Evaluation of the Phytochemical, Antioxidant, Enzyme Inhibition, and Wound Healing Potential of *Calotropis gigantea* (L.) Dryand: A Source of a Bioactive Medicinal Product

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Traditionally, plants of the genus *Calotropis* have been used to cure various common diseases. The present research work explores the chemical and biological characterization of one of the most common species of this genus, i.e., *Calotropis gigantea* (L.) Dryand (syn. *Calotropis gigantea* (L.) Dryand.), having multiple folklore applications. The ethanolic extract of leaves of *Calotropis gigantea* (L.) Dryand was analyzed for the phytochemical composition by determining the total bioactive (total phenolic and total flavonoid) contents and UHPLC-MS secondary metabolites analysis. For phytopharmacological evaluation, in vitro antioxidant (including DPPH, ABTS, FRAP, CUPRAC, phosphomolybdenum, and metal chelation antioxidant assays) activities, enzyme inhibition potential (against AChE, BChE, α-amylase, and tyrosinase enzymes), and in vivo wound healing potential were determined.

The tested extract has been shown to contain considerable flavonoid (46.75 mg RE/g extract) and phenolic (33.71 mg GAE/g extract) contents. The plant extract presented considerable antioxidant potential, being the most active for CUPRAC assays. Secondary metabolite UHPLC-MS characterization, in both the positive and negative ionization modes, indicated the tentative presence of 17 different phytocompounds, mostly derivatives of sesquiterpene, alkaloids, and flavonoids. Similarly, the tested extract exhibited considerable inhibitory effects on tyrosinase (81.72 mg KAE/g extract) and phenolic (33.71 mg GAE/g extract) contents. The plant extract presented considerable antioxidant potential, being the most active for CUPRAC assays. Secondary metabolite UHPLC-MS characterization, in both the positive and negative ionization modes, indicated the tentative presence of 17 different phytocompounds, mostly derivatives of sesquiterpene, alkaloids, and flavonoids. Similarly, the tested extract exhibited considerable inhibitory effects on tyrosinase (81.72 mg KAE/g extract), whereas it showed weak inhibition ability against other tested enzymes. Moreover, in the case of in vivo wound healing assays, significant improvement in wound healing was observed in both the tested models at the doses of 0.5 percent w/w (p < 0.001) and 2.0 percent w/w (p < 0.01) on the 16th day. The outcomes of the present research work suggested that *C. gigantea* (L.) Dryand plant extract...
INTRODUCTION

The kingdom of plants is a source of a vast spectrum of medicinally active compounds. Plants have synthesized these compounds using different metabolic pathways. Therefore, it is essential to establish a quick and reliable method for screening plant extracts. Cuthbertson et al., (2013). Plants, with a huge number of bioactive ingredients, are the primary suppliers of natural herbal medicines that are effective against different diseases, including cancer, and these bioactive components are associated with the biological activities of plants (Ahmad et al., 2016; Mahomoodally et al., 2019). Over the last few decades, the importance of herbal medicines for the treatment of various diseases has risen exponentially because a vast number of people belonging to diverse cultures rely on the use of phytomedicines due to a shortage of primary health facilities (Shanmugam and Bhavani, 2014). According to the World Health Organization reports on phytomedicines, more than 25 percent of the drugs prescribed in recent years come from various plant sources (Khurm et al., 2016). Interestingly, due to the beneficial health effects of medicinal herbs, herbal pharmaceutical manufacturing has evolved into an ever-growing industry (Suroowan and Mahomoodally, 2019). Accordingly, there is an increased interest in exploring the folklore and conventionally used medicinal flora to discover new bioactive compounds (Aumeeruddy and Mahomoodally, 2019).

The Calotropis gigantea (L.) Dryand (C. gigantea) or sweet akand of the Asclepiadaceae family is native to India and grows well at 900 m in the lower hills (Ghule et al., 2014). Various parts of C. gigantea (L.) Dryand are documented for the treatment of sprain, agitation, fatigue, epilepsy, mental conditions, diarrhea, analgesic intervention, and the interchangeable properties of pregnancy, toothache, and earache (Argal and Pathak, 2006; Pathak and Argal, 2007; Ghule et al., 2014). Regarding the secondary metabolites, the different species of the Calotropis genus have been reported for the presence of several important classes of phytochemicals, including glycosides, flavonoids, fatty acids, and triterpenoids (Srivastava et al., 2015).

Taking into consideration the dietary and herbal uses of this plant as reported earlier, the current research plans to evaluate the detailed phytochemical composition (total phenolic and flavonoid content, ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS), secondary metabolites characterization) and conduct in vitro and in vivo biological evaluation (antioxidant, enzyme inhibition, and wound healing activities) of C. gigantea (L.) Dryand leaves extract. Antioxidant potential was appraised by testing free radical scavenging activity (DPPH and ABTS), reducing power (FRAP, ferric reducing antioxidant power; CUPRAC, cupric reducing antioxidant power), phosphomolybdenum total antioxidant capacity, and ferrous ion chelation (metal chelation) assays. Similarly, the capacity for inhibition against clinically important enzymes involved in major pathologies, including neurological disorders (acetylcholinesterase, AChE; butyrylcholinesterase, BChE), skin conditions (tyrosinase), and diabetes (α-amylase), were also examined. Additionally, in vivo wound healing activity determined by excision wound and burn wound models were also assessed.

MATERIAL AND METHODS

Plant Collection and Extraction

Calotropis gigantea (L.) Dryand (syn. Calotropis gigantea (L.) Dryand.) leaves were obtained and authenticated from Leaf Cleantech Private Limited. Approximately 50 g of powdered leaves with 400 ml of ethanol was extracted for 8 h using the Soxhlet unit. The resultant extract was concentrated and evaporated into dryness at a low temperature by distillation. Then, the extract was weighed, and the extractive percentage was measured in terms of the air-dried weight of the plant material.

Animals

Seven-week-old male Wistar albino rats (180–200 g) purchased from Bioneeds, Nelamangala, Tumkur, were maintained in the animal house, maintained in a clean room with a light-dark cycle of 12/12, 18–26°C temperature, and 30–70 percent relative humidity. Rats are initially acclimatized for one week and served water ad libitum with a daily chow diet. In solid-bottom caging, rats were caged with bedding with enough room and washed regularly. In accordance with international guidelines, all laboratory experiments (Garber et al., 2011), including animal care and handling, feeding, and scarification, were approved by the institute (IAEC/2019). After the experiment, all the animals in the treated group were euthanized with excessive isoflurane and CO2 inhalation.

Total Bioactive Contents

The total bioactive contents of C. gigantea (L.) Dryand leaves ethanolic extract were evaluated by analyzing total phenolic and flavonoid contents. Using a well-established Folin–Ciocalteu reagent process, the total phenolic content was estimated (Kähkönen et al., 1999), and the values were presented as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). Similarly, total flavonoid content was measured using the colorimetric method of aluminum chloride (Chew et al., 2009), and the results were expressed as milligrams of rutin equivalent per gram of extract (mg RE/g extract).

UHPLC-MS Phytochemical Analysis

After determining the total bioactive contents, the individual secondary metabolites profiling of C. gigantea (L.) Dryand leaves
ethanolic extract was achieved via UHPLC-MS analysis in both the positive and negative ionization modes. Agilent Zorbax Eclipse XDB-C18 column with a narrow bore of 2.1 × 150 mm, 3.5 μm (P/N: 930990-902) was utilized. The temperature of the autosampler and column was kept at 4°C and 25°C, respectively. The mobile phases with 0.1% formic acid solution in water (A) and 0.1% formic acid and acetonitrile solution (B) were used. The flow rate of the mobile phase was maintained at 0.5 ml/min. The extract solution was prepared by mixing, and 1.0 μl in HPLC grade methanol was injected for 25 min and post-run time was 5 min. The gas used as the nebulizing source was nitrogen at the flow rate of 25 and 600 L/hour and drying gas was also used. The temperature optimized for analysis was 350°C. The capillary voltage used for analysis was 3,500 V, and fragmentation voltage was maintained at 125 V (Saleem et al., 2019; Saleem et al., 2020). The data were further processed with Agilent Mass Hunter, and the tentative qualitative identification of phytochemicals was achieved by METLIN database library search.

**Antioxidant Assays**

The antioxidant methods as described earlier by Grochowski et al. (2019) were utilized to estimate the radical scavenging activity (DPPH and ABTS), reduction power (FRAP and CUPRAC), total antioxidant capacity (phosphomolybdenum assay), and metal chelation activity of the C. gigantea leaves ethanolic extract. The results of all the antioxidant assays were reported as milligrams of Trolox equivalents per gram of extract (mg TE/g extract), while metal chelating activity was expressed as milligrams of ethylenediaminetetraacetic acid equivalent (mg EDTAE/g extract) per gram of extract.

**Enzyme Inhibition Assays**

*C. gigantea* (L.) Dryand leaves ethanolic extract was tested for its enzyme inhibition potential against AChE, BChE, tyrosinase, and α-amylase enzymes utilizing standard *in vitro* methods, as mentioned earlier (Mollica et al., 2017). Galantamine was the standard used for AChE and BChE, and inhibitory action was measured as mg GALAE/g extract (milligrams of galantamine equivalent per gram of extract). Likewise, acarbose was used as a standard for inhibition of the α-amylase enzyme, and the findings were reported in millimoles of acarbose equivalent per gram of extract (mmol ACAE/g extract). The effects of the inhibition of the tyrosinase enzyme were reported as milligrams of kojic acid equivalents per gram of extract (mg KAE/g extract) using kojic acid as a standard.

**In Vivo Wound Healing Activity**

*Ointment Preparation*

The extract ointment was formulated in a simple ointment, namely, with two different extract concentrations, 0.5 percent (w/w) and 2.0 percent (w/w), respectively. The simple ointment containing wool fat (5 gm), hard paraffin (5 gm), cetostearyl alcohol (5 gm), and white soft paraffin (85 gm) was prepared as per Gemeda et al. (2018); (Gemeda et al., 2018). Nitrofurazone ointment (0.2 percent w/w) was used as a reference drug to compare the extract wound healing ability in the various animal models.

**Experimental Design**

 Albino Wister male rats weighing 180–200 g were divided into four groups, each consisting of 6 rats following the previously established protocols (Harish et al., 2008):

- **Group 1:** Control group treated with simple ointment
- **Group 2:** Standard group treated with 0.2 percent nitrofurazone
- **Group 3:** Test group 1 treated with 0.5 percent w/w ECGLD (0.5 g extract in simple, 100 g ointment)
- **Group 4:** Test group 2 treated with 2 percent w/w ECGHD (2 g extract in simple, 100 g ointment)

**Excision Wound Model**

The rats were depilated on the back, and an area of 500 mm² of maximum skin thickness was excised in the interscapular dorsal region as defined by Morton and Malone (1972). The ointment was applied topically every day until the day of the procedure, and complete epithelization was achieved. On the 4th, 8th, 12th, and 16th days, wound closure was assessed to see the percentage of wound closure and epithelization, indicating the development of new epithelial tissue to cover the wound. Epithelization time is the number of days needed to minimize the scar without having traces of the raw wound (Balakrishnan et al., 2006). Percent wound contraction was calculated by the following formula:

\[
\text{Percent wound contraction} = \left(\frac{A_0 - A_n}{A_0}\right) \times 100,
\]

where \(A_0\) and \(A_n\) are initial wound area and wound area after “n” days, where n is day 4, day 8, day 12, and day 16.

**Burn Wound Model**

In overnight fasting animals under anesthesia with pentobarbitone (30 mg/kg IP), partial-thickness burn wounds were inflicted by pouring 80°C hot molten wax. The wax was

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**TABLE 1** Total bioactive contents, antioxidant, and enzyme inhibition activities of ethanolic extract of C. gigantea (L.) Dryand leaves.

| Total bioactive contents | C. gigantea (L.) Dryand leaves ethanolic extract |
|--------------------------|---------------------------------------------|
| Total phenolic content (mg GAE/g extract) | 33.71±0.13 |
| Total flavonoid content (mg RE/g extract) | 46.75±1.30 |

**Antioxidant assays**

| DPPH (mg TE/g extract) | 67.90±0.75 |
| ABTS (mg TE/g extract) | 89.67±2.06 |
| FRAP (mg TE/g extract) | 118.8±0.35 |
| CUPRAC (mg TE/g extract) | 207.29±1.18 |
| Phosphomolybdenum (mg TE/g extract) | 1.71±0.05 |
| Metal chelating (mg EDTAE/g extract) | 27.74±0.63 |

**Enzyme inhibition assays**

| AChE (mg GALAE/g extract) | 4.63±0.26 |
| BChE (mg GALAE/g extract) | 5.78±0.62 |
| α-Amylase (mmol ACAE/g extract) | 0.66±0.01 |
| Tyrosinase (mg KAE/g extract) | 81.72±0.36 |

Data from three repetitions with a mean ± standard deviation. GAE, gallic acid equivalent; RE, rutin equivalent.
poured on the animal shaved back through a circular opening nozzle of 300 mm². The wax had been allowed to stay on the skin until it solidified. The ointment or vehicle was applied topically every day on the injury and wound contraction was measured as described on the following days (Rashed et al., 2003).

\[
\text{Percent wound healing} = \frac{A_0 - A_n}{A_0} \times 100
\]

where \(A_0\) and \(A_n\) are initial wound area and wound area after \(n\) days, where \(n\) is day 4, day 8, day 12, and day 16.

**Statistical Analysis**

All results were the mean of three equivalent experiments and were expressed as average ± SD of value. Unpaired \(t\)-tests were used to assess the \(p\) value and significance compared to the control group with Graph Pad Prism version 6.04 for Windows.

**RESULTS AND DISCUSSION**

**Phytochemical Composition**

In the present study, the total bioactive content of *C. gigantea* (L.) Dryand leaves ethanolic extract was measured by evaluating total phenolic and total flavonoid contents. As presented in Table 1, the tested extract was found to have higher total flavonoid contents (46.75 mg RE/g extract) than phenolic contents (33.71 mg GAE/g extract). Similarly, to gain more insight into the chemical composition, the secondary metabolites profiling of *C. gigantea* (L.) Dryand leaves ethanolic was carried out by UHPLC-MS-MS in positive and negative ionization modes. The tentatively identified compounds (as per METLIN database) are listed in Tables 2, 3, respectively, while their total ion chromatograms (TICs) are presented in Figures 1A, B. As seen in Table 2, the UHPLC-MS chromatograms base peak analysis tentatively identified 12 and 5 distinct phytocompounds in positive and negative ionization modes, respectively.

**Antioxidant Activity**

Related antioxidant protocols have been performed to investigate the antioxidant ability of studied plant extract, including radical scavenging activity (DPPH and ABTS), accompanied by antioxidant reduction (FRAP and CUPRAC), phosphomolybdenum, and metal chelation assays to confirm and evaluate antioxidant findings (Table 1). The results highlighted the potential of the tested extract as considerable DPPH (67.90 mg TE/g extract) and ABTS (89.67 TE/g extract) radical scavenger (Table 1). Similarly, to measure the reduction potential of the studied plant extract, reducing power assays, i.e., FRAP and CUPRAC methods, have been used, and the results are shown in Table 1. The tested extract exhibited the FRAP and CUPRAC reducing potential with values of 118.8 and 207.29 mg TE/g extract, respectively. Likewise, the tested extract capacity for phosphomolybdenum assay is depicted in Table 1, and presented the total antioxidant capacity with a value of 1.71 mg TE/g extract. Moreover, the chelating capacity of the extract was evaluated by the metal chelation assay, and the extract

| S.No | RT (min) | B. peak (m/z) | Proposed compound | Compound class | Mol. Formula | Mol. Mass |
|------|----------|--------------|------------------|----------------|-------------|----------|
| 1    | 0.708    | 272.10       | Deidadolin       | Glucoside      | C₂₃H₂₇NO₆  | 271.10   |
| 2    | 7.46     | 422.21       | Armilline        | Sesquiterpene  | C₂₄H₂₆O₇  | 420.21   |
| 3    | 9.327    | 479.11       | Tamariarin       | Flavonoid      | C₂₅H₂₆O₁₂ | 478.11   |
| 4    | 10.693   | 212.15       | Elaeokanine C    | Alkaloid       | C₂₃H₂₂O₇  | 211.15   |
| 5    | 10.975   | 247.19       | Isonovacardenuran | Furan deriv | C₁₇H₂₆O₇  | 246.19   |
| 6    | 11.264   | 227.15       | Dihydrojasmonic acid, methyl ester | Jasmonic acid deriv | C₂₃H₂₄O₄  | 226.15   |
| 7    | 11.575   | 317.05       | Nodiflorin       | Flavonoid      | C₁₇H₂₂O₇  | 316.05   |
| 8    | 12.263   | 273.23       | 2-Hydroxyhexadecanoic acid | Fatty acid | C₁₈H₃₀O₄  | 272.23   |
| 9    | 14.023   | 295.18       | Gingerol         | Phenol         | C₁₈H₂₀O₂  | 294.18   |
| 10   | 15.814   | 235.16       | Curcumenol       | Sesquiterprenoid | C₁₈H₂₀O₄ | 234.16   |
| 11   | 16.185   | 299.25       | cis-9,10-Epoxyacrylic acid | Fatty acid | C₁₈H₂₀O₄  | 298.25   |
| 12   | 16.73    | 279.15       | Emmotin A        | Sesquiterprenoid | C₁₈H₂₀O₄ | 278.15   |

| RT, retention time; B. peak: base peak. |

| S.No | RT (min) | B. peak (m/z) | Proposed compound | Compound class | Mol. Formula | Mol. Mass |
|------|----------|--------------|------------------|----------------|-------------|----------|
| 1    | 0.627    | 215.04       | Isobergaptene    | Coumarin       | C₁₈H₁₄O₄  | 216.04   |
| 2    | 0.658    | 341.12       | Dictyosin A      | Alkaloid       | C₁₇H₂₀N₅O₅ | 342.12   |
| 3    | 9.533    | 187.10       | Norcacid        | Terpenoid      | C₂₀H₂₆O₆  | 188.10   |
| 4    | 11.449   | 329.24       | 5,8,12-Trihydroxy-9-octadecenoic acid (6S)-Dehydrovormilfolioli | Fatty acid | C₁₈H₂₀O₅  | 330.24   |
| 5    | 12.329   | 221.12       |                  | Sesquiterpenoid | C₁₈H₂₀O₃  | 222.12   |

| RT, retention time; B. peak: base peak. |
was found to be considerably active (27.74 mg EDTAE/g extract) (Table 1).

**Enzyme Inhibition Assays**

In the current study, *C. gigantea* (L.) Dryand leaves ethanolic extract was screened against AChE, BChE, α-amylase, and tyrosinase, and the findings are reported in Table 1. The tested plant extract exhibited higher AChE inhibition (4.63 mg GALAE/g extract). The observed reduction of AChE can be due to phenolic behavior, and this evidence is confirmed as previous studies validate similar associations (Hasnat et al., 2013; Bilal et al., 2016). For the BChE inhibition, the extract was found to present the inhibition values of 5.78 mg GALAE/g extract (Table 1). As for α-amylase enzyme inhibition, the tested extract was found to exhibit weak inhibitory potential with the value of 0.66 mmol ACAE/g extract, while, in the case of tyrosinase enzyme, significant inhibitory activity was noted, i.e., 81.72 mg KAE/g extract (Table 1).
Wound Healing Activity
Excision Wound Model
The effects of wound healing as noted by the excision wound model are given in Table 4 and Figures 2, 3, 4, which represent 4, 8, 12, and 16 days of wound healing percentage for control (group treated with simple ointment), standard (nitrofurazone 0.2 percent w/w–treated group) and the test groups, namely, the ECPLD (C. gigantea (L.) Dryand extract low dose 0.5 percent w/w) and ECPHD (C. gigantea (L.) Dryand extract high dose 2 percent w/w). It was found that the capacity of the extract ointment to contract wound in both concentrations was significantly greater than that of the control (i.e., group treated with simple ointment). The wound contracting potential of animals treated with an ointment containing 0.5 percent (w/w) ethanolic extract on day 16 (94.33 ± 0.43) was found to be highly significant ($p < 0.001$) compared to the control group. The 2 percent (w/w) ointment–treated groups showed significant wound healing from the fourth day onwards, and the capacity to contract the wound was found to be significant ($p < 0.001$) on the 12th and 16th days (50.83 ± 1.94, 81.00 ± 3.26, respectively). Compared to control, both ECGLD (0.5 percent w/w) and ECGHD (2 percent w/w) provided a substantial decrease in the epithelization period. ECGLD therapy has achieved a significant decrease ($p < 0.001$) in epithelization time (20.20 ± 0.82). Some of the essential phytochemicals that have been reported for wound healing activity are related to flavonoids (Iyyam Pillai et al., 2010). Secondary metabolite analysis shows the existence of flavonoids (tamarixin) that may be accountable for their role in healing wounds.

Burn Wound Model
The results of burn wound healing activity are depicted in Table 5 and Figures 5, 6, 7, showing a percentage of wound healing at 4, 8, 12, and 16 days for control (simple ointment–treated group), standard (nitrofurazone, 0.2 percent w/w–treated group), and...
test groups, namely, the ECGLD (0.5 percent w/w) and ECGHD (2 percent w/w). Substantial improvement has been observed in percentage contractibility in ECGLD-treated rats from day 4 onwards, and even in later days, the closure rate is much higher than that in control groups. In contrast to the control group, the wound contracting capacity of animals treated with an ointment containing (0.5 percent w/w) ethanol extract was observed to be significantly higher ($p < 0.001$) on the 16th day ($78.75 \pm 1.74$). On the 16th day ($70.67 \pm 1.32$), the percentage of wound contraction in ECGHD 2 percent w/w-treated animals demonstrated moderate activity ($p = 0.05$). A stronger healing trend and decrease in the epithelization period were found in ECGLD 0.5 percent w/w-treated group with extremely significant activity ($p < 0.001$) (24.10 ± 1.01) and ECGHD 2 percent w/w showed modest significant activity ($p < 0.01$) (25.30 ± 0.54) compared with the control group. In our study, flavonoids and terpenoids have been identified to have astringent and antimicrobial properties that promote the healing and epithelization of wounds (Iyyam Pillai et al., 2010). It is known that flavonoids also inhibit lipid peroxidation not only through blocking or minimizing cell necrosis but also by enhancing vascularity and improving the viability of collagen fibrils (Getie et al., 2002).

**DISCUSSION**

Plants bioactive compounds, including phenolic and flavonoids, have several beneficial effects on human health (Dirir et al., 2017). Mostly these compounds were found to be derivatives of sesquiterpene, flavonoids, alkaloids, fatty acids, and phenols. The two identified sesquiterpenes (armillane and emmotin A), flavonoids (tamarixin), alkaloids (elaeokanine C), phytocomponents (isoavocadenofouran, dihydrojasmonic acid, methyl ester, nodiflorterin, curcumenol, isobergapten, dictyoquinazol C (6S)-dehydrovomilfoliol), fatty acid (2-hydroxyhexadecanoic acid, cis-9,10-epoxysteaeric acid, 5,8,12-trihydroxy-9-octadecenoic acid), terpene (nonic acid), phenol (gingerol), glucoside (dehydrolignan). The presence of these phytochemical classes, specifically alkaloids, phenolics, terpenes, flavonoids, is in accordance with the previous studies that have reported the presence of these classes as major phytochemical groups in this plant species (Kumar et al., 2011).

Free radicals such as DPPH and ABTS are widely used to test various plant samples for their radical scavenging ability (Sarıkürkçü et al., 2017). The former higher content of total phenolic and flavonoids may be ascribed and associated with

TABLE 5 | Effect of topical application of an ointment containing ethanolic leaf extract of *C. gigantea* leaves on wound contraction of the burn wound. Mean percentage of wound contraction ±SEM.

| Groups          | Treatment                        | 4th day | 8th day | 12th day | 16th day | Period of epithelization |
|-----------------|----------------------------------|---------|---------|----------|----------|--------------------------|
| Group 1         | Control (simple ointment)        | 23.09 ± 1.15 | 33.00 ± 1.45 | 57.19 ± 0.83 | 65.18 ± 0.92 | 28.09 ± 0.47 |
| Group 2         | Standard (nitrofurazone) 0.2% w/w | 28.12 ± 2.03*** | 53.02 ± 0.20*** | 73.07 ± 0.95*** | 81.38 ± 0.81*** | 22.93 ± 1.57*** |
| Group 3         | ECGLD 2% w/w                     | 27.00 ± 0.95*** | 32.83 ± 2.76 ns | 61.33 ± 0.99*** | 78.75 ± 1.71*** | 24.10 ± 1.00*** |
| Group 4         | ECGHD 4% w/w                     | 25.25 ± 0.95 ns | 44.17 ± 2.55*** | 50.78 ± 1.70*** | 70.67 ± 1.31*** | 25.30 ± 0.54*** |

ECGLD: extract of *C. gigantea* low dose; ECGHD: extract of *C. gigantea* high dose. n = 6 animals in each group. The treated groups are analyzed by two-way ANOVA for wound closure and -way ANOVA for the period of epithelization by comparing with the control group. ***$p < 0.001$; ns: not significant.
this observed higher antioxidant potential of these extracts. Interestingly, the increased antioxidant function of methanol extracts was also illustrated by previous researchers (Li et al., 2017; Sumczynski et al., 2017). Previously, the antioxidant effects of the methanolic, chloroform, hot water, and ethyl acetate extracts of the leaves of C. gigantea (L.) Dryand were via DPPH, reducing power, and inhibition of nitric oxide radical, and the chloroform extracts showed the highest antioxidant activity (Mohanraj and Usmani, 2012). Total antioxidant activity was calculated using phosphomolybdenum assays based on molybdenum (VI) reduction to molybdenum (V) compounds with antioxidant potential and the subsequent development of molybdenum (V) complex (green in color) (Prieto et al., 1999). The phenolic and flavonoid content could be related to the observed antioxidant ability of the tested extract, and our findings are consistent with several previous studies suggesting that the amount of bioactive content in plant extracts plays an important role and accounts for the capacity of antioxidants (Patro et al., 2016; Nordin et al., 2018). Therefore, our findings could be regarded as the results of a preliminary investigation to analyze further the use of C. gigantea (L.) Dryand as a possible source of natural antioxidant molecules.

In recent years, enzymes have been studied as an important research area to prevent common health-related issues worldwide (Rauf and Jehan, 2017). AChE and BChE (cholinesterases) enzyme inhibitors are presently being utilized for the treatment/management of Alzheimer’s diseases (AD). Likewise, α-amylase is an enzyme responsible for releasing glucose from starch and is also involved in the carbohydrates catabolism (Kubinova et al., 2014). Consequently, the compounds with inhibition potential against α-amylase and cholinesterases could be considered key molecules for treating the diseases linked with these enzymes (Yerlikaya et al., 2017). Tyrosinase is a crucial enzyme in melanin synthesis, so it can be used as the primary target for controlling hyperpigmentation problems (Pillaiyar et al., 2017). Tacrine, acarbose, and kojic acid are some of the available compounds that have been used as inhibitors for cholinesterases, glucosidases, and tyrosinase, respectively. However, these commercially available inhibitors have reported adverse effects (Mishra et al., 2019; Milovanovic et al., 2021). Henceforward, the search for new enzyme inhibitors from natural flora may therefore be an appropriate alternative to overcome the above-mentioned deficiencies.

**CONCLUSION**

In this study, the phytochemical profile, antioxidants, enzyme inhibition effects, and secondary metabolites profile of C. gigantea (L.) Dryand leaves ethanolic extract are explored. The plant was found to have a substantial phenolic and flavonoid content. As identified by the UHPLC-MS study, the secondary metabolite profile indicated the existence of several bioactive metabolites belonging to important phytochemical groups. Overall, the plant extract under study was found to have notable antioxidant and enzyme inhibitory ability, which could be attributed to its total bioactive and secondary metabolite composition. Thus, this plant...
should be viewed as a perspective origin for bioactive phytochemicals having antioxidant and enzyme inhibition potential. In addition, the current research justifies the use of *C. gigantea* (L.) Dryand leaves to facilitate wound healing in the traditional medicinal system. However, further studies on the isolation of responsible lead compounds and their toxicity are highly recommended.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the Vydehi Institute of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore, India.

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AhA was responsible for the conceptualization and wrote the original draft. SS contributed to the methodology. HS contributed to the methodology and writing of the manuscript. AA was responsible for the conceptualization. SYA conducted the formal analysis and edited the manuscript. SZ contributed to the methodology. SAA was responsible for the conceptualization. AbA was responsible for the conceptualization and project supervision. NA wrote, reviewed, and edited the work. SiA was responsible for the conceptualization, data curation, and writing of the original draft. UK contributed to the methodology and writing of the manuscript.

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