"Wound Healing Activity of *Andrographis paniculata* Using Experimental Animal Models"

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ABSTRACT

Objective:

The current study was aimed to evaluate the wound healing activity of *Andrographis paniculata.* extract on experimental animal.

Materials and Methods:

An ethanolic extract of *Andrographis paniculata* leaves was for assessment of wound healing activity. The plant material was standardized by employing macroscopical features, phytochemical analysis, and ash value calculation methods.

Results:

Phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, glycosides, tannins, phenolic compounds, and flavonoids. Furthermore, the total ash value, water-soluble ash, and acid-insoluble ash values were found to be acceptable range. The results of wound healing activity studies confirmed that ethanolicic extract of *Andrographis paniculata* leaves wound healing process by decreasing the surface area of the wound and increases the muscle tensile strength, out of tested concentration showed comparative activity with the standard. The extract exhibited highly significant ($P < 0.001$) wound healing activity.

Conclusion

The results of the study indicate the presence of potent wound healing activity in the ethanolic extract of *Andrographis paniculata* leaves. Further investigation, including compound isolation, is needed.

Keywords: Andrographis paniculata, wound healing activity, phytochemical analysis, alkaloids, glycosides, tannins, phenolic compounds, flavonoids

INTRODUCTION

Wound healing, is a specific process leading to the restoration of injured tissues. Wound healing process can be described as regeneration of the injured connective tissue of wounds in order to fill the wound gap followed by proliferation and migration of dermal and epidermal cells and matrix synthesis.1 Proliferation follows with the wound being rebuilt with new granulation tissue formed mostly by fibroblast and a new network of blood cells develop through the process of angiogenesis. Maturation involves remodeling and an improvement in collagen fiber components, leading to an increase in tensile strength. Parallel to this, scar formation occurs along with the accumulation of fibrous tissue at the edges of the wound, which eventually merges with surrounding tissue (Attinger et al., 2006).

Synthetic agents and natural wound healing agents act to accelerate and achieve the healing of wounds. Wound healing drugs are still unsatisfactory because of their low availability, high cost, and several detrimental side effects. The natural plants have been used as the remedy for various skin and dermatological disorders especially cut, wounds, burns etc.4 Many plant drugs have been used in management and treatment of wounds over the years. Plants and their extracts have immense potential in the management and treatment of wounds. Therefore, medicinal plant derived drugs are under great demand due to common belief that they are safe, reliable, clinically effective, low cost and better tolerated by patients (Okhuarobo et al., 2014, Hossain et al., 2014, Hosseinkhani et al., 2017, Guo et al., 2010). *Andrographis paniculata* is an annual herb native to Sri Lanka and India that belongs to the Acanthaceae family. It is also known as creat or green chiretta. It is frequently grown in Southern and Southeast Asia, where it is reputed to be beneficial in treating a variety of diseases including bacterial infections. Primarily the leaves and roots have been used for these purposes; in other cases, the entire plant has also been used (Okhuarobo et al., 2014, Hossain et al., 2014)). The current study was aimed to evaluate the wound healing activity of Andrographis paniculata

MATERIALS AND METHODS

Collection and Authentication of the Plant

In light of recent scientific findings, plants have been investigated for their medicinal properties all over the world due to their potent pharmacological effects, low toxicity, and commercial viability. On the basis of information acquired from regional vaidhyas and other traditional anti-inflammatory medicine practitioners, the current study assesses the activity of Andrographis paniculata. The aerial section of *Andrographis paniculata* has been cleaned, rinsed, and dried for six days. The dried plant material was mixed into a coarse powder and kept at room temperature for future investigation.

Soxhlet Extraction

The soxhlet method is widely used to extract organic compounds from solid or semi-solid materials. It is widely employed in many sectors, including those related to food, medicine, and the environment. The freshly collected plant materials were washed, dried in the shadow, then dried in a hot air oven at a maximum temperature of 50°C. Hexane, petroleum ether, chloroform, and ethanolic were used as the nonpolar and polar solvents, respectively, in a sequential extraction process in a soxhlet apparatus to extract the dry powder of the plant's aerial parts. Each solvent was extracted for a total of 72 hours at 40 to 45 0 C. The extraction process was started with hexane and finished using petroleum ether, chloroform, and ethanolic. After the extraction, the solvent was put out, and the leftover material was stored in a dessicator for future study (Bajaj et al., 2012, Mehta et al., 2018, Mahto et al., 2022, Tiwari et al., 2020, Pandey et al., 2016, Chauhan et al., 2019).

At the commencement of this process, the tree's bark and leaves are first taken off, dried, and the fruits are then ground into a fine powder. In contrast to organic solvents, the amount of powder was measured in a conical carafe. In the rotary shaker, it was rotated at 190–220 rpm for 24 hours. After then, the mushroom-based fabric was separated and centrifuged. The recovery of a dissolvable refinery component allowed for.

The % Yield in different solvents plant extracts were calculated by using the following formula:

*% Yield = (*Net weight of powder in gram after extraction /Total weight of powder in gram taken for extraction*) ×100*

Physicochemical Evaluation

The following physicochemical properties were assessed using conventional technique using the powdered plant material of *Andrographis paniculata* (Bajaj et al., 2012, Mehta et al., 2018, Mahto et al., 2022, Tiwari et al., 2020, Pandey et al., 2016, Chauhan et al., 2019).

Loss on drying

Mass loss as a percentage of mass/matter is referred to as loss on drying. A Petri plate containing 5–6g of medication powder is precisely weighed and stored in a hot-air oven with the temperature set at 105°C for 4-5 hours. Each case's weight loss was noted after chilling in a dessicator. The process was repeated until the weight remained consistent.

Loss on drying (%) = loss in weight X 100/ W

W= weight of the drugs in grams.

Determination of Ash Value

Ash value is a method for evaluating the purity and quality of unprocessed powdered pharmaceutical materials. Given that the natural problem has been burnt, the trash is actually rough material without any natural components, which tells us more clearly about the existence of active ingredients in unrefined medicine.

Total ash value

Take 2 to 3g of precisely weighed powdered extract and place it in a platinum or silica plate that has been tarred, lighted, and weighed. Disperse the medication powder on the plate's base. Increase the heat gradually, never reaching dull red heat, and incinerate until the material is free of carbon. Then let it cool and weigh it. If a carbon-free ash cannot be produced in this manner, the charred mass should be extinguished with hot water, the residue collected on an ashless filter paper, the filtrate added, the residue evaporated, and the residue ignited at a low temperature. First, an empty silica crucible was placed into the muffle furnace with a tong for 30 minutes of 6000C ignition. 2g of powdered drug was placed to the silica crucible after it had been removed and weighed. Finally weighed it, with an air-dried sample, the percentage of total ash was determined.

Total ash value = (z-x/y) × 100

Where,

X = weight of the silica crucible

Y = weight of the drug powder (g)

Z = weight of the silica cruicible with powder ash

Acid-insoluble ash

The insoluble material was removed from the ash after it had been heated for 10 to 15 minutes with around 30 millilitres of diluted hydrochloric acid. It was lit, cleaned with hot water, and weighed. In order to quantify the proportion of acid-insoluble ash, the air-dried medication was used as a reference. To 25 ml of dil HCl, the ash generated by the aforementioned technique was added. For five minutes, boil it. The residue was then collected on filter paper with less ash. Then, it was heated to 5600C for 1 hour in a muffle furnace, calculating the proportion of acid-insoluble ash using an air-dried sample.

Acid insoluble ash value % = (a /y) × 100

Where,

A = weight of the remaining residue

Y = weight of crude powder taken (g)

Water-soluble ash

The ash from the total ash value was boiled in 25 cc of water for 5 minutes. The insoluble material was added to filter paper with no ash. Then, a low temperature ignition was used to maintain a steady weight. By deducting the weight of water insoluble ash from the overall ash value, the weight of water soluble ash was calculated. Calculations were used to determine the amount of water-soluble ash in relation to air-died value.

Extractive Value

Procedure A medication that had been air dried and ground into a coarse powder was macerated in a closed flask for 24 hours with 100 cc of solvent (chloroform, ethanol, and water), shaking

regularly for the first six hours, and then left to stand for the last 18 hours. After that, it was quickly filtered to prevent alcohol loss. A 25 ml sample of the filtrate was dried to dryness in a shallow dish with a flat bottom, dried at 1050°C, and weighed. The proportion of extractive that is soluble in alcohol was estimated using the air-dried medication as a base. The amount of soluble components needed for extraction in that particular solvent is referred to as the extractive value.

Extractive value was determined using the formula

$$\text{Extractive value (\%)} = \frac{weight\ of\ residue}{weight\ of\ dry\ powder} \times 100$$

Determination of foreign matter

Prescriptions made at home shouldn't contain pesticides, moulds, animal waste, or other unnecessary components like glass and metal. Plant parts intended for human consumption are among the harmful substances, which should be acknowledged and should not violate WHO regulations. Picked plant material was examined for novel materials with an unassisted eye and an amplified focus point. The chemical was identified by gauging. The amount of unfamiliar material was incredibly minimal since all of the plant materials were manually collected, ensuring that no unidentified material was ever present (Bajaj et al., 2012, Mehta et al., 2018, Mahto et al., 2022, Tiwari et al., 2020, Pandey et al., 2016, Chauhan et al., 2019).

Phytochemical Screening

Standard procedures were followed for the phytochemical analysis of the powdered seed and/or aqueous seed extract (made by Soxhlet extraction). The next sections give a quick explanation of the methodologies. Three copies of every analysis were performed.

For the qualitative analysis of alkaloids, carbohydrates, fixed oils, flavonoids, glycosides, phytosterol/terpenoids, saponins, and tannins/phenols, preliminary phytochemical screening was carried out following the prescribed methods. The following screening exams:

Test for Glycosides

On a watch glass, the extract and a little amount of anthrone were combined. Conc. H_2SO_4 was added in one drop, formed into a paste, and then slowly warmed over a water bath. Glycosides are present, as indicated by the dark green coloration warm 200 mg of the medication in 5 ml of diluted H_2SO_4 before extracting. Filter, then add 5% NaOH solution to the acid extract to neutralise it. When it becomes alkaline, add 0.1ml of Fehling's solutions A&B, and then heat on a water bath for 2 minutes. Take note of the amount of red precipitate that was produced and compare it to test B's.

Instead of using H_2SO_4, extract 200 mg of the medication using 5 ml of water by warming on a water bath. Add the same quantity of water that was guaranteed for NaOH in the test above after boiling. until alkaline, add 0.1 ml of Fehling's solutions A and B. then cook it for two minutes in a water bath. Take note of how much red precipitate produced. Compared to test A, compare the

amount of precipitate that was produced in test B. The presence of glycosides is shown by the precipitate in Test A being larger than Test B.

Test for flavonoids

i.The Shinoda test

5ml of 90% alcohol, 0.5g of magnesium turnings, and conc. HCl were added to the extract and heated for a short while. The presence of flavonoids is indicated by pink or red coloration.

ii.Alkaline test

Alcohol and 10% NaOH solution, or ammonia, were added to the extract. Flavonoids are present as shown by the dark yellow tint.

iii. Test for Zinc Hydrochloric Acid

Add a combination of Zn dust and strong HCl to this test solution. After a short period of time, the coloration becomes red, indicating the presence of flavonoids.

Test for Alkaloids

i) Dragendorff's test, first

A few drops of acetic acid and Dragendroff's reagent were added to the extract and thoroughly mixed. Alkaloids are present when an orange-red precipitate forms.

ii) The Mayer test

Mayer's reagent and a few drops of diluted hydrochloric acid were added to the extract. Alkaloids are present when white precipitate forms.

iii).Wagner's test

A few drops of Wagner's reagent were applied along the test tube's sides to a few ml of filtrate. The test is positive when a reddish brown precipitate forms.

iv) The Hager test

One or two ml of Hager's reagent were added to a few millilitres of filtrate. A noticeable yellow precipitate signals a positive test result.

Animal study

Wistar rats weighing between 220 and 270 grams, of both sexes, were used in the experiment. The rats were kept in pairs and kept at 24°C, with plenty of food and water available from Hindustan Lever in India. The animals were starved for four hours prior to any acute food-only experiments. The studies were carried out from 08:00 to 16:00 during the light phase.

The research complied with the standards for the treatment and care of animals employed in scientific research established by the IAEC (Institutional Animal Ethics Committee) and the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The Wistar rats were kept in sterile, tidy facilities created with animal welfare in mind."

According to accepted standards for animal care, the paragraph details the experimental setup and the precautions taken to assure the safety and well-being of the Wistar rats used in the study.

To maintain a clean and well-ventilated atmosphere, the animal room and polypropylene cages underwent routine and intensive cleaning. In order to encourage air circulation, proper aeration was made.

To keep the cages clean and hygienic, extra food and waste were regularly removed.

To give the rats a comfortable and stable environment, the temperature in the animal room was carefully maintained at 26 2°C (Elzayat et al., 2018, Murti et al., 2011, Sawant et al., 2016, Goyal et al., 2012).

Daily feeding pellets were given to the rats, which were purchased from Chennai's Poultry Research Station. They had unrestricted access to hygienic water.

The rats were handled gently and carefully, being careful not to put them under undue strain or pressure.

The animals were divided into 4 groups each consisting of six animals.

Group I - Received only vehicle (Normal saline)

Group II-((Normal saline) + Povidone iodine (Used as a positive drug)

Group III - (Normal saline) + with *Andrographis paniculata* Formulation with low dose

Group IV - (Normal saline) + with *Andrographis paniculata* Formulation with high dose

Pharmacological Screening (Okhuarobo et al., 2014, Hossain et al., 2014)

Excision wound model

On the depilated area of the skin, an excision incision was formed. The dorsal surface of the anaesthetized rats was marked with an 8 mm2 standard wound area, and skin in full thickness was removed to create the wound. By dabbing an ulcerated region with a cotton swab soaked in normal saline, homeostasis was achieved. On different days, the wound diameter was measured using callipers, and at the conclusion of the research, the epithelialization time was noted. The time of epithelization was determined by the number of days needed for the scar to come off without any remaining raw wound. From the day of injury (day zero) to the complete epithelialization, the time was expressed in days. Every third day, the wound area was measured and traced using a transparent piece of paper. As a percentage of the decrease in wound size, the wound's contraction was indicated.

Percentage of Wound contraction = [(initial wound area – specific day wound area)/initial wound area] x 100

Four groups of six rats each with the same wounds were created from a total of 24 rats. Groups 3 and 4 were, respectively, treatment groups; Group 1 was the control group, which contained untreated rats; the second group served as a positive control.

Evaluation of oxidative stress marker

1. GSH, or glutathione a. Making a Tissue or Cell Extract: i. Gather the tissue sample or separate the desired cells. ii. Mix the tissue or cells well in an appropriate buffer (such as phosphate-

buffered saline). iii. Centrifuge the homogenate to get the cellular extract-containing clear supernatant.

Use a spectrophotometric test kit based on the reaction of GSH with a chromogenic reagent, such as 5,5'-dithiobis(2-nitrobenzoic acid; DTNB), for the total glutathione assay. ii. After mixing the sample and reagent according to the kit's instructions, wait for GSH to react and produce a coloured product. Utilising a spectrophotometer, determine the reaction mixture's absorbance at a certain wavelength. iv. Calculate the GSH concentration by comparing the absorbance to a standard curve made using known values of GSH.

Catalase (CAT): Making a Tissue or Cell Extract:

i. Gather the tissue sample or separate the desired cells.

ii. Mix the tissue or cells well in an appropriate buffer (such as phosphate-buffered saline).

iii. Centrifuge the homogenate to get the cellular extract-containing clear supernatant.

Use a spectrophotometric test kit based on catalase's breakdown of hydrogen peroxide (H2O2) for the catalase activity assay. ii. Combine the sample with a predetermined amount of H2O2, then track the drop in absorbance at a particular wavelength when H2O2 is oxidised by catalase. iii. To assess the catalase activity, compare the sample's rate of H2O2 oxidation to a standard curve produced using known catalase concentrations (Badmanaban et al., 2009, Ambrekar et al., 2011).

SOD (Superoxide Dismutase):

i. Collect the tissue sample or isolate the target cells for the tissue or cell extract.

ii. Mix the tissue or cells well in an appropriate buffer (such as phosphate-buffered saline).

iii. Centrifuge the homogenate to get the cellular extract-containing clear supernatant.

Use a spectrophotometric test kit based on the suppression of a particular process by SOD for the SOD activity assay

(B). ii. Mix the sample with the reaction ingredients according to the kit's instructions, then check the absorbance change at a certain wavelength. iii. To calculate the SOD activity, compare the sample's inhibition rate to a standard curve built using known SOD concentrations.

Secondly, lipid peroxidation (LPO): a. Making a Tissue or Cell Extract: i. Gather the tissue sample or separate the desired cells. ii. Mix the tissue or cells well in an appropriate buffer (such as phosphate-buffered saline). iii. Centrifuge the homogenate to get the cellular extract-containing clear supernatant.

LPO Test: Use an assay kit for measuring lipid peroxidation that relies on the interaction of lipid peroxides with a particular reagent, such as thiobarbituric acid (TBA). ii. To create a coloured complex, combine the sample with the reagent according to the kit's instructions and incubate at a certain temperature. Utilising a spectrophotometer, determine the reaction mixture's absorbance at a certain wavelength. iv. Evaluate the absorbance against a standard curve prepared with known lipid peroxide values

RESULTS AND DISCUSSIONS

It may be inferred from the extensive literature review and experimental data analysis that *Andrographis paniculata* is a traditional treatment for fever, colds, and other illnesses. Additionally, it uses a variety of immunological techniques in the treatment of cancer, immunomodulatory activities, and viral illnesses like HIV, among others. The herb is also useful for treating heart illness and reducing liver toxicity, which enhances the activities of the heart and liver. According to conventional phytochemical procedures, the plant extract used in our experiment was tested for the presence of important secondary metabolite classes as Alkaloides, Flavonoides, Saponin, Terpenoide, Tannin, Glycosides, Phytosterol, and Proteins. The tests relied on visual observation of the colour change or precipitate development following the addition of a particular reagent.

Additionally, it is quite helpful for issues with the central nervous system, the functioning of the brain, respiratory issues, allergic responses, snake bites, stomach pain, and body pains. According to some reports, andrographis paniculata reduces both animal and human fecundity. Given the plant's beneficial properties, it may be strongly recommended as a secure, crucial medicinal herb for people.

Using standard phytochemical techniques, the plant extract was checked for the presence of important secondary metabolite classes as Alkaloides, Flavonoides, Saponin, Terpenoide, Tannin, Glycosides, Phytosterol, and Proteins. The tests were based on visual observation of the colour change or precipitate development following the addition of a particular reagent.

The outcomes of phytochemical studies conducted on Andrographis paniculata using various solvents are shown in Table. In each solvent extract, the current investigation demonstrated the presence or lack of phytochemical components. Ethanolic was discovered to exhibit the greatest amount of phytochemicals. To speed up the healing of wounds, herbal medicines have been utilised for ages in traditional medical practises. By having antibacterial, anti-inflammatory, antioxidant, and regenerative qualities, a number of herbal medicines have demonstrated potential for assisting the healing process of wounds.

Physical Test of Crude Drugs (Table 1)

Insights on the nature, colour, odour, and taste of Andrographis paniculata extract can be gained from knowledge of its physical features in their basic pharmacological form. The Andrographis paniculata extract physical test findings are as follows:

Table 1: The Organoleptic properties of the plant extract were evaluated for Andrographis paniculata

S.no	Parameter	Result
1.	Odour	Pleasant
2.	Powder as such	Fine
3.	Colour	Greenish-brown
4.	Texture	Coarseness
5.	Taste	Savory taste
6.	Consistency	Powdered

Table 2: Extractive Values (Table 2)

The Extractive Values of the plant extract were evaluated for alcoholic and aqueous solutions *Andrographis paniculata*

Crude drugs	Ethanolic% w/w	Aqueous% w/w
Andrographis paniculata Extract	**12.55**	**10.20**

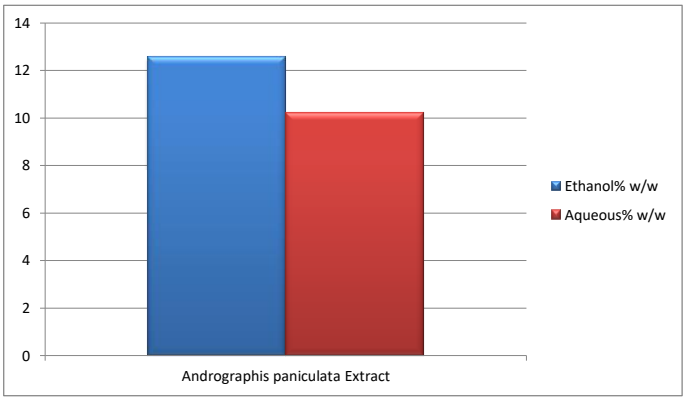

Fig 1: Graph of the Extractive Values

Table 3: Loss on Drying and Foreign Organic Matter Andrographis paniculata fruit extract

S.No	Physical Constant	Values (% W/W)
1.	Moisture content	1.5
2.	Foreign matter	1.64
3.	Extractive value(%w/w)	15.35

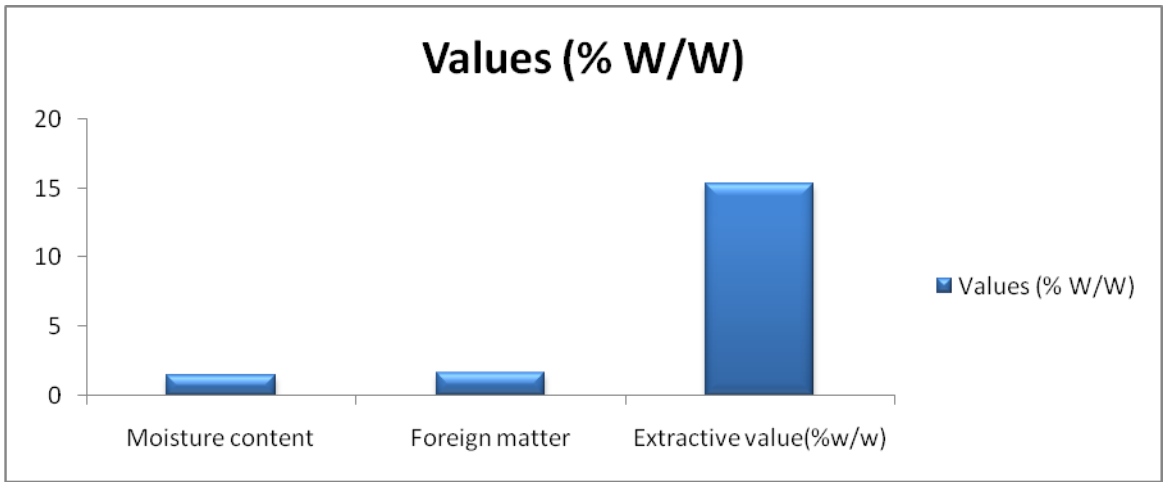

Fig:2 Graph of Loss on Drying and Foreign Organic Matter *Andrographis paniculata* fruit extract

Table 4: Total Ash, Acid Insoluble Ash and Water Soluble Ash Values *Andrographis paniculata* leaves extract

S.no	Properties	Leave extract (w/w)
1.	Total ash	4.55
2.	Acid insoluble ash	6.25
3.	Water insoluble ash	5.50
4.	Loss on Drying	4.25

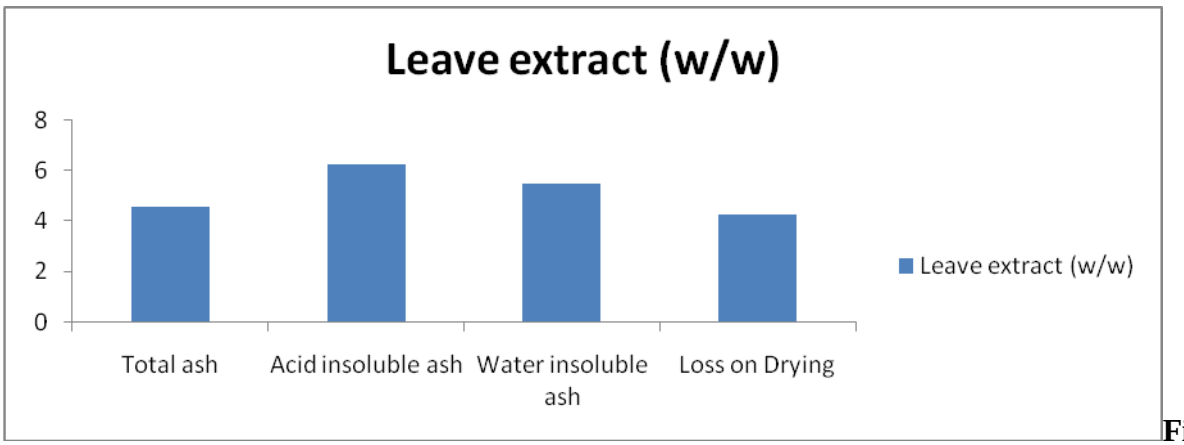

Fig 3: Total Ash, Acid Insoluble Ash and Water Soluble Ash Values *Andrographis paniculata* leaves extract

Phytochemical Screening

Phytochemical screening was performed on the fraction of the dry extract using the technique developed by Trease, Evans, and Harbourne. Alkaloids, saponins, glycosides, proteins, phytosterols, flavonoids, triterpenoids, tannins, fixed oil, and fats were all tested for throughout the phytochemical screening. Below is a list of outcomes:

Table 5: Phytochemical screening for extract of Andrographis paniculata Extract (Table 5)

S.No	Chemical Tests	Andrographis paniculata Extract
1.	Tests for Steroids and Triterpenoids:	
	• Liebermann's Burchard Test	+
	• Salkowski Test	+
2.	Test for Saponins:	
	• Foam Test	+
3.	Tests for Alkaloids:	
	• Hager's Test	+
	• Mayer's Test	+
4.	Tests for Glycosides:	
	• Borntrager's Test	+
	• Keller Killiani Test	+
5.	Tests for Tannins and Phenolic compounds:	

	• Gelatin Test	+
	• Ferric Chloride Test	+
	• Lead Acetate Test	+
	• Dilute Nitric acid Test	+
6.	Tests for Flavonoids:	
	• Ferric chloride Test	+
	• Alkaline reagent Test	+
	• Lead acetate Test	+
7.	Tests for Proteins:	
	• Biuret Test	+
	• Xanthoproteic Test	+
8.	Test for Carbohydrates:	
	• Fehling Test	-

"+"Found "-" Not Found

Pharmacological Screening

The measurements of the wound's surface area show how the ethanol medication extract causes the wound to shrink over time. Over the course of 16 days, the wound area in the control group decreased gradually from 8 mm^2 to 5.5 mm^2. By the 16th day, the wound area in the standard group (probably a positive control) had decreased more noticeably, to 1.5 mm^2. Although not as much as the control group, both Formulation-1 and Formulation-2 show a reduction in the size of the wound. These findings imply that the ethanol medication extract formulations may contribute to the process of wound contraction, perhaps promoting wound healing.

Table 6: Effect of ethanol drug extract on wound contraction (mm^2)

S. No	Sample	0^{th} dy	4^{th} day	8^{th} day	12^{th} day	16^{th} day
1	Control	8	8	7.5	6.5	5.5
2	Standard	8	6	4.5	2.8	1.5
3	Formulaton-1	8	7.5	6.5	5.5	4.7
4	Formulation-2	8	7.2	6.1	4.7	3.5

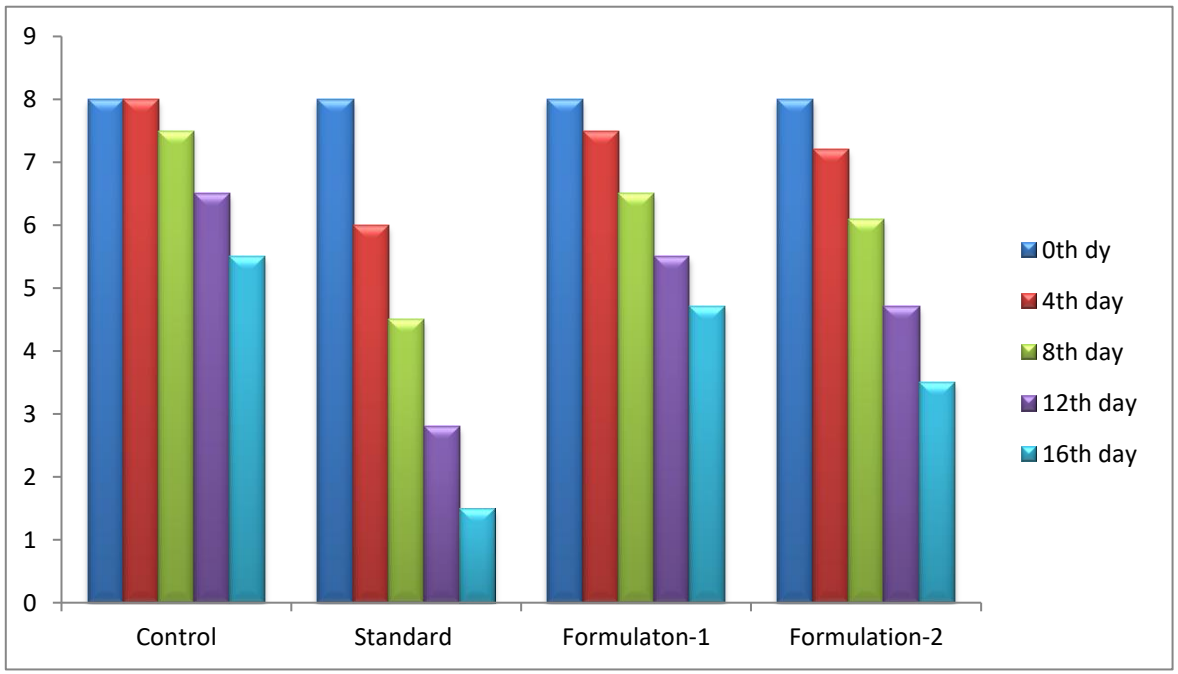

Fig 4: Graph of Effect of ethanol drug extract on wound contraction (mm^2)

Evaluation of oxidative stress marker

The measurements of GSH, SOD, CAT, and LPO levels in the different groups indicate the following:

The control group shows relatively lower levels of GSH (22 units/mg), SOD (0.5 mmol/mg), and CAT (22 U/mg), but higher levels of LPO (7.5 mmol/mg).

The standard drug group exhibits slightly higher levels of GSH (26 units/mg) and SOD (0.7 mmol/mg), similar levels of CAT (23 U/mg), and significantly lower levels of LPO (1.8 mmol/mg). The treated groups (both treated group 1 and treated group 2) show higher levels of GSH (28 units/mg and 27.5 units/mg, respectively), SOD (0.8 mmol/mg and 0.75 mmol/mg, respectively), and CAT (25 U/mg and 24 U/mg, respectively), and moderately lower levels of LPO (5.5 mmol/mg and 4.5 mmol/mg, respectively).

These results suggest that the treated groups, along with the standard drug group, exhibit increased antioxidant levels (GSH, SOD, and CAT) and reduced lipid peroxidation (LPO) in the wound tissue compared to the control group. This indicates that the treatment or standard drug may have a positive impact on oxidative stress and inflammation, potentially aiding in the healing process of the wounds.

Table 7: GSH, SOD, CAT and LPO levels in wound tissue

S. No	Groups	GSH (Units/mg)	SOD (mmol/mg)	CAT (U/mg)	LPO (mmol/mg)
1	Control group	22	0.5	22	7.5
2	Standard drug group	26	0.7	23	1.8
3	Treated group	28	0.8	25	5.5
4	Treated Group	27.5	0.75	24	4.5

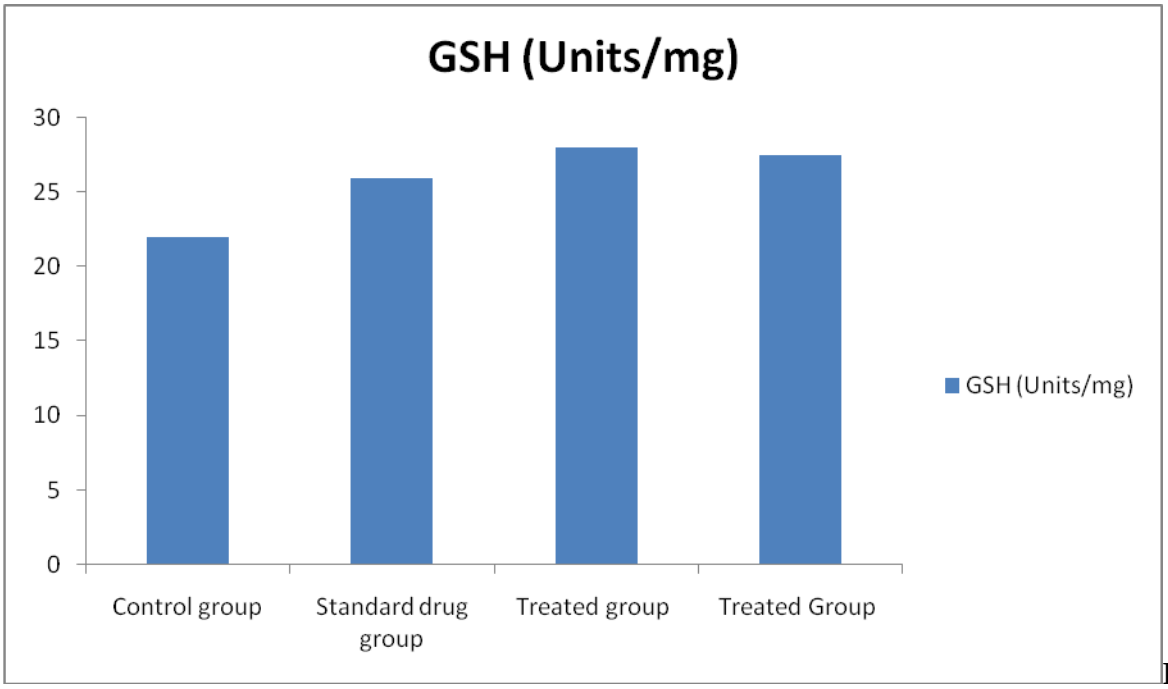

Fig 5: The effect of formulation for GSH levels in wound tissue

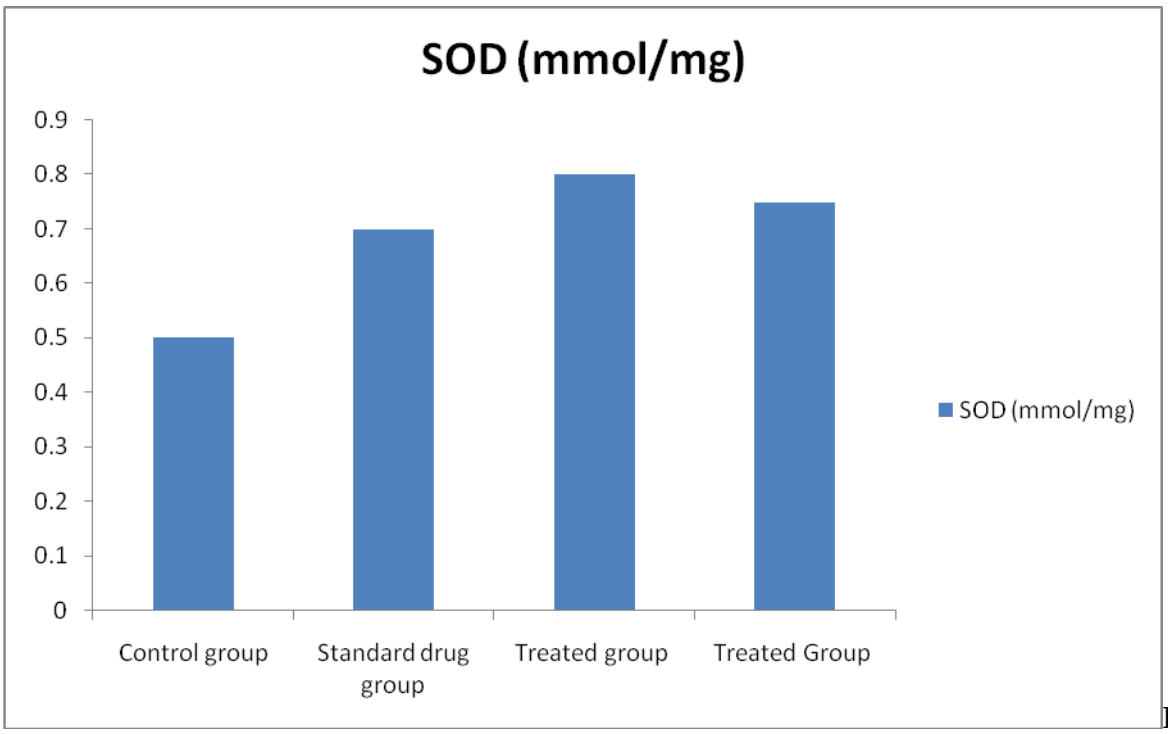

Fig 6: The effect of formulation for SOD levels in wound tissue

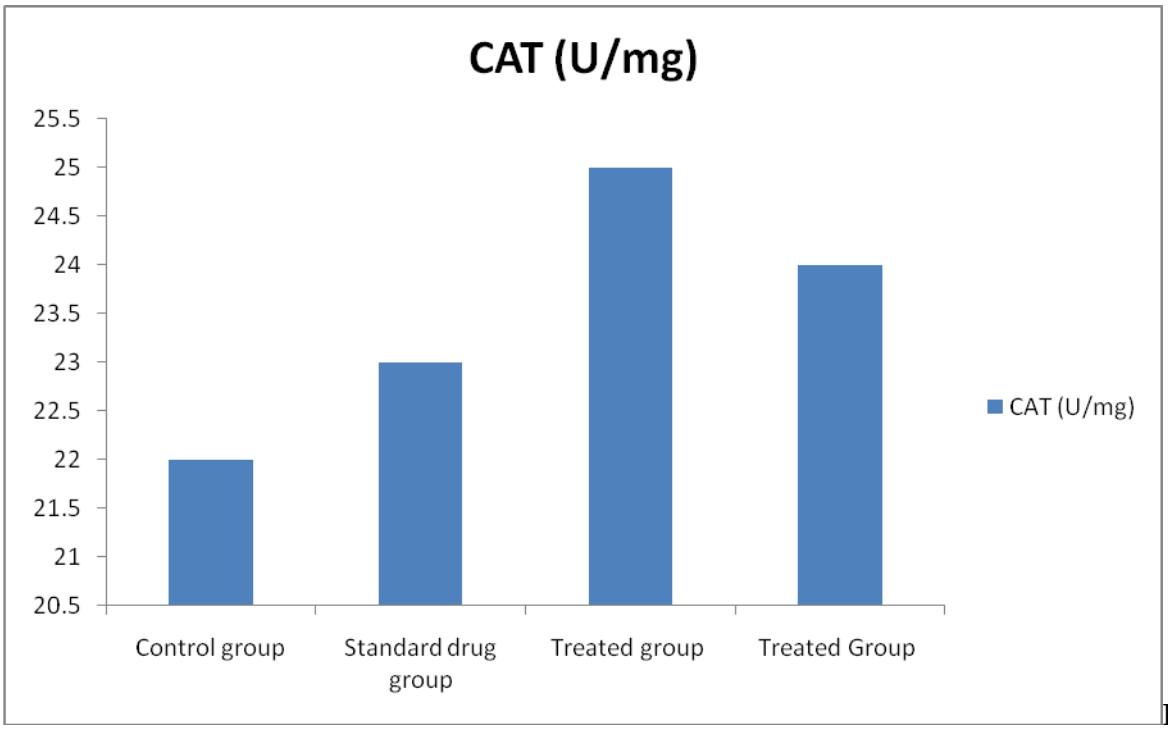

Fig 7: The effect of formulation for CAT levels in wound tissue

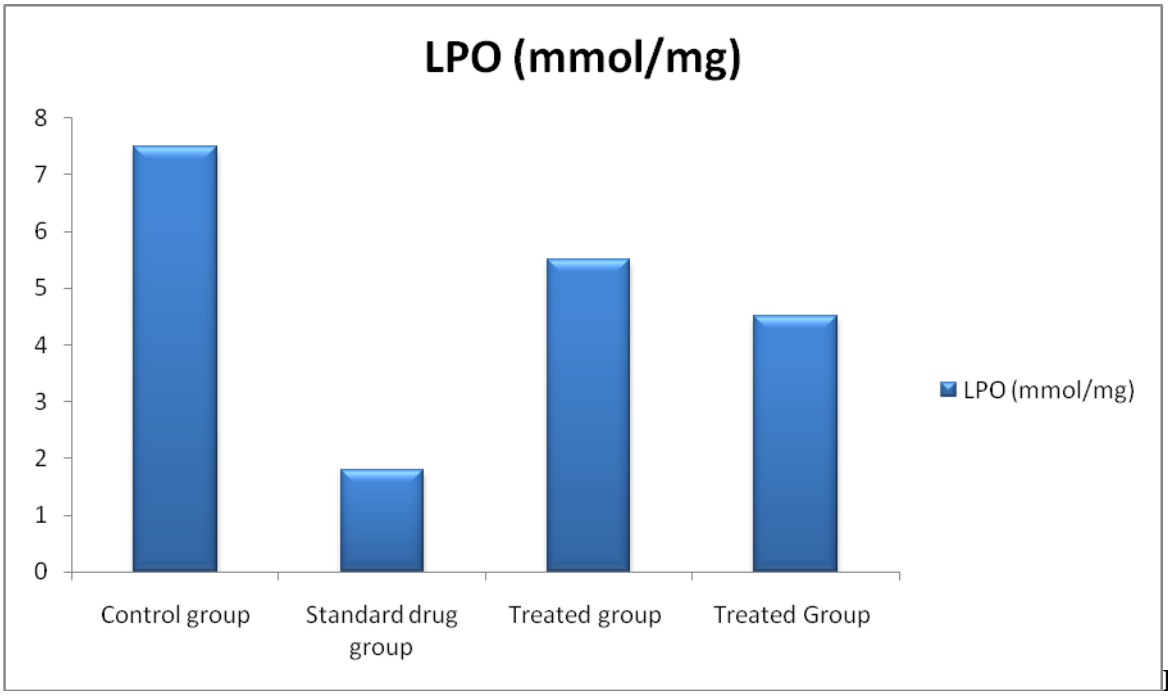

Fig 8: The effect of formulation for GSH, SOD, CAT and LPO levels in wound tissue

Acute toxicity studies

No clinical symptoms, no fatalities, notable weight changes, or gross necropsy were discovered during the acute toxicity investigation, which is summarised in the table below. Studies on acute toxicity were conducted for groups -1 to -4 with a dosage of 200 mg/kg, and 400 mg/kg (Table 7) was confirmed to be safe.

Table 8: Acute toxicity studies

S.No	Treatment	Dose mg/kg	No. of animals	Mortality			Toxicity profile
				7 Days	14 days	21 days	
1	Group -1,2	200	5	0	0	0	Safe
2	Group – 3,4	400	5	0	0	0	Safe

SUMMARY AND CONCLUSIONS

In summary, the *Andrographis paniculata* plant extract has a pleasant odor, fine powder consistency, greenish-brown color, coarse texture, savory taste, and is in a powdered form. These organoleptic properties provide sensory information about the extract, aiding in its characterization and potential applications.

In summary, the *Andrographis paniculata* extract is composed of approximately 12.55% ethanol and 10.20% water. These percentages represent the relative amounts of Ethanolic and water present in the extract.

In summary, the *Andrographis paniculata* fruit extract has a moisture content of 1.5%, 1.64% foreign matter, and an extractive value of 15.35%. These physical constants provide information about the water content, presence of foreign matter, and the extractive efficiency of the fruit extract.

In summary, the phytochemical screening of the *Andrographis paniculata* extract reveals the presence of steroids, triterpenoids, saponins, alkaloids, glycosides, tannins, phenolic compounds, flavonoids, and proteins. However, no carbohydrates were detected in the extract. These results provide information about the phytochemical composition of the extract and the presence of bioactive compounds that contribute to its potential therapeutic properties.

In summary, the measurements of wound area demonstrate the effect of the ethanol drug extract on wound contraction over time. The control group shows a gradual reduction in wound area from 8 mm^2 to 5.5 mm^2 over 16 days. The standard group (presumably a positive control) shows a more pronounced reduction in wound area, reaching 1.5 mm^2 by the 16th day. Both Formulation-1 and Formulation-2 also exhibit a decrease in wound area, although not as substantial as the standard group. These results suggest that the ethanol drug extract formulations may contribute to the process of wound contraction, potentially aiding in the healing of wounds.

In summary, the measurements of GSH, SOD, CAT, and LPO levels in the different groups indicate the following:

The control group shows relatively lower levels of GSH (22 units/mg), SOD (0.5 mmol/mg), and CAT (22 U/mg), but higher levels of LPO (7.5 mmol/mg).

The standard drug group exhibits slightly higher levels of GSH (26 units/mg) and SOD (0.7 mmol/mg), similar levels of CAT (23 U/mg), and significantly lower levels of LPO (1.8 mmol/mg).

The treated groups (both treated group 1 and treated group 2) show higher levels of GSH (28 units/mg and 27.5 units/mg, respectively), SOD (0.8 mmol/mg and 0.75 mmol/mg, respectively), and CAT (25 U/mg and 24 U/mg, respectively), and moderately lower levels of LPO (5.5 mmol/mg and 4.5 mmol/mg, respectively).

In acute toxicity study no clinical signs, no deaths, remarkable body weight changes or gross necropsy was found.

The present study demonstrated that the ethanolic extract aerial parts of *Andrographis paniculata* promotes wound healing activity in rats in the preclinical study. The aerial parts of *Andrographis paniculata* showed remarkable wound healing activity and it may be suggested for treating various types of wounds and injuries in animals. The enhanced wound healing activity of aerial parts of *Andrographis paniculata* could possibly be made use clinically in healing of open wounds. However, confirmation of the study has to be done through well designed clinical evaluation

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