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Comparative Study of Conventional Extract and Nanoparticles of *Cynodon dactylon* for Gastric Acid Neutralization

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ABSTRACT:

Peptic ulcer disease and gastric hyperacidity are common gastrointestinal disorders caused by excessive gastric acid secretion, stress, irregular food habits, and prolonged use of non- steroidal anti-inflammatory medicines. Hence, herbal medication s with better safety and efficacy have gained significant attention. The present study aimed to evaluate and compare the gastric acid neutralizing potential of conventional extract and nanoparticle formulation of *Cynodon dactylon*. Fresh plant material was collected, authenticated, shade dried, powdered, and extracted by decoction method. UV-visible spectroscopy and FTIR analysis were used to characterise the produced nanoparticles in order to verify their production and pinpoint the functional groups involved in stabilisation. ANC was evaluated for both conventional extract and nanoparticle. The findings revealed that the nanoparticle formulation exhibited significantly higher ANC than the conventional extract, indicating enhanced antacid activity.

INTRODUCTION:

A significant worldwide health burden is caused by gastrointestinal illnesses, especially those linked to excessive stomach acid output, such as gastritis, peptic ulcers, and GERD. When this equilibrium is upset, the stomach mucosa is vulnerable to harm, which can result in inflammation and ulcers. Synthetic medications are frequently used to treat acid-related gastrointestinal conditions. These medications are very successful at relieving symptoms and encouraging mucosal healing, but prolonged use of them is frequently linked to a number of drawbacks and negative effects. [1-5] The need for herbal alternatives is further strengthened by the growing interest in integrating

traditional medicine with modern scientific approaches. Recent developments in nanotechnology have made it possible to create herbal formulations based on nanoparticles, which can enhance the stability, bioavailability, and targeted administration of chemicals obtained from plants. These developments lower the necessary dosage and related negative effects while simultaneously improving therapeutic efficacy. [6-13] Thus, investigating herbal medications especially when combined with cutting-edge drug delivery systems represents a viable and sensible strategy for the efficient and secure treatment of acid-related illnesses. Herbal medicines are promising substitutes for manmade drugs because of their many benefits. Since they come from natural sources, they typically have higher tolerance and fewer adverse effects, making them appropriate for long-term use. Additionally, a wide range of bioactive substances found in herbal remedies have synergistic therapeutic benefits through a variety of pathways, including gastroprotective, antibacterial, anti-inflammatory, and antioxidant properties. This multi-targeted strategy addresses the disease's symptoms as well as its underlying causes by boosting mucosal defence, promoting healing, and lowering gastric acid output. [14-20]

Cynodon dactylon, a perennial creeping herb of the Poaceae family, is also referred to as "Doob grass" or Bermuda grass. Ayurvedic and traditional medical systems have traditionally used *Cynodon dactylon* to treat a variety of ailments, such as diabetes, gastrointestinal problems, wounds, infections, and inflammation. Its broad therapeutic potential is due to the presence of numerous bioactive phytoconstituents. Certain phytochemicals present in *Cynodon dactylon* are believed to neutralize excess gastric acid and promote the secretion of protective mucus.

Silver nanoparticles (AgNPs) are nanoscale silver particles that have distinct physicochemical and biological characteristics that set them apart from bulk silver. Their size typically ranges from 1 to 100 nm. AgNPs have better surface energy, increased reactivity, and better interaction with biological systems because of their minuscule size and high surface area-to-volume ratio. Their nanoscale size (1–100 nm) allows for effective targeting of the stomach mucosa because to its huge surface area, enhanced reactivity, and increased contact with biological membranes. Nanoparticles have two functions in the context of gastric acid neutralization: they either directly buffer excess acid or improve the transport and effectiveness of therapeutic medicines that lower acid secretion and shield the stomach lining. This is particularly beneficial in conditions such as Gastritis and Gastroesophageal reflux disease. [21-30]

OBJECTIVES [31,32]

- To synthesize nanoparticles (especially silver nanoparticles) utilizing the plant extract through a green synthesis method.
- To study the physical and chemical properties of the prepared nanoparticles using standard characterization techniques.
- To evaluate the acid neutralizing ability of both the plant extract and its nanoparticle formulation.
- To compare the results obtained from conventional extract and nanoparticle formulation.
- To apply statistical methods to validate and interpret the experimental data.
- To determine whether nanoparticle formulation provides better efficiency than the conventional extract.

Plant Profile:

Fig. no. 1: Arial parts/leaves of *Cynodon dactylon*

Botanical Name: *Cynodon dactylon*

Family: Poaceae (Grass family)
Common Name: Bermuda grass, Doob, Durva, Harali

Worldwide, *Cynodon dactylon* is found in many tropical and subtropical areas. It grows abundantly in India, Africa, Asia, Europe, and America. [11-20]

Table no. 1: Morphological Characteristics of *C. dactylon*

Color	Green (fresh), pale green (dried)
Odor	Characteristic, slightly grassy
Taste	Slightly bitter and astringent
Texture	Smooth, fibrous
Appearance	Creeping grass with nodes and narrow leaves

Cynodon dactylon's important pharmacological qualities are attributed to a variety of bioactive phytoconstituents that are dispersed throughout its different portions. The plant's antioxidant and anti-inflammatory properties are mainly due to its abundance of flavonoids, alkaloids, glycosides, tannins, and phenolic chemicals. In particular, the leaves include vital minerals, proteins, carotenoids, chlorophyll, and flavonoids like luteolin and apigenin. [14-16]

Table no. 2: Chemical Constituents in Different Parts of *Cynodon dactylon*

Sr. no.	Plant Part	Chemical Constituents
1	Whole Plant	Flavonoids, alkaloids, glycosides, tannins, phenolic compounds
2	Leaves	Flavonoids (apigenin, luteolin), chlorophyll, carotenoids, proteins, minerals
3	Stem	Carbohydrates, fibers, phenolic compounds
4	Roots	Alkaloids, saponins, glycosides, triterpenoids
5	Rhizomes/Stolons	Sugars, starch, essential nutrients

Methodology:

1. Collection and Authentication

Fresh *Cynodon dactylon* entire plant material was gathered from Sangli, Maharashtra, the plant specimen was authenticated by a qualified botanist, and the authenticated sample was preserved for future reference. [11-13]

2. Preparation of Aqueous Extract

About 100 grams of dried powdered *C. dactylon* were added to a clean beaker containing
1.0 liter of distilled water. The mixture was boiled on a water bath at 60–70°C for two to three hours while being continuously stirred to ensure proper phytoconstituent extraction. The combination was heated, let to cool to ambient temperature, and then left undisturbed for a full day in order to achieve full extraction. The extract was initially filtered using muslin cloth to remove coarse plant particles in order to obtain a clean filtrate. Whatman No. 1 filter paper was then used to filter it again. The filtrate was concentrated in a water bath with a controlled temperature. The concentrated extract was transferred into a sterile glass container and refrigerated at 4°C for further study. [8-10]

3. Phytochemical Screening

The aqueous extract was first screened for phytochemicals using standard qualitative chemical assays. [8-10]

3.1 Test for Alkaloids

After adding diluted hydrochloric acid to around 2 mL of the extract, it was filtered. A few drops of Mayer's reagent were added to the filtrate. When a cream-colored precipitate developed, alkaloids were present.

3.2 Test for Flavonoids

Two ml of extract were mixed with a few drops of strong hydrochloric acid and magnesium turnings. The emergence of pink or reddish coloring indicated the presence of flavonoids.

3.3 Test for Tannins

A few drops of ferric chloride solution were added to two ml of extract. Tannins were present when a dark blue or greenish-black coloration formed.

3.4 Saponins

In a test tube, around 5 mL of extract was quickly shaken with distilled water. The presence of saponins was shown by persistent froth formation.

3.5 Glycosides

The extract was carefully mixed with glacial acetic acid, ferric chloride solution, and strong sulphuric acid. The

development of a brown ring at the intersection confirmed the presence of glycosides.

3.6 Phenolic Compounds

A few drops of ferric chloride solution were combined with the extract. The emergence of a bluish-black colour indicated the presence of phenolic compounds.

3.7 Carbohydrates

After adding the Molisch reagent to two ml of extract, the test tube's sides were treated with concentrated sulphuric acid. A violet ring indicated the presence of carbohydrates.

3.8 Proteins

The extract was heated after few drops of ninhydrin reagent were added. The violet hue of proteins proved their existence.

4. Characterization of Aqueous Extract

4.1 Organoleptic Evaluation

Under typical lighting circumstances, the produced aqueous extract was visually inspected for colour, odour, texture, and appearance. [8-9]

4.2 pH

A pH meter that had been calibrated was used to determine the extract's pH. The electrode was submerged in a beaker containing around 10 mL of extract in order to measure the pH.

5. Synthesis of AgNPs

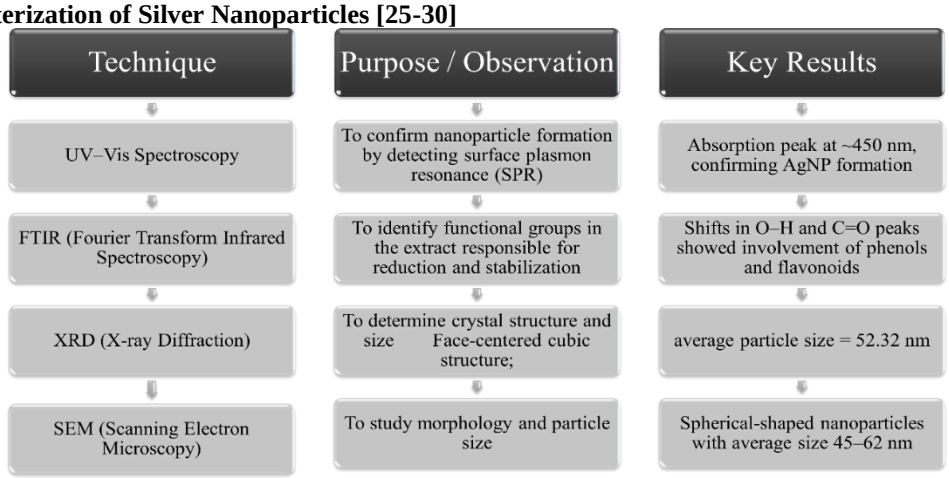
A 1 mM silver nitrate solution was made by dissolving precisely weighed silver nitrate in distilled water. 10 ml of aqueous plant extract were progressively added to ninety ml of silver nitrate solution while being continuously stirred magnetically. The reaction mixture was shielded from direct light and kept at ambient temperature. For thirty to sixty minutes, stirring was done continuously. The transition from pale yellow to dark brown signified the reduction of silver ions and the synthesis of silver nanoparticles. The reaction mixture was incubated for an entire day to ensure complete nanoparticle production. [21-30]

Formulation table:

Table no. 3: Formulation table for *C. dactylon's* AgNPs Synthesis

Sr. No.	Ingredients	Quantity	Purpose
1	Plant extract of *Cynodon dactylon*	10 ml	Reducing and stabilizing agent
2	Silver nitrate ($AgNO_3$) solution (For 100 ml: 0.0169 g $AgNO_3$)	90 ml	Silver ion source
3	Distilled water	q.s.	Solvent/preparation medium
4	Temperature	40–60°C	Enhances nanoparticle formation
5	pH	7–9	Stabilizes nanoparticlesynthesis

6. Characterization of Silver Nanoparticles [25-30]



7. Stability Study of Extract and Nanoparticles

Stability studies were performed for both aqueous extract and synthesized nanoparticles under refrigerated conditions. Samples were stored in tightly closed containers and evaluated periodically at 0, 15, 30, and 60 days. For nanoparticle formulations, particle size and zeta potential were also measured at regular intervals to evaluate aggregation and stability behavior during storage. Any significant physical or physicochemical changes observed

during the study period were documented systematically. [31,32]

8. Acid Neutralizing Capacity (ANC) Study

8.1 ANC Study for Solid Nanoparticles

The acid neutralization capacity (ANC) study of solid nanoparticle formulation was carried out by accurately weighing a specific quantity of the nanoparticle sample (250,500,1000mg) and mixing it with a fixed volume of standardized hydrochloric acid solution (until pH reaches to 3). After constant stirring, the mixture was left to react for a predetermined amount of time. Following the reaction's completion, phenolphthalein was used as an indicator to titrate the surplus acid against a standardized sodium hydroxide solution until a persistent pale pink endpoint was reached. The ANC was computed in mEq/g and the amount of sodium hydroxide consumed was recorded. [39]

8.2 ANC Study for Liquid Nanoparticles

The acid neutralization capacity (ANC) study of liquid nanoparticle formulation was performed by taking a measured volume of the liquid sample (2,5,10 ml) and adding it to a fixed volume of standardized hydrochloric acid solution (10 ml). The mixture was stirred and allowed to react completely. The excess acid remaining after the reaction was titrated with standardized sodium hydroxide solution using phenolphthalein as an indicator until the endpoint was reached. The volume of sodium hydroxide used was recorded, and the ANC was calculated in terms of mEq/ml.

8.3 Formulae for ANC calculation: [39]

1. $\text{ANC (mEq/g)} = \frac{(V1-V2) \times N}{W}$

2. $\text{ANC (mEq/g)} = \frac{(V1-V2) \times N}{V}$

9. Comparative Evaluation

Comparative evaluation between aqueous extract and synthesized silver nanoparticles was carried out based on characterization parameters, stability profile, and acid neutralizing capacity. The obtained ANC values of both formulations were compared to determine enhancement in gastric acid neutralizing activity. Physical stability and nanoparticle characteristics were also analyzed comparatively to evaluate formulation effectiveness.

10. Statistical Analysis

To guarantee that the results could be repeated, each experiment was conducted at a varied concentration. Appropriate analytical techniques were used for statistical analysis in order to compare various formulations. Comparative statistical evaluation was used to determine the significance of differences between aqueous extract and nanoparticle formulations.

11. Molecular Docking

Protein, ligand preparation, and validation

The molecular docking study was performed using the H^+/K^+ ATPase pump (PDB ID: 5YLU) as the target receptor. The protein structure was downloaded, purified by removing water molecules and bound ligands, and prepared by adding polar hydrogen atoms using BIOVIA Discovery Studio. The phytoconstituent apigenin was obtained from the PubChem database. Molecular docking was carried out using AutoDock Vina in PyRx 0.8 after converting both protein and ligand structures into PDBQT format and performing energy minimization. The best docking pose was selected based on binding energy, and protein– ligand interactions were visualized using BIOVIA Discovery Studio. [34–38]

RESULTS AND DISCUSSION

Collection, drying and Authentication of Plant Material. (*Cynodon dactylon*)

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Fig. No. 2: Collection of leaves of *C. dactylon*

Fig. no. 3: Drying

Fig.no. 5: Authentication letter

Fig. no. 4: Powder

2. Preparation & Characterization of Aqueous Extract

Fig.no. 6: Decoction

Fig.no. 7: Filtration

Fig. no.9: Stored in container

Fig. no. 8: Obtained Extract

2.1 Percentage Yield calculation:

$$\text{Percentage yield (\%)} = \frac{\text{Volume of Liquid extract Obtained}}{\text{Weight of the powder drug taken}} \times 100$$

$$\text{Percentage yield (\%)} = \frac{32}{100} \times 100$$

$$\text{Percentage yield (\%)} = 32\%$$

2.2 Visual (organoleptic) Observation of *Cynodon dactylon* Extract

Table no. 4: Visual (organoleptic) Observation of *Cynodon dactylon* Extract

Sr. No.	Parameter	Observation
1	Color	Yellowish -Brown
2	Odor	Characteristic grassy odor
3	Appearance	Clear solution
4	Consistency	Thin liquid
5	Taste	Slightly bitter
6	pH	Slightly acidic to neutral

These findings support the initial assessment prior to additional investigation and validate the existence of phytoconstituents in *Cynodon dactylon*.

2.3 Phytochemical screening:

Several significant bioactive components were found in the aqueous extract of *Cynodon dactylon*, according to preliminary phytochemical screening.

Table no. 5: Phytochemical Screening of *Cynodon dactylon* Extract

Sr. No.	Phytochemical Class	Test	Observation	Result
1	Alkaloids	Mayer's test Dragendorff's	Cream ppt Orange red ppt	+ +
2	Flavonoids	Alkaline reagent test Shinoda test	Yellow disappears after HCL Pink / Red coloration	+ +
3	Phenols	FeCL3 test	Dark green coloration	+
4	Tannins	FeCL3 test Lead Acetate test	Blue-green / Black coloration Yellow-White ppt	+ +
5	Saponins	Foam test	Persistent froth	+
6	Glycosides	Keller–Killiani test	Brown ring formation	+
7	Terpenoids	Salkowski test	Reddish-brown interface	–
8	Steroids	Liebermann–Burchard test	Green / Blue coloration	–
9	Carbohydrates	Molisch's test	Violet ring formation	+
10	Proteins	Biuret / Ninhydrin test	Violet / Blue coloration	+

"+" indicates presence of phytoconstituents "–" indicates absence of phytoconstituents

Fig. no. 10: Phytochemical tests

2.4 FTIR of *Cynodon dactylon* Extract:

The FTIR spectrum of *Cynodon dactylon* confirmed the presence of phenols, flavonoids, proteins, aromatic compounds, and carbohydrates, indicating bioactive phytochemicals responsible for its therapeutic activity.

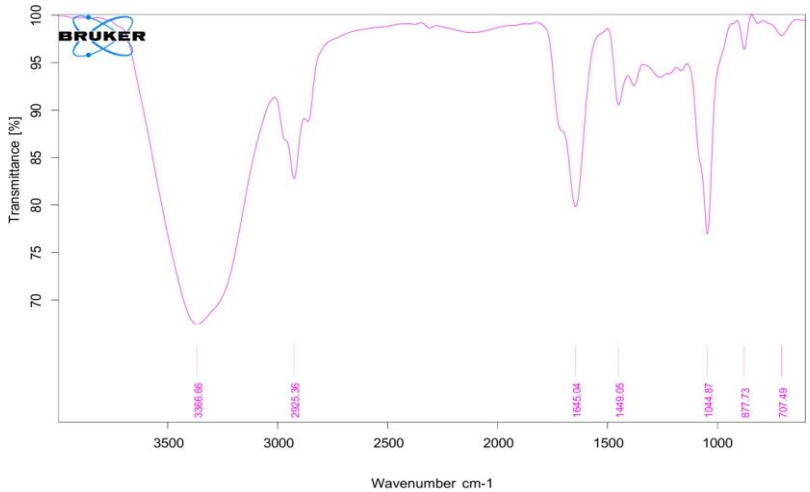

Fig. no. 11: FTIR of *Cynodon dactylon* Extract

Table no. 6: Interpretation of FTIR of *C. dactylon* Extract

Wavenumber (cm^{-1})	Functional Group	Type of Vibration	Possible Compounds	Interpretation
3366.66	O–H (Alcohols/Phenols)	Stretching (broad)	Flavonoids, Phenolic compounds	Indicates antioxidant and gastroprotective compounds
2925.36	C–H (Alkanes)	Stretching	Organic compounds	Represents plant metabolites
1645.04	C=O / C=C	Stretching	Proteins, Amides, Conjugated compounds	Suggests presence of bioactive molecules
877.73	C–H (Aromatic)	Bending	Aromatic compounds	Supports presence of secondary metabolites
707.49	C–H (Aromatic)	Bending	Flavonoids, Phenolic compounds	Indicates antioxidant and gastroprotective compounds

3. Synthesis & Characterization of AgNPs:

3.1 Synthesis of AgNPs:

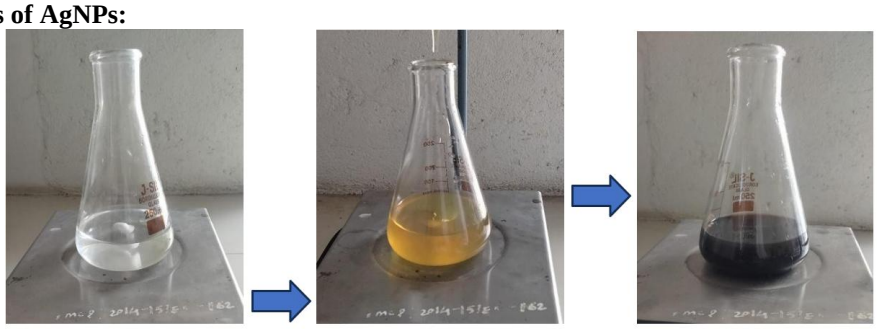

Fig. no. 12: Synthesis of AgNPs

Successful synthesis is indicated by a noticeable hue shift from bright yellow to dark brown. A hue shift indicates the reduction of Ag^{+} ions to silver nanoparticles.

3.2 Characterization of AgNPs

a) UV–Visible Spectroscopy

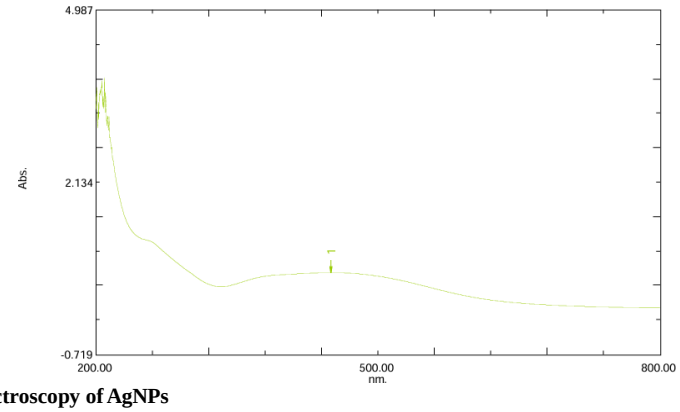

Fig. no. 13: UV–Visible Spectroscopy of AgNPs

Peak usually occurs in the 450 nm range. verifies the production of silver nanoparticles.

b) FTIR Analysis

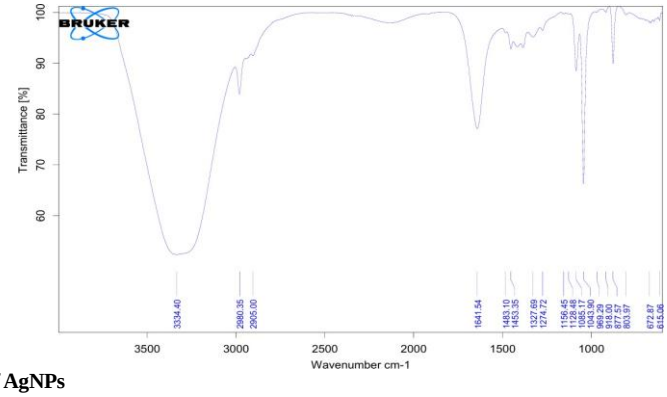

Fig. no. 14: FTIR Analysis of AgNPs

The presence of phenols, proteins, and carbohydrates—which serve as stabilizing and reducing agents—is confirmed by FTIR, indicating that the synthesis of silver nanoparticles from *Cynodon dactylon* was effective.

Table no. 7: Interpretation of FTIR of *C. dactylon* AgNPs

Wavenumber (cm^{-1})	Functional Group	Type of Vibration	Possible Compounds	Interpretation
3334.40	O–H (Alcohols/Phenols)	Stretching (broad)	Flavonoids, phenolics	Reducing &stabilizing agents
2980.35	C–H (Alkanes)	Stretching	Organic compounds	Plant metabolitespresent
1641.54	C=O (Amide I)	Stretching	Proteins, enzymes	Capping & stabilization
1453.35	C–H bending	Bending	Organic compounds	Structural components
1043.90	C–O	Stretching	Polysaccharides	Supports carbohydrates
615.06	Ag–O	Stretching	Silver nanoparticles	Confirms AgNPs

c) Particle Size Analysis

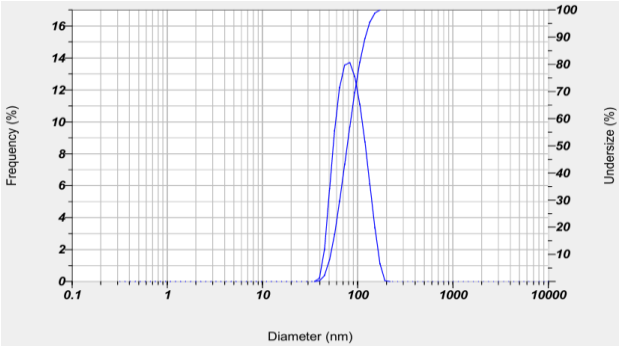

Fig. no. 15: Particle Size Analysis of AgNPs

Nanoparticles observed in nano range 82.2nm (typically 10–100 nm).

d) Zeta Potential

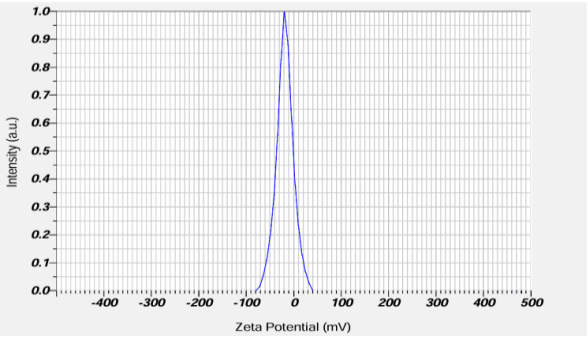

Fig.no. 16: Zeta Potential of AgNPs

Zeta potential of –37.2 mV indicates highly stable nanoparticles with strong repulsion and minimal aggregation.

e) SEM Analysis

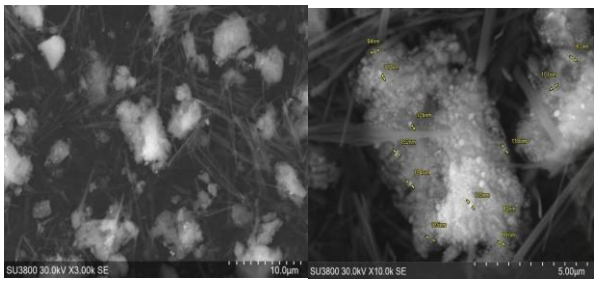

Fig. no. 17: SEM Analysis of AgNPs

The SEM micrograph shows the formation of nanoparticles with particle sizes predominantly in the range of 80–140 nm, confirming successful size reduction. The measured values (e.g., 80 nm, 91 nm, 104 nm, 118 nm, 126 nm, 132 nm, and 135 nm) indicate a narrower size distribution, suggesting improved control over the synthesis process.

4. Stability Study

The stability study of the prepared extract and synthesized nanoparticles was carried out over a specified period. The formulation showed no significant change in physical appearance, with no visible aggregation, precipitation, or color change observed throughout the study. The pH remained nearly constant, indicating chemical stability of the system. In the case of nanoparticles, particle size analysis revealed no considerable increase in size, confirming the absence of aggregation. Zeta potential values were found to be relatively stable, suggesting good dispersion stability. Overall, the formulation remained stable during the study period, with no signs of degradation or instability under the tested conditions.

4.1 Stability Study of *Cynodon dactylon* Aqueous Extract

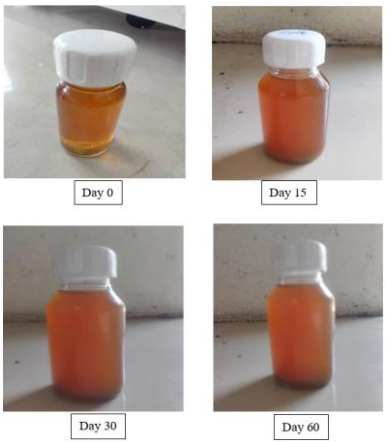

Fig. no. 18: Visual Appearance of *C. dactylon* Extract at day 0, 15, 30 & 60

Table no. 8: Observation Table for Stability study of *C. dactylon* Extract

Parameter	Color	Odor	Appearance	pH	Precipitation
Day 0	Bright yellowish Brown	Characteristic	Clear	6.5	Absent
Day 7	No change	No change	Clear	6.4	Absent
Day 15	Slight dull	No change	Slight turbidity	6.3	Slight trace
Day 30	Dull yellow	Slightly reduced	Slight turbidity	6.1	Slight trace
Day 60	Brown	Slightlyreduced	Turbid	5.8	Trace

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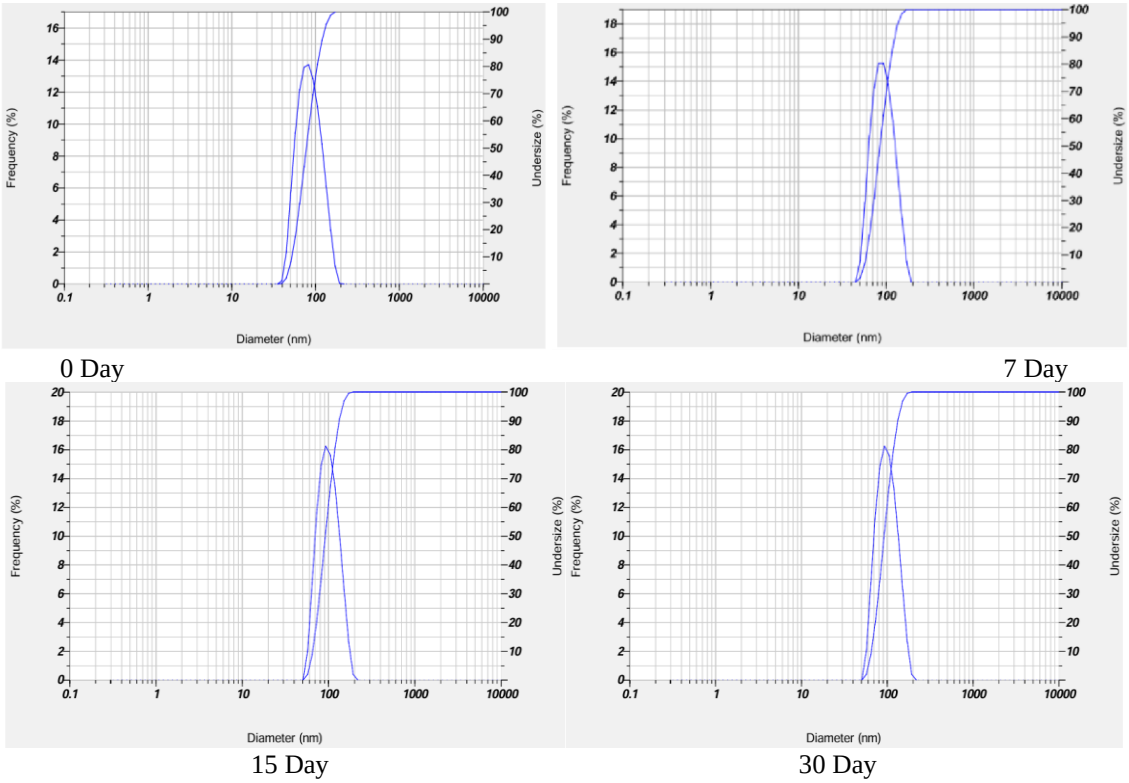
4.2 Stability Study of Silver Nanoparticles

Fig. no. 19: Visual Appearance of C. dactylon AgNPs at day 0, 15, 30 & 60

Table no. 9: Observation Table for Stability study of *C. dactylon* NPs

Parameter	Color	Appearance	pH	Particle Size (nm)	Zeta Potential (mV)
Day 0	Brown	Clear	7.0	82.2	-37.2
Day 7	No change	Clear	6.9	84.0	-32.4
Day 15	No change	Clear	6.9	89.2	-30.1
Day 30	No change	Clear	6.8	91.7	-28.8
Day 60	No change	Slight turbidity	6.3	102.6	-25.3



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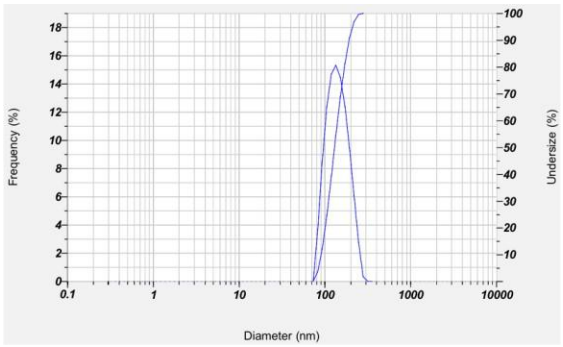
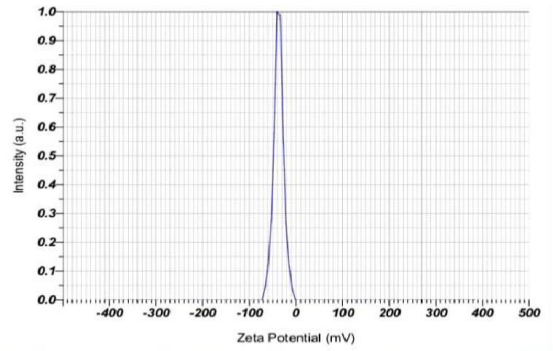
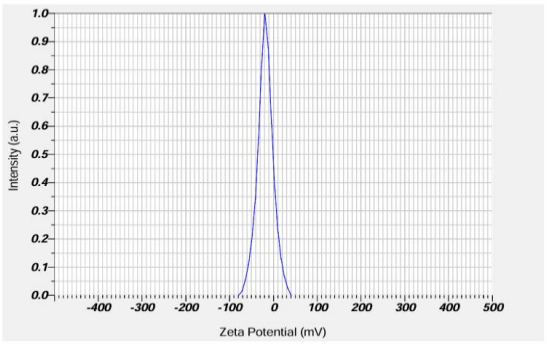


60 Day

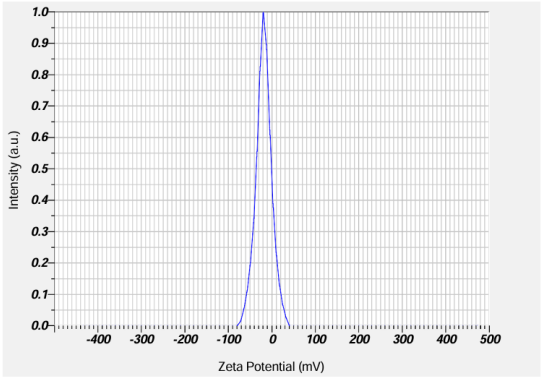
Fig. no. 20: Particle size analysis of AgNPs at Day 0, 7, 15, 30 & 60



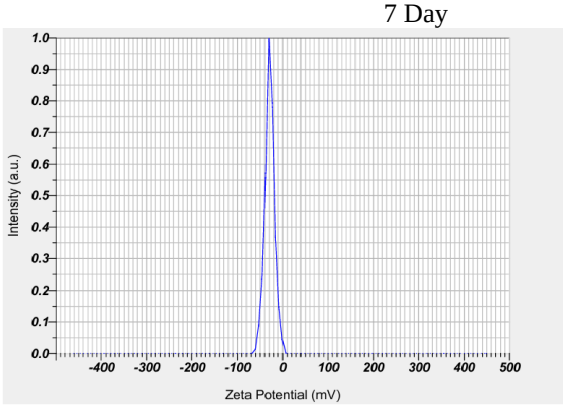
0 Day



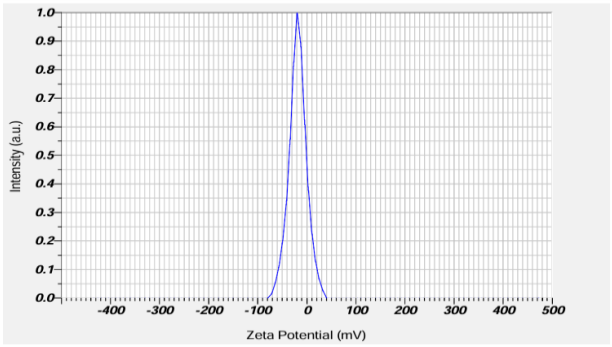
7 Day



15 Day



30 Day



60 ay

Fig. no. 21: Zeta Potential analysis of AgNPs for Stability study

5. Gastric acid neutralization Capacity (ANC Value)

The acid neutralization capacity (ANC) of *Cynodon dactylon* extract and its silver nanoparticles was evaluated and compared with a standard antacid. The nanoparticles showed higher ANC than the extract at all concentrations, indicating improved acid- neutralizing ability. Although the standard exhibited maximum activity, the nanoparticles showed performance closer to it. The enhanced activity of nanoparticles may be due to increased surface area and better interaction with gastric acid, confirming their potential as an effective alternative.

5.1 ANC results (Solid nanoparticles) along with extract and standard

Table no. 10: Observation Table for ANC results (Solid nanoparticles)

Sr.no	Sample	Conc. (mg)	NaOH (mL)	mEq Acid Consumed	ANC (mEq/g)
1	Control	–	35.6	0.44	N/A
2	$Al(OH)_3 + Mg(OH)_2$	250	8.2	3.18	12.72
		500	4.1	3.59	7.18
		1000	2.6	3.74	3.74
3	*Cynodon dactylon* AgNPs	250	18.6	2.14	8.56
		500	15.8	2.42	4.84
		1000	13.2	2.68	2.68
4	*Cynodon dactylon* Extract	250	24.3	1.57	6.28
		500	21.5	1.85	3.70
		1000	19.2	2.08	2.08

Fig. no. 22: *Cynodon dactylon* Extract results

Fig.no. 23: *Cynodon dactylon* AgNPs

The results demonstrate that *Cynodon dactylon* AgNPs possess superior acid neutralization capacity compared to the conventional extract and exhibit activity closer to the standard antacid, highlighting their potential as an effective alternative for gastric acid neutralization. AgNPs showed higher ANC than extract and closer performance to standard, confirming enhanced effectiveness of nanoparticle formulation.

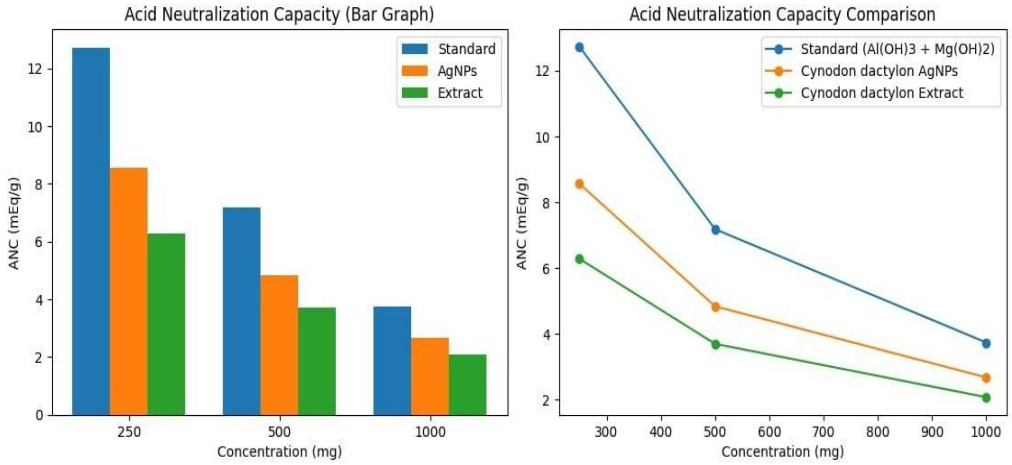


Fig. no. 24: Graphical representation of ANC results (Solid nanoparticles)

5.2 ANC results (Liquid nanoparticles) along with extract and standard:

Table no. 11: Observation Table for ANC results (Liquid nanoparticles):

Sample	Initial Acid Volume (mL)	NaOH Used (mL)	ANC (mEq/mL)	Observation
Extract (2 ml)	10	4.3	5.7	Moderate activity
Extract (5 ml)	10	3.7	6.3	Increased activity
Extract (10 ml)	10	2.9	7.1	Good neutralization
Liquid NPs (2 ml)	10	3.4	6.6	Better than extract
Liquid NPs (5 ml)	10	2.6	7.4	Enhanced activity
Liquid NPs (10 ml)	10	1.8	8.2	Highest activity
Standard (Antacid)	10	1.5	8.5	Maximum neutralization

Fig. no. 25: *Cynodon dactylon* Extract results Fig.no. 26: *Cynodon dactylon* AgNPs results

The liquid nanoparticle formulation showed activity close to the standard, indicating its potential as an effective alternative for gastric acid neutralization. Liquid nanoparticles showed higher ANC than extract and near-standard activity, indicating improved acid neutralization efficiency.

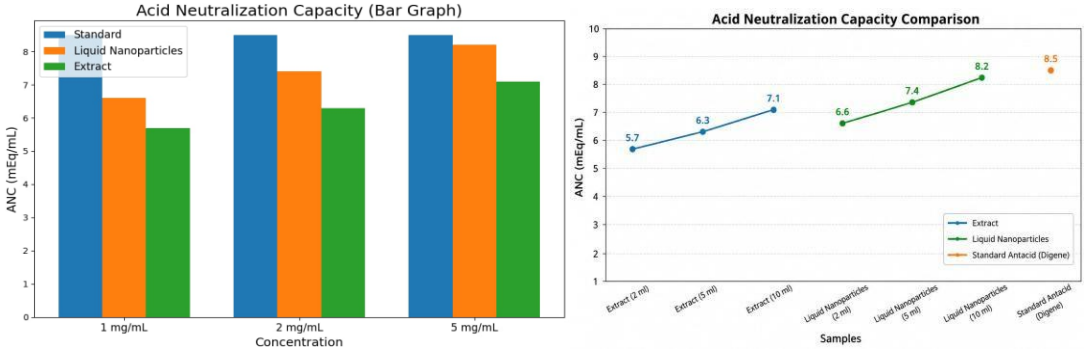


Fig. no. 27: Graphical representation of ANC results (Liquid Nanoparticles)

6. Comparative Evaluation of Aqueous Extract and Silver Nanoparticles of *Cynodon dactylon*

Table no. 12: Comparative Evaluation of Aqueous Extract and AgNPs of *Cynodon dactylon*

Sr. no.	Parameter	Aqueous Extract	Silver Nanoparticles
1	Color	Yellowish-brown solution	Dark brown solution
2	Particle Size	Large and irregular particles	Uniform nano-sized particles (1–100 nm)
3	Stability	Slightly decreased over time	Comparatively stable
4	pH Stability	Minor variation in pH over time	pH remained nearly constant
5	Bioavailability	Normal absorption of phytoconstituents	Enhanced absorption and bioavailability
6	Therapeutic Activity	Moderate pharmacological activity	Improved therapeutic effectiveness
7	Acid Neutralizing Capacity	Lower ANC values	Higher ANC values
8	Toxicity	Safe but may require high dose	Effective at lower concentrations
9	Overall Effectiveness	Effective	More effective and stable

7. Statistical Analysis of ANC Results

7.1 For Extract & Solid nanoparticles

The ANC results of the standard antacid, *Cynodon dactylon* AgNPs, and *Cynodon dactylon* extract were analyzed descriptively based on different concentrations (250, 500, and 1000 mg).

Table no. 13: Statistical Analysis of ANC Results (For Extract & Solid nanoparticles)

Sample	Mean ANC (mEq/g)	Standard Deviation (SD)	Observation

$Al(OH)_3 + Mg(OH)_2$	7.88	4.51	Highest ANC activity
Cynodon dactylon AgNPs	5.36	3.00	Better activity than extract
Cynodon dactylon Extract	4.02	2.13	Lowest ANC among test samples

The statistical comparison revealed that *Cynodon dactylon* AgNPs possessed higher mean ANC values than the conventional extract, indicating improved antacid activity due to nanoparticle formulation. However, the standard antacid exhibited the greatest neutralizing capacity overall.

7.2 For Extract & Liquid Nanoparticles:

The Acid Neutralizing Capacity (ANC) of *Cynodon dactylon* liquid extract, liquid nanoparticle formulation, and standard antacid was statistically analyzed using mean and standard deviation values.

Table no. 14: Statistical Analysis of ANC Results (For Extract & Liquid nanoparticles)

Sample	ANC Values (mEq/mL)	Mean ± SD	Observation
Liquid Extract	5.7, 6.3, 7.1	6.37 ± 0.70	Lowest ANC among test samples
Liquid AgNPs	6.6, 7.4, 8.2	7.40 ± 0.80	Better activity than extract
Standard Antacid	8.5	8.5	Highest ANC activity

Statistical evaluation showed that the liquid NPs formulation of *Cynodon dactylon* possessed superior acid neutralization capacity compared to the conventional extract, while the standard antacid exhibited the highest overall activity.

8. Molecular docking study

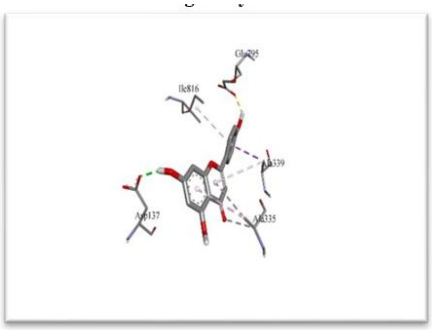


Fig. no. 28: YLU Docked 3D

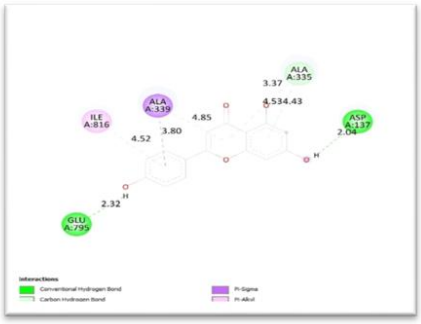


Fig. no. 29: YLU-Ligand 1 2D

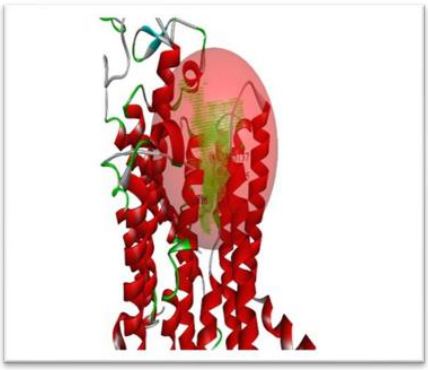


Fig. no. 30: YLU docked 3D

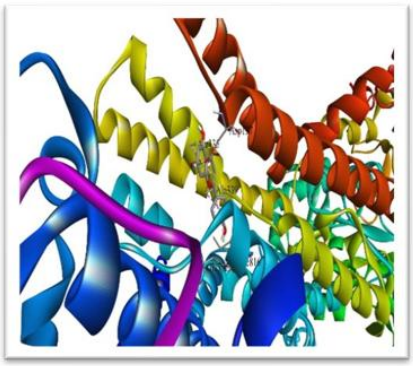


Fig. no. 31: YLU-docked 3D

Table no. 15: Observations of Binding affinity

Sr. no.	Ligand	Binding Affinity	rmsd/ub	rmsd/lb

1	**5YLU_5280443_uff_E=233.26**	**-9.1**	**0**	**0**
2	5YLU_5280443_uff_E=233.26	-8.8	2.936	1.189
3	5YLU_5280443_uff_E=233.26	-8.6	2.993	1.403
4	5YLU_5280443_uff_E=233.26	-7.9	3.022	1.954
5	5YLU_5280443_uff_E=233.26	-7.9	17.38	15.74
6	5YLU_5280443_uff_E=233.26	-7.5	7.783	2.63
7	5YLU_5280443_uff_E=233.26	-7.4	8.697	4.1
8	5YLU_5280443_uff_E=233.26	-7.4	18.107	16.627
9	5YLU_5280443_uff_E=233.26	-7.3	4.808	3.235

Table no. 16: Showing Observations for Binding energy and Type of interaction

Name of phytochemical constituent	5YLU			
	Binding energy (Kcal/mol)	Interacting residue	Distance	Type of interaction
Apigenin	-9.1	GLUA:795	2.32	Conventional HB
		ILEA:816	4.52	π-alkyl
		ALAA:339	3.80	π-σ
		ALAA:339	4.85	π-alkyl
		ALAA:335	4.53	π-alkyl
		ALAA:335	4.43	π-alkyl
		ASPA:137	2.04	Conventional HB

The molecular docking study of Apigenin with the target protein having PDB ID 5YLU demonstrated a strong binding affinity with a docking score of −9.1 kcal/mol, indicating favorable ligand–protein interaction and stable complex formation. The ligand formed conventional hydrogen bonds with key amino acid residues GLU A:795 and ASP A:137 at bond distances of 2.32 Å and 2.04 Å, respectively, contributing significantly to complex stabilization. Additional interactions included carbon hydrogen bonding with ALA A:335 and hydrophobic Pi-Sigma and Pi-Alkyl interactions with ALA A:339 and ILE A:816.

These interactions suggested that apigenin was well accommodated within the active binding pocket of the receptor through both hydrogen bonding and hydrophobic interactions. The presence of multiple stabilizing interactions along with low binding energy supports the potential biological and therapeutic significance of apigenin.

Summary:
This study compared the gastric acid neutralization activity of conventional extract and nanoparticle formulations of *Cynodon dactylon*. Silver nanoparticles were successfully synthesized using a green synthesis method and characterized by UV-Visible spectroscopy and FTIR analysis. The Acid Neutralization Capacity (ANC) study showed that both the extract and nanoparticles possessed antacid activity, with nanoparticle formulations exhibiting significantly higher ANC values. Molecular docking studies also demonstrated favorable interactions of phytoconstituents with the H^+/K^+ ATPase target protein, indicating potential gastroprotective activity. Overall, nanoparticle formulations showed enhanced gastric acid neutralization compared to the conventional extract.
Conclusion

When compared to the traditional plant extract, the current study found that the *Cynodon dactylon* nanoparticle formulation exhibited better gastric acid neutralization action. Because of their larger surface area and better bioavailability, the nanoparticles demonstrated superior physicochemical characteristics and increased functional activity. The ANC study clearly indicated that both solid and liquid nanoparticle formulations showed greater acid neutralizing capacity than the conventional extract and produced results comparable to standard antacid preparations. Furthermore, molecular docking studies supported the experimental findings by demonstrating effective interaction of phytoconstituents with gastric disorder-related target proteins. The combined results of characterization studies, ANC evaluation, and molecular docking confirmed the potential of the nanoparticle formulation as an effective herbal antacid system. Thus, the study emphasizes the significance of nanotechnology in augmenting the therapeutic value of herbal remedies and proposes that formulations of *Cynodon dactylon* nanoparticles could be a viable substitute for neutralizing gastric acid and treating gastric disorders with enhanced efficacy and possible safety.

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