conc. H2SO4	An orange colour +ve
6 .Zinchydrochloride reduction test	Plant extract + pinch of zinc dust + conc. HCl along the side of test tube	Magenta colour +ve
5 .Conc. H2SO4 test	Plant extract + conc. H2SO4	An orange colour +ve

Fig.no.07 Phytochemcal

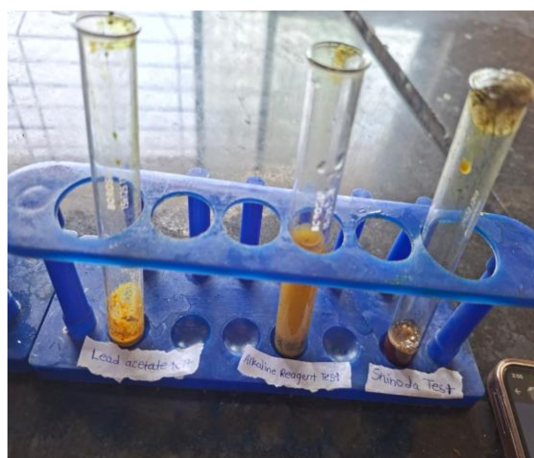

Fig.no.08 Phytochemical

Observation Table Anti-ulcer Activity Assay:- [36,37]
For Acid Neutralizing Capacity (ANC) assay:

- o Ethanolic plant extract (at concentrations: 100 mg, 500 mg, 1000 mg, 1500 mg)
- o Aluminium hydroxide + Magnesium hydroxide (500 mg, standard antacid)

- N Hydrochloric acid (HCl
- o 0.5 N Sodium hydroxide (NaOH) (for titration)
- o Phenolphthalein indicator
- o Distilled water

- For H+ /K+ -ATPase inhibition assay:
- o Fresh goat stomach (fundus region) – source of parietal cells
- o Tris buffer (16 mM, pH 7.4)
- o Triton X-100 (10%) – for cell lysis
- o Adenosine triphosphate (ATP, 2 mM) – substrate
- o Magnesium chloride ($MgCl_2$, 2 mM) – cofactor
- o Potassium chloride (KCl, 10 mM) – activator
- o Ammonium molybdate (4.5% for stopping reaction; 2.5% for Pi estimation)
- o Perchloric acid (60%) – to stop reaction/precipitate protein
- o ANSA reagent (1-amino-2-naphthol-4-sulfonic acid reagent) – color reagent for Pi
- o BSA (Bovine Serum Albumin) – standard for protein estimation (Bradford method)
- o Bradford reagent – for protein estimation
- o Distilled water

H+/K+ -ATPase Inhibition Activity[36,37]

The inhibitory effect of the aqueous extract on the gastric proton pump (H+/K+ -ATPase) was assessed at concentrations of 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg, with Conyza canadensis serving as the standard drug. The extract exhibited significant, dose-dependent inhibition of H+/K+ -ATPase activity. The maximum inhibition of 65.27 ± 0.76% was recorded at 100 µg, compared with 70.14 ± 1.68% observed for the standard Conyza canadensis. These findings indicate that the extract possesses potent acid neutralizing and proton pump inhibitory properties, supporting its potential as a natural antiulcer and gastroprotective agent. These findings suggest that the potent antiulcer effect of Conyza canadensis may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and saponins, which contribute to mucosal protection and reduction of gastric acidity.

Effect Of ethanolic Extract Of Conyza canadensis On Acid Neutralizing Capacity [3839,40]

Sr.No	Conc. (mg)	V. NaOH (mL)	mEq acid neutralized	Moles (mol)	ANC (mol/g)	ANC (mmol/g)
01	100	5.0	1.0	0.0010	0.0100	10.0

02	500	12.0	2.6	0.0026	0.0052	4.8
03	1000	22.0	4.6	0.0046	0.0046	4.6
04	1500	34.0	7.4	0.0074	0.0048	4.8
05	**500 (std)**	**38.0**	**7.6**	**0.0076**	**0.0152**	**15.2**

- 100mg, 500mg, 1000mg, 1500mg Of ethanolic Extract Of Conyza canadensis
- 500mg Of Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2]

Effect of Ethanolic Extract of Conyza canadensis on In-Vitro H+/K+ - Atpase Inhibition Activity[3839,40]

Sr. No.	Concentration (µg)	Percentage Inhibition (%)(Mean ± SEM) Standard (Omeprazole)	Percentage Inhibition (%)(Mean ± SEM) ethanolic extract of Conyza canadensis
01	**20**	**50.92 ± 0.82**	**28.46 ± 0.42**
02	**40**	**55.78 ± 1.18**	**34.72 ± 0.88**
03	**60**	**57.12 ± 1.46**	**42.18 ± 1.12**
04	**80**	**59.04 ± 0.31**	**57.63 ± 1.04**
05	**100**	**70.14 ± 1.68**	**65.27 ± 0.76**

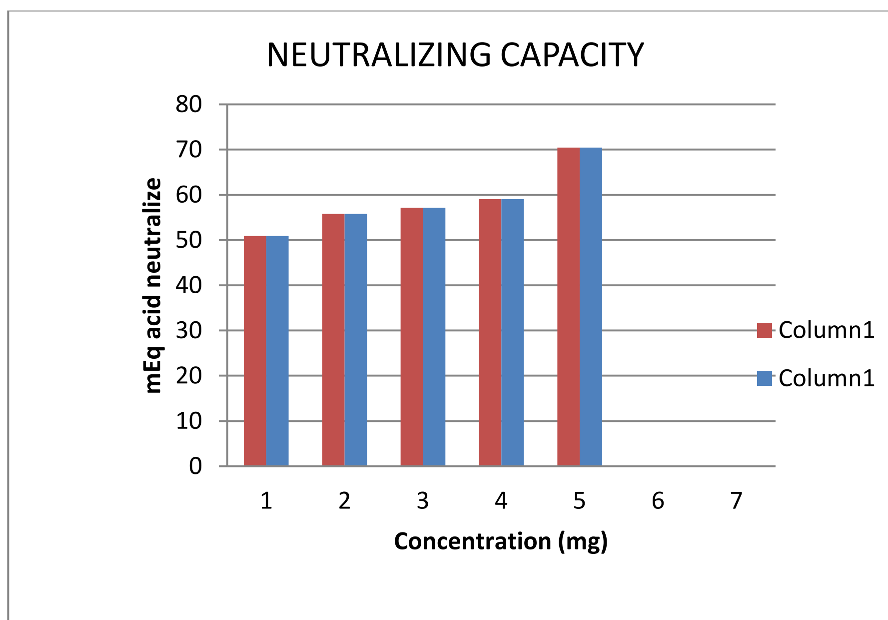

Figure 11.Effect of Ethanolic Extract of Conyza canadensis on Acid Neutralizing Capacity

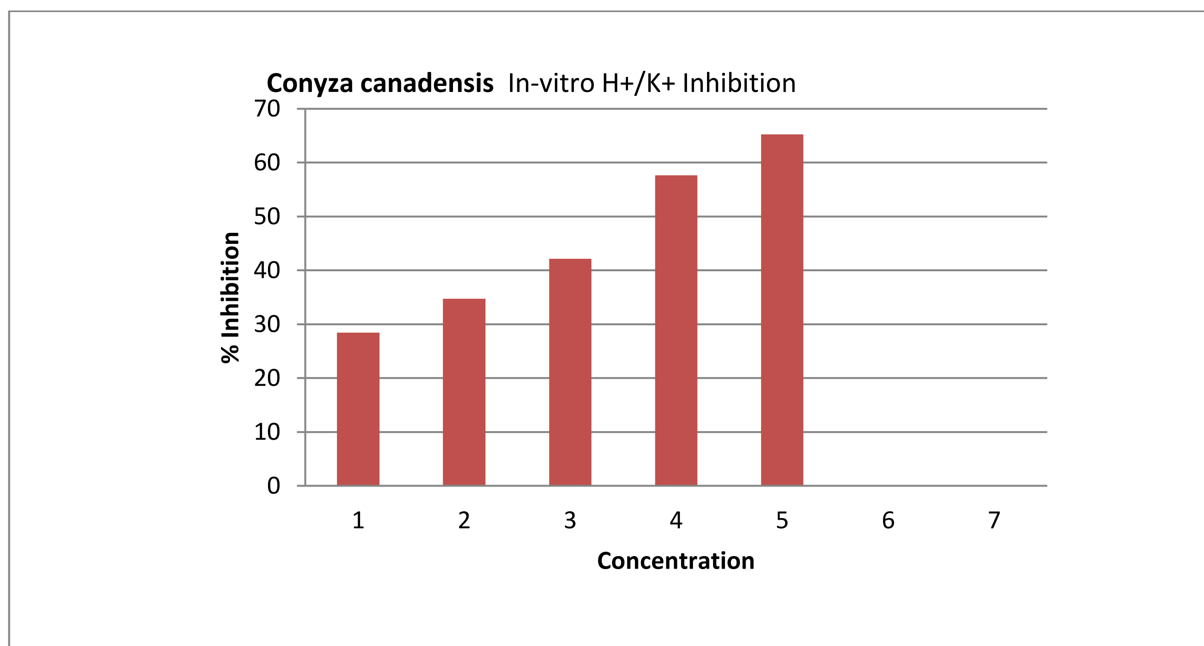

Figure 12. Effect Of Conyza Canadensis On In-Vitro H+/K+ - Atpase Inhibition Activity

Results:-

Anti-ulcer Activity

The leaf extract of Conyza canadensis exhibited significant anti-ulcer activity in experimental models. A dose-dependent reduction in ulcer index was observed in treated groups compared to the control. The extract significantly decreased gastric volume and acidity while increasing gastric pH, indicating antisecretory effects. Additionally, enhanced mucus production and histopathological findings confirmed protection of the gastric mucosa with reduced inflammation and tissue damage.

Conclusion:-

Anti-ulcer Activity

The present study concludes that the ethanolic extract of Conyza canadensis Linn. exhibits significant in vitro antiulcer activity, as demonstrated by its performance in the acid-neutralizing capacity (ANC) assay and the H^+/K^+-ATPase inhibition assay. The extract effectively neutralized gastric acid and inhibited proton pump activity, showing results comparable to, and in certain parameters exceeding, those of the standard drug omeprazole. The observed antiulcer potential of Conyza canadensis may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and saponins, which are known to contribute to gastric mucosal protection and reduction of gastric acidity. However, to substantiate its therapeutic applicability, further detailed in vitro, in vivo, and clinical studies are warranted to isolate the active constituents, elucidate their mechanisms of action, and evaluate their safety and efficacy, thereby supporting the development of Conyza canadensis as a potential natural antiulcer agent.

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