# Phytochemical Analysis and Evaluation of Wound Healing Properties of *Cucurbita pepo* Linn, *Colocasia esculenta* (L) Schott and *Amaranthus graecizans* Linn

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**Abstrat**

Wound healing is a typical biological process. It involves four intricate steps: homeostasis/coagulation; inflammation, migration, and proliferation; reepithelialization; and restoration. A number of mediators, including platelets and cytokines, inflammatory cells, cellular and extracellular matrix, proteinases, growth factors, and inhibitors, have an impact on each stage of the healing process for wounds. In this study, the methanolic leaf extracts of *Cucurbita pepo* Linn (CP), *Colocasia esculenta* (L.) Schott (CE), and *Amaranthus graecizans* Linn (AG) were evaluated for their ability to promote wound healing. An established test technique that is documented in the literature was used to determine the qualitative analysis of different phytochemical elements. Rats of either sex were used to test an excision- and incision-based wound model. Five groups of six Wistar albino rats were created; group I (left untreated) was used as the negative control, group II was given 5% (w/w) povidone iodine ointment (Intadine USP) as the standard, group III received 5% (w/v) CP extract, group IV received 5% (w/v) CE extract, and group V received 5% (w/v) AG extract as the test group. Each therapy was administered once daily. The percentage wound contraction, epithelialization time, hydroxyproline content, and histoarchitecture studies in the excision wound model and tensile strength in the incision wound model were used to measure the wound healing effect. In both of the investigated animal models, extracts greatly aided wound healing activity. The methanolic extracts of the leaves of CP, CE, and AG contained alkaloids, terpenoids, flavonoids, carbohydrates, tannins, and saponins, according to a phytochemical screening. In CP-treated rat, there was a high rate of wound contraction, a reduction in the time needed for epithelialization, and an increase in the hydroxyproline level. In the incision model, animals treated with CP extract had higher skin-breaking strengths than the animals in the negative control group. Studies on the histopathology of the CP-treated groups demonstrated the efficiency in promoting wound healing. As a result, CP extract has a wound-healing action. The quick wound contraction that shortens the distance for migrating keratinocytes may have caused the sample CP to shorten the epithelialization period. The results of this investigation showed that CP leaf extract has superior wound healing activity to CE and AG, and it may also be utilised to treat various types of

wounds in people.

**Keywords:** Wound healing, *Cucurbita pepo* Linn, *Colocasia esculenta* (L.) Schott, *Amaranthus graecizans* Linn, Histopathological studies, Epithelialization period

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## Introduction

In many parts of the world, wounds have a major role in morbidity and death. According to studies, there are 10,000 microbial infections related mortality for every million wound patients [1, 2]. In addition to causing pain, loss of function and mobility, depression, distress, and anxiety, embarrassment, and social isolation, chronic morbidity, and even death due to the low rate of complete healing, chronic wounds have a significant negative impact on the health and quality of life of patients and their families [2]. Debridement, irrigation, the use of antiseptics, antibiotic and corticosteroid therapy, and tissue grafts are some of the current methods for treating wounds. These therapeutic methods come with undesirable side effects, too, including bleeding, tissue damage, contact dermatitis, a delay in wound healing, and the possibility of bacterial resistance [3]. Just 1-3 percent of the medications listed in western pharmacopoeias are intended for use on wounds, despite the enormous advancements made in the pharmaceutical drug industry [4]. More so in developing nations, infection-related morbidity and mortality have grown due to a rise in resistance bacteria, high costs, and a lack of next generation medications [5]. In order to create nontoxic and efficient wound healing agents, there is a huge need for scientific study of medicinal plants. Strong bioactive chemicals produced by plants enable them to communicate with other creatures in their surroundings. These bioactive substances play a crucial role in defence systems and help people avoid sickness. The bioactivity of plant extracts and their constituent parts against harmful pathogenic organisms has been assessed by numerous researchers [6]. Recent years have seen an increase in the use of the plants studied in Chhattisgarh as a natural source of therapeutic chemicals. Plants and products derived from plants from the Chhattisgarh region are used to treat and relieve a variety of physical and mental disorders. Several tribal, rural, and local groups in the state of Chhattisgarh ate a variety of leafy vegetables. These Chhattisgarh tribal and indigenous groups, especially those that live near a forest, rely on green vegetables as a source of food for their diet. They make the connection that

Chhattisgarh's native vegetation is a rich source of nutrients for leafy vegetables. Leafy vegetables are abundant in dietary fibre, vitamin C, pro-vitamin A, carotenoids, folate, manganese, and vitamin K. They also have high protein content per calorie. Secondary metabolites are also present in the leaf portion of leafy vegetable plant cells and are helpful in treating human illnesses as well. Steroids, flavonoids, tannins, alkaloids, and saponins are some of the secondary metabolites found in green leaves [7-9]. There are fifteen species in the cucurbitagenus of the family Cucurbitaceae, including *Cucurbita pepo* Linn (CP). While it is more popularly recognized as squash in English, it is known as kadoo in Urdu and Hindi. This plant, which is developing into a massive vine perinial, features large, yellow-orange, insect-pollinated blooms as well as circular, lobed leaves [10, 11]. Due to the inclusion of numerous chemical elements like terpenoids, cucurbitacin glycosides, flavonoids, and cardiac glycosides, CP has therapeutic properties like anti-inflammatory, anti-diabetic, antibacterial, antiulcer, antioxidant, anticancer, as well as antihyperlipidemic [12]. Its seeds comprise a range of elements such as proteins, gamma amino buticacid, polysaccharides, para amino benzoicacid, carotenoids e.g., β-carotene, lutein epoxide,violaxanthin, α-carotene, lutein, α-cryptoxanthin,luteoxanthin, auroxanthin epimers, chrysanthemaxanthin andflavaxanthin [13]. Taro is another name for *Colocasia esculenta* (L.) Schott (CE, Araceae). Common names for them include Keladi Sarawak, Keladi Pinang, KeladiMinyak, KeladiCina, and KeladiMawar [14]. They are manufactured for commercial uses. They have a significant role as staple foods in many regions of the world, especially in Asia and the Pacific Islands. Ayurveda and Unani medicine have traditionally employed CE leaf extract to treat a variety of illnesses. Traditional uses of CE include treating high blood pressure, hepatic disorders, rheumatic pain, lung congestion, ulcers, and more. Anti-inflammatory [15], hypolipidemic [16], anti-cancer [17], antioxidant [18], and antibacterial [19] actions have all been linked to the CE. The flavonoids vitexin, isovitexin, orientin, isoorientin, schaftoside, and isoschaftoside, as well as the vitamins A, B, and C, thiamine riboflavin, niacin, oxalic acid, and minerals iron, zinc, copper, and boron, are all found in the leaves of CE [20-22]. Several of these phytoconstituents have been linked to ACE inhibitor [23], hypotensive, anti-inflammatory, antispasmodic, vasodilatory, Ca2+ channel blocking, and diuretic activity [24-26] effects. *Amaranthus graecizans* (AG), sometimes known as green amaranth is a member of the more complicated Amaranthaceae family. Greek defines amaranthus or amaranth as never fading. While some species in the genus are frequently regarded as weeds, other species are widely consumed as leaf vegetables. Earlier studies have shown that various plant components are abundant in some phytonutrients that contribute to the suppression of free radicals. Many species of this genus, including the AG, have had their antioxidant capacities assessed. The presence of phytochemicals such as flavonoids, alkaloids,

tannins, phenolics, saponins, and glycosides that have been linked to radical scavenging activity was discovered through phytochemical screening of these vegetables. Further pharmacological effects such as antibacterial, anti-inflammatory, anti-malaria, anti-diabetes, anti-carcinogenic, and hepatoprotective importance have been identified [27]. These actions are in addition to their antioxidant activity. Practitioners of traditional medicine utilize AG to treat inflammatory diseases. The leaves are applied as an emollient to soothe stings from scorpions and snakes, as well as irritated or itching rashes. The entire plant is used as a treatment for mastitis and to treat generalized oedema. Astringent AG is applied topically as a wash and poultice for ulcers and sores, as well as a gargle for mouth and throat ulcers [28]. According to a review of the literature, no systematic strategy has been taken to research the ability of the leaves of these three plants to cure wounds. In the current study, we examined the topical wound-healing properties of methanolic extracts of CE, CP, and AG leaves against povidion-iodine ointment in wistar albino rats.

## Materials and methods

### Plant material

In August 2021, Bilaspur, Chhattisgarh's local region was scoured for leaves of CP, CE, and AG. Dr. Ashwini Kumar Dixit, an associate professor in the department of botany at Guru Ghasidas Vishwavidhyalaya in Bilaspur, Chhattisgarh, India, recognised the sample. For the Department of Botany at the Guru Ghasidas Vishwavidhyalaya in Bilaspur, Chhattisgarh, India, a herbarium of plants was submitted. The specimen voucher numbers for the CP, CE, and AG were Bot/GGV/2021/04a, Bot/GGV/2021/04b, and Bot/GGV/2021/04c, respectively. The plant material (part of the leaves) chosen for the study was properly cleaned under running water, rinsed in distilled water, and then let to dry for a while at room temperature. The plant material was then dried in the shade for three to four weeks. An electronic grinder was used to ground dried plant matter. Color, smell, taste, and texture of the powdered plant material were noted. The dried plant material was placed in an airtight container and kept for future biological and phytochemical research.

### Chemical reagents

The HiMedia Labs Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. provided all the chemicals used in this study (Mumbai, India). The investigation only employed analytical-grade compounds.

### Extraction

Separately, 50gm of dried, crushed AG, CE, and CP leaves were added to the thimble of the soxhlet apparatus. At first, n-hexane was used as a non-polar solvent for soxhlation at 70°C. Plant waste (marc) was dried before being extracted with methanol and chloroform. Soxhlation

was continued for each solvent until no colour was seen in the syphon tube. A gathering of colorless solvent in a syphon tube serves as proof that all of the plant material has been extracted. The entire extract was concentrated, dried using a rotary flash evaporator (Lab Systems) at decreased pressure, and then put away until use in an airtight container free from contamination. The dried extracts' percentage yields were then determined [29].

% yield = Weight of dry extracted/ Weight of sample used x100

**Qualitative phytochemical analysis of plant extract**

The CE, CP and AG leaves extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [30, 31]. The extract was tested for a number of active ingredients, including phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, lipids or fixed oils, protein and amino acids, and tannins.

**Animals**

For the study, healthy albino wistar rats of either sex, weighing 180 to 230 grammes, were chosen. There were five groups of six animals apiece. The animals were housed in temperature ranges of 18 to 20°C under 12:12 h day and light schedules. They spent the experimental time in a large, airy, clean cage. Up until the conclusion of the study, animals had unrestricted access to water and a regular pellet diet. The Institutional Animal Ethics Committee of PBRI, Bhopal (Reg. No. 1824/PO/RcBi/S/15/CPCSEA), which was established for the management and oversight of experimental animals, approved the animal experiments. The approval code for the protocol was PBRI/IAEC/10-09-22/013.

**Wound-healing activity**

Excision and incision wound models were used to evaluate the wound-healing activityof CP, CE and AG leaves.

**Excision wound model**

The animals were lightly sedated with diethyl ether vapour inhalation before the dorsal thoracic region's hairs were cut. Cut-out skin fragments from the shaved area were used to create excision incisions that were 200 mm2 in size and 1 mm deep. The wound was left completely open. The animals were carefully examined for any signs of infection, and those that did were removed from the study, quarantined, and replaced. Thereafter, during the following 18 days, the designated groups received daily applications of the test sample (5% w/v; CP, CE, and AG) and standard povidine iodine. On days 0, 2, 4, 6, 8, 10, 12, 14, and 18 for all groups, wound areas were measured using a transparency sheet and a permanent marker. The epithelization process began on the day that the scar went off without leaving a trace of a raw wound. This model was used to track the rate of epithelization and wound contraction. The percentage wound contraction calculated using formula:

Percentage of wound contraction = Wound Area on Initial day - Wound Area on Test day/ Wound Area on Initial day×100

The end of complete epithelization was determined to be the moment the wound's scab fell off, and the number of days needed to achieve this was determined to be the epithelization period. Each group of rats had a tissue sample taken from the healed wound for histopathological analysis.

***Epithelialization period***

It was measured by keeping track of how long it took the eschar to remove itself from the wound surface without also leaving a raw wound.

***Hydroxyproline estimation***

0.1 millilitres of hydrolysate sample were pipetted into sterile test tubes and the volume was increased to 0.5 millilitres with distilled water. 1.6 ml of the standard hydroxyproline stock solution was collected and diluted up to 100 ml. A clean test tube was used to pipette 0.5 ml, or 8µg, from this. This received 1ml additions of 6% hydrogen peroxide, 0.01M copper sulphate, and 2.5N sodium hydroxide. The tubes were immediately submerged in a water bath set at 80°C for 16 minutes, followed by a 5-minute cooling period. This was mixed with 4ml of 3N sulfuric acid and 2ml of freshly made 5% para-dimethylamino-benzaldehyde in n-propanol. Once more, test tubes were heated to 80°C in a water bath for 15 minutes, and then cooled for 5 minutes. For the purpose of estimating the amount of hydroxyproline, the optical density (O.D.) of the pink hue of these test samples was compared to that of standard samples of hydroxyproline with known concentrations at 540 nm.

**Incision wound model**

Diethyl ether was used to anaesthetize the rats both before and during the formation of the wounds. The animals' dorsal fur was clipped with an electric clipper. On the back, a 5-cm-long longitudinal paravertebral incision was created through the skin and cutaneous tissue. A surgical thread and curved needle were used to stitch the skin that had been divided 1 cm apart after the incision. The injuries were not covered. Animals were kept in separate cages by using cotton swabs dipped in 70% alcohol to disinfect their wounds. Once each day, the 5% w/v extract was topically administered to the wound. All of the animals' sutures were removed on the seventh post-wounding day, and the tensile strength was assessed using a tensiometer on the tenth post-wounding day [32, 33].

**Histopathology**

On the final day of the experiment, deep granulation tissues and cross-sectional full-thickness skin specimens from the implanted tube were obtained to examine the histological changes. Pieces of skin tissue were cut, cleaned, and transferred in 10% formalin solution during the

collection of the tissue sample for histology. To dehydrate the pieces, they were taken out of the 10% formalin solution and put in pure alcohol. Pieces were put into a 1:1 alcoholxylene mixture after pure alcohol and left there for 15 to 20 minutes. Tissue fragments were taken from the first embedding, added to the second infiltration, and held there at a predetermined temperature. Filtered wax that had reached maturity was poured into the lid to a height of 4/5ths. The tissues were swiftly taken out of the infiltration and carefully inserted into the lid. It was left to stand until it crystallised at room temperature. Using a microtome, the block was subsequently divided into sections that resembled ribbons. Using xylol, the region on the slide was de-waxed. The staining solution was aqueous hematoxylin. The sections were carefully mounted on the slides with Canada balsam and covered with a cover slip before being examined under a microscope.

**Statistical analysis**

The data is presented as Mean±SD (n=6). One-way analysis of variance (ANOVA) was used to statistically examine the results, and then the Bonferroni t-test. $P< 0.05$ was regarded as the threshold for significance when comparing the groups.

**Results**

Table 1 displays the findings of a qualitative phytochemical examination of crude powdered leaves of CP, AG, and CE. Terpenoids, flavonoids, alkaloids, saponins, gums/mucilage, carbohydrate, and tannin were all detected in the methanolic extract of all three plants, but terpenoids, carbohydrates, and gums/mucilage were predominantly present in the chloroform and hexane extracts of all three plants. In an excision wound model, a better healing pattern with full wound closure was seen in the standard and treated groups between 14 and 16 days, compared to roughly 18 days in control rats, as shown in Tables 2 & 3. Animals treated with extracts plus the conventional medication had shorter epithelization times than the untreated group, according to data shown in (Table 4 and Figure 1). The duration of epithelialization for the negative control group, standard medication, CP, CE, and AG extract, respectively, was determined to be 18.80±0.837, 12.80±0.837, 15.00±0.707, 15.60±1.140, and 16.80±1.194 days in table 4. In comparison to the control group, the treated group displayed significantly higher levels of hydroxyproline (Table 5). On the 18th day, histological examinations of the excision wound's tissue were conducted, and Figures 2(A)-2(E) illustrate the histopathological characteristics of the tissue from all groups of animals. In contrast to CP and CE treated groups, which demonstrated complete reepithelialization, significant cell migration, and dermal proliferation of fibroblasts along with scattered lymphoplasmacytic infiltrates and thin walled congested vesicles, wound tissues in the negative control group demonstrated incomplete epithelialization and damage, delayed wound healing, and minimal cellular infiltration. Epidermal thickening, collage deposition, and regeneration of the skin's appendages in the control group were all seen.

The skin-breaking strength of CP-treated animals in the incision wound model reached 501.83±13.348 Table 6 and Figure 3.

**Discussion**

In order to return damaged tissue's cellular structures and tissue layers as closely as possible to their pre-damage form, wound healing is a complicated and dynamic process. Wound contracture is a process that starts in the fibroblastic stage of the healing process and causes the area of the wound to decrease. The wound contracts during the maturational phase, the last stage of wound healing, result in less visible scar tissue. Granulation tissue is made up mostly of fibroblasts, collagen, edoema, and new, small blood vessels when the proliferative phase is at its most advanced stage. The test-treated animal's increase in dry granulation tissue weight shows a higher protein content. Two models were employed in this experiment to evaluate the impact of the CP, CE, and AG. The percentage wound contraction, epithelialization time, hydroxyproline content, and histoarchitecture studies in the excision wound model and tensile strength in the incision wound model were used to measure the wound healing effect. In CP-treated mice, there was a high rate of wound contraction, a reduction in the time needed for epithelialization, and an increase in the hydroxyproline level. In the incision model, animals treated with CP extract had higher skin-breaking strengths than the animals in the negative control group. Studies on the histopathology of the CP-treated groups demonstrated the efficiency in promoting wound healing. As a result, CP extract has a wound-healing action. The quick wound contraction that shortens the distance for migrating keratinocytes may have caused the sample CP to shorten the epithelialization period. The hydroxyproline level of the granulation tissue significantly increased when the CP was extracted with methanol, indicating an increase in collagen turnover. The amino acid hydroxyproline, which has been employed as a biochemical marker for tissue collagen, is a key component of collagen, the substance that builds and supports extracellular tissue [34]. The ability of CP to heal wounds could be due to any of its phytochemical components. Astringent and antimicrobial properties of phytochemicals like flavonoids [35] and triterpenoids [36] are known to promote the wound-healing process in part because they appear to be responsible for wound contraction and an increased rate of epithelization. Recent studies with other plant extracts have demonstrated this. Our prior research demonstrated the presence of triterpenoids, which were in charge of the powerful wound-healing properties. The phytoconstituents in the plant may be responsible for CP's ability to heal wounds, and the phytoconstituents' additive or individual effects on wound healing may determine how quickly a wound heals. To isolate, describe, and identify the precise active substances responsible for wound-healing activity, more phytochemical research are being conducted. The effect of the extract on angiogenesis, epithelization, or collagen deposition can be determined by electron

microscopy. Plant extracts' ability to heal wounds may also follow from a corresponding antibacterial impact [37]. In contrast to placebo and standard treatment controls, the current investigation has shown that a methanol extract of CP, CE, and AG contains features that make it capable of increasing wound-healing activity. In addition to wound contraction, higher tensile strength, and increased hydroxyproline content, CP therapy of wounds has the added benefit of encouraging wound healing.

**Table 1: Result of phytochemical screening of extracts of CP, AG and CE**

| Test | *Cucurbita pepo* Linn | | | *Amaranthus graecizans* Linn | | | *Colocasia esculenta* (L.) Schott | | |
|---|---|---|---|---|---|---|---|---|---|
| | Methanol | Chloroform | Hexane | Methanol | Chloroform | Hexane | Methanol | Chloroform | Hexane |
| Glycosides | - | - | - | - | - | - | - | - | - |
| Alkaloids | + | - | - | + | - | - | + | - | - |
| Terpenoids | + | + | + | + | + | + | + | + | + |
| Flavonoids | + | - | - | + | + | - | + | - | - |
| Phlobatannins | - | - | - | + | + | - | + | - | - |
| Carbohydrates | + | + | + | + | + | + | + | + | + |
| Saponins | + | - | - | + | + | + | - | - | - |
| Tannins | + | - | - | + | - | - | + | - | - |
| Gums/mucilage | + | + | + | + | + | + | + | + | + |

**Table 2: Effect of crude extracts on wound contraction (mm) of an excision wound in rat**

| S. No. | Group | Time (Days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| I | Negative control | 206.0±6.603 | 176.50±8.735 | 161.67±5.922 | 138.67±6.772 | 117.83±4.401 | 78.67±6.890 | 64.17±4.355 | 27.50±4.037 | 16.00±4.00 | 0±0 |
| II | Std | 201.00±5.099$^{NS}$ | 168.67±8.959$^{NS}$ | 152.00±15.620$^{NS}$ | 98.50±15.834** | 51.50±8.019** | 10.17±2.137** | 1.83±1.472** | 0±0** | 0±0$^{NS}$ | 0±0 |
| III | CP | 206.67±7.840$^{NS}$ | 177.67±9.668$^{NS}$ | 149.00±9.550$^{NS}$ | 103.50±8.479** | 81.00±4.336** | 60.67±2.733** | 34.00±4.336** | 12.83±2.483** | 1.33±0.516$^{NS}$ | 0±0 |
| IV | CE | 202.50±3.886$^{NS}$ | 182.17±4.401$^{NS}$ | 160.00±5.865$^{NS}$ | 122.50±5.099* | 98.67±2.160** | 67.83±11.583$^{NS}$ | 38.33±8.042** | 14.67±2.805** | 3.33±1.033$^{NS}$ | 0±0 |
| V | AG | 197.33±6.623$^{NS}$ | 185.33±9.564$^{NS}$ | 154.17±5.947$^{NS}$ | 120.00±2.449* | 97.00±7.694** | 79.33±1.366$^{NS}$ | 58.50±12.988$^{NS}$ | 34.00±7.376$^{NS}$ | 11.50±4.593* | 0±0 |

Values are expressed as MEAN±SD at n=6, Oneway ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and $^{NS}$P>0.001 compared to the negative control

**Table 3: Effect of crude extracts on % wound contraction of an excision wound in rat**

| S. No | Group | Time (Days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| I | Negative control | 0±0$^{NS}$ | 14.33±2.913 | 21.49±2.882 | 32.63±3.885 | 42.75±2.875 | 61.76±3.695 | 68.92±2.760 | 86.73±1.718 | 92.55±1.888 |
| II | Std | 0±0$^{NS}$ | 16.04±4.948$^{NS}$ | 24.46±6.561$^{NS}$ | 51.11±6.990** | 74.43±3.575** | 94.95±0.983** | 99.09±0.730** | 100** | 100** |
| III | CP | 0±0$^{NS}$ | 13.96±5.274$^{NS}$ | 27.86±4.727$^{NS}$ | 49.95±3.139** | 60.79±1.847** | 70.62±1.509** | 83.53±2.184** | 93.80±1.122** | 99.36±0.224** |
| IV | CE | 0±0$^{NS}$ | 10.05±0.676$^{NS}$ | 21.01±1.430$^{NS}$ | 39.76±1.811** | 51.26±1.560** | 66.46±5.949$^{NS}$ | 81.02±4.173** | 92.75±1.406** | 98.35±0.521** |
| V | AG | 0±0$^{NS}$ | 6.12±2.025* | 21.88±0.898$^{NS}$ | 39.16±1.287$^{NS}$ | 50.82±3.887** | 59.77±1.058$^{NS}$ | 70.43±5.988$^{NS}$ | 82.84±3.339** | 94.22±2.199$^{NS}$ |

Values are expressed as MEAN±SD at n=6, One way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and $^{NS}$P>0.001 compared to the negative control

**Table 4: Effect of crude extracts on epithelization period of an excision wound in rat**

| S. No. | Group | Period of Epithelization (days) |
|---|---|---|
| I | Negative control | 18.80±0.837 |
| II | Std | 12.80±0.837** |
| III | CP | 15.00±0.707** |
| IV | CE | 15.60±1.140** |
| V | AG | 16.80±1.194* |

Values are expressed as MEAN±SD at n=6, One way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and $^{NS}$P>0.001 compared to the negative control

**Table 5: Effect of crude extracts on hydroxyproline content of an excision wound in rat**

| S No. | Group | Hydroxyproline Content (µg/ml) |
|---|---|---|
| I | **Negative control** | 10.58±0.864 |
| II | **Std** | 38.38±0.910** |
| III | **CP** | 26.64±1.008** |
| IV | **CE** | 21.23±0.961** |
| V | **AG** | 17.84±1.073 ** |

Values are expressed as MEAN±SD at n=6, One way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and $^{NS}$P>0.001 compared to the negative control

**Table 6: Effect of crude extracts on breaking strength of an incision wound in rat**

| S No. | Group | Tensile strength (g) |
|---|---|---|
| I | **Negative control** | 243.83±5.636 |
| II | **Std** | 550.67±14.459** |
| III | **CP** | 501.83± 13.348** |
| IV | **CE** | 458.17±18.476** |
| V | **AG** | 400.00±12.215** |

Values are expressed as MEAN±SD at n=6, One way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and $^{NS}$P>0.001 compared to the negative control

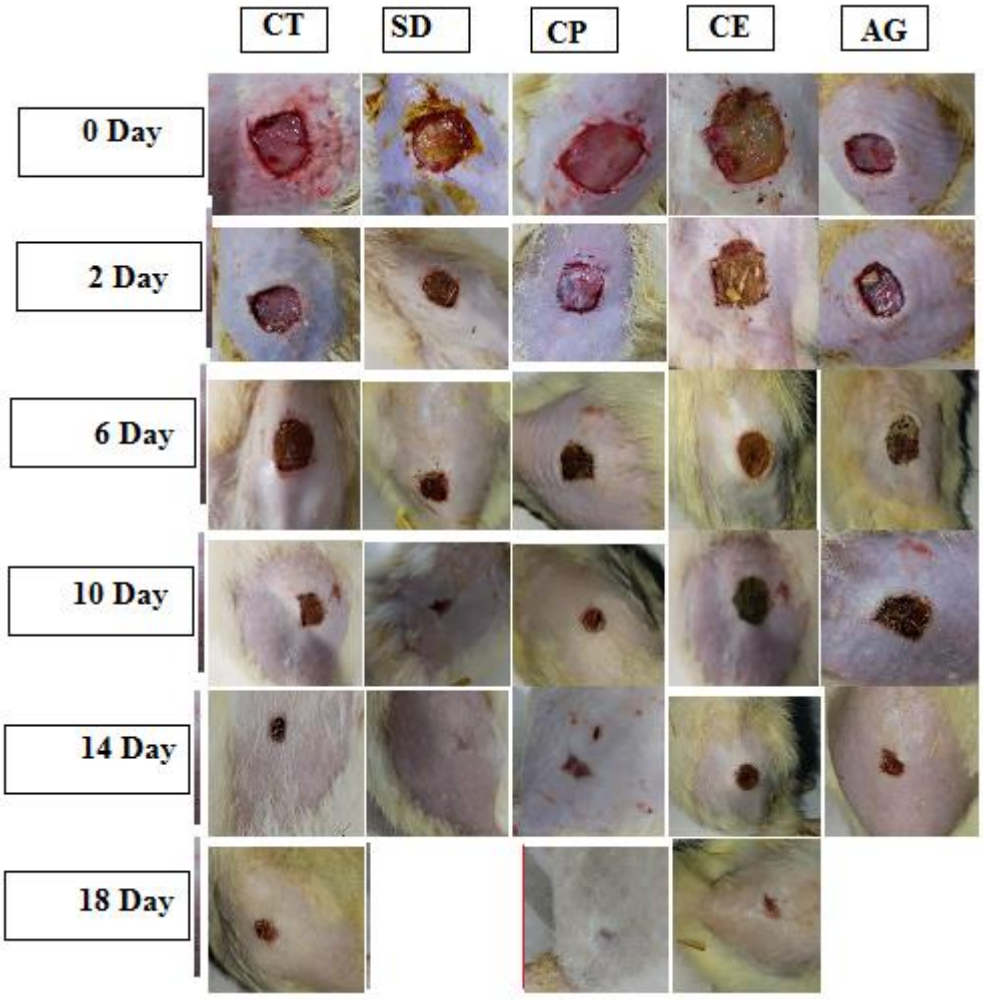
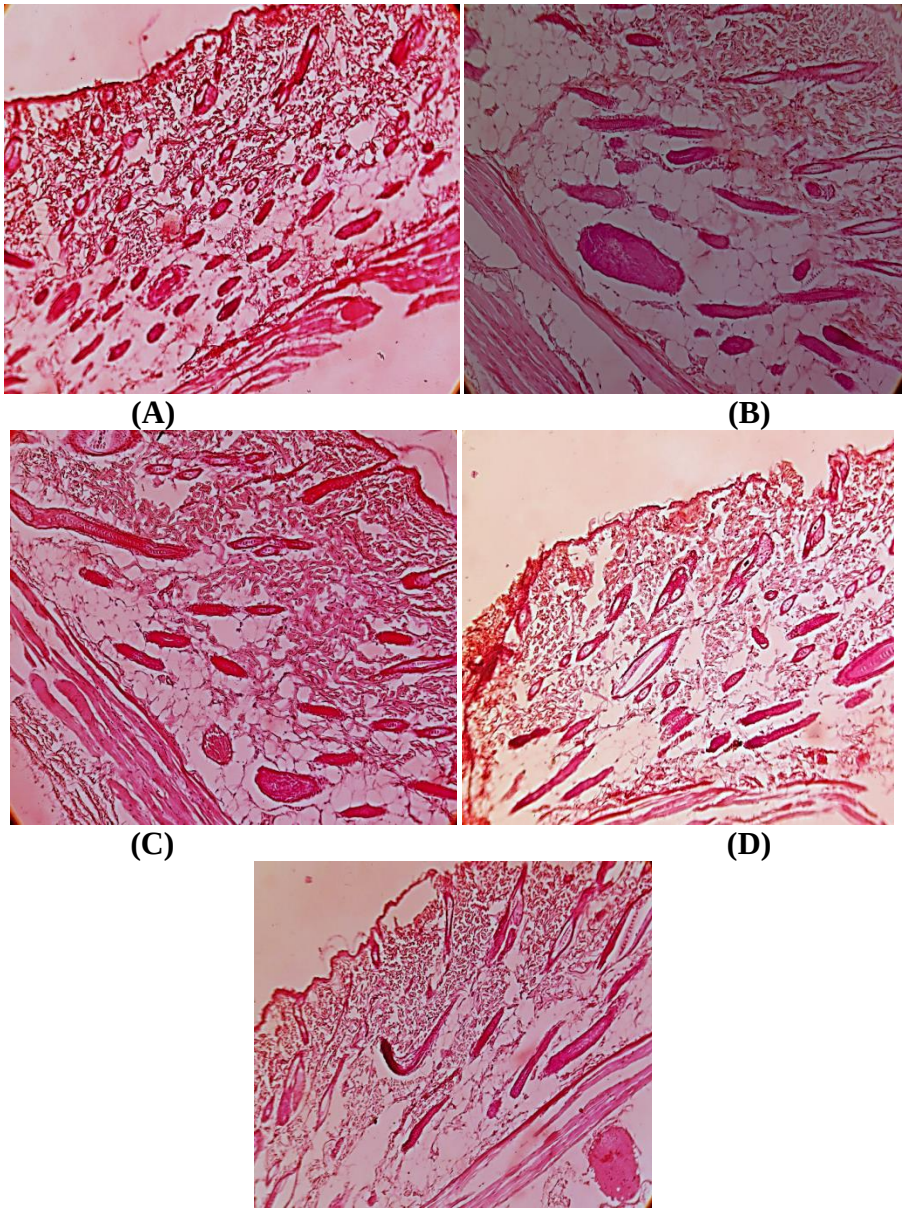



**Figure 1 Wound repair at different post wounding days in excision wound of mice.**



**(A)** **(B)**

**(C)** **(D)**

**(E)**

**Figure 2 Histopathology of the skin tissue (A) Control (B) Standard, (C) CP, (D) CE, (E) AG**

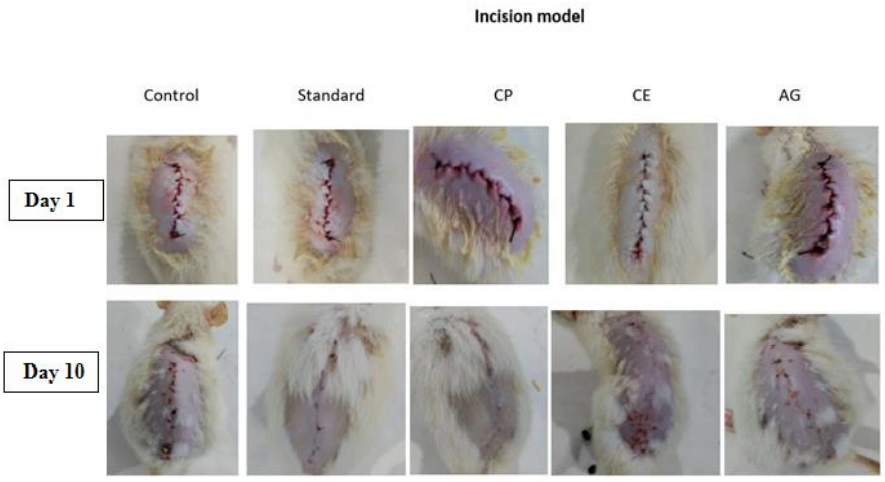



**Figure 3The images represent incision wounds evaluated in the 1st and 10th days after**

**surgery treatment with standard and test samples**

## Conclusion

It is a typical biological procedure for a wound to heal. In contrast to CE and AG, the current study has shown that a methanolic extract of CP leaves contains features that enable it to promote rapid wound-healing activity. Further research into the use of CP in the topical management and treatment of wounds is supported by wound contraction, higher tensile strength, and increased hydroxyproline content. It is reasonable to draw the conclusion that the 5%w/v extract of CP leaves has significant wound-healing activity based on the findings of the current study.

## Abbreviation:

CP= *Cucurbita pepo* Linn

CE= *Colocasia esculenta* (L.) Schott

AG= *Amaranthus graecizans* Linn

USP= United States Pharmacopeia

ACE= Angiotensin-converting enzyme

BP= Blood pressure

Bot= Botany

PBRI=Pinnacle Biomedical Research Institute

IAEC =Institutional Animal Ethic Committee

CPCSEA= Committee for the Purpose of Control and Supervision of Experiments on Animals

SD= Standard deviation

ANOVA= Analysis of variance

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