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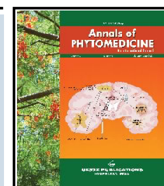

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Assessment of biological activity of ethanolic leaf extract of *Zaleya decandra* L.

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Abstract

The healthcare sector has a special place for natural products. *Zaleya decandra* L., is a member of the genus Trianthema in the family Aizoaceae. It has been used to treat a variety of chronic and challenging illnesses, including cancer, cardiovascular disease, diabetes, rheumatism, and anti-inflammatory, analgesic, and microbiological conditions. The present study aims to assess the antioxidant, analgesic, and anti-inflammatory activities of an ethanolic leaf extract of *Z. decandra* (EEZD) in an experimental animal model. The DPPH scavenging assay and the hydrogen peroxide radical scavenging assay were both used to measure *in vitro* antioxidant activity. The tail immersion method was employed to investigate analgesic activity in Wistar albino rats, with pentazocin as the drug of choice at a dose of 30 mg/kg of body weight. The acute and chronic anti-inflammatory activities were determined by carrageenan-induced paw edoema and cotton pellet granuloma tests. The extract was taken at doses of 200 mg/kg and 400 mg/kg of body weight, and indomethacin was used as a standard for both acute and chronic anti-inflammatory models at a dose of 10 mg/kg of body weight. A positive correlation (p-value <0.01) was observed between EEZD and free radical (DPPH• and •OH) scavenging efficiencies. EEZD significantly diminished both the early and late phases of the inflammatory response and the edoema retained between the two phases. The data showed that the ethanolic leaf extract of *Z. decandra* is a potential source of natural antioxidants, analgesics, and anti-inflammatory agents and serves as an effective free radical scavenger and anti-inflammatory agent. Hence, *Z. decandra* might be a good plant-based pharmaceutical product for several diseases caused by free radicals and inflammation.

1. Introduction

Inflammation is a typical phenomenon that everyone is familiar with, and it is the body's way of dealing with infections and tissue damage. Inflammation is one of the leading causes of morbidity in the modern era. Inflammatory cascades and their potential for long-term tissue damage are kept in check, and any imbalance leads to acute and chronic debilitating disorders. Though, an inflammatory response is required for survival, excessive and uncontrolled inflammation can lead to a variety of disorders, including rheumatoid arthritis, allergic asthma, allergic rhinitis, chronic sinusitis, diabetes, cancer, atherosclerosis, and cardiovascular disease (Lisa *et al*., 2020; Thangavelu *et al*., 2019). Inflammation is characterised by redness, pain, and swelling (Ezeja et *al*., 2011; Fiorucci *et al*., 2001; Apu *et al*., 2012; Fan *et al*., 2014; Yasmen *et al*., 2018). One of the major signs of inflammation is the pain that can be triggered by the action of inflammatory mediators (Hijazi *et al*., 2017). These mediators, cytokines, histamine, serotonin, and prostaglandins, are used to increase vascular permeability (Ishola *et al*., 2014; Ali *et al*., 2022).

Pain relievers such as asprin, opioids, and nonsteroidal anti-inflammatory medications (NSAIDS) have major side effects such as physical dependence, addiction, and gastrointestinal problems (Mishra *et al*., 2011; Karpakavallie *et al*., 2013). As a result, researchers are still looking for a new, safe analgesic and anti-inflammatory medicine. Medicines can be found in abundance in nature (Sri Bhuvaneswari *et al*., 2022). Pharmaceuticals, healthcare items, and nutraceuticals are all made from medicinal plants. Medicinal plants provide low-cost, easily accessible alternatives to pharmaceuticals with little or no side effects (Leelaprakash *et al*., 2011; Pushpangadan *et al*., 2015; Sameemabegum *et al*., 2022).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals (Mahdi-Pour *et al*., 2010; Sameemabegum *et al*., 2022). Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognised as agents involved in the pathogenesis of illnesses such as asthma, inflammatory arthropathies, diabetes, cancer, and Alzheimer's disease (Malarvizhi *et al*., 2015; Sahgal *et al*., 2009).

The plant, *Z. decandra*, popularly known as Purslane in English, is a member of the Aizoaceae family. In India, it is a succulent annual herb with branched stems (Geethalakshmi *et al*., 2010). *Z. decandra* and its species are used for antidiabetic (Meenakshi *et al*., 2010), anti-inflammatory, antihyperglycemic, hepatoprotective, antioxidant, and diuretic purposes in traditional medicine systems such as ayurveda and unani (Thirupathi *et al*., 2014; Arya *et al*., 2022). To cure a partial headache, put 2-3 drops of leaf juice into the nostrils (Malarvizhi *et al*., 2015).

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The outcomes of ethnobotanical and ethnopharmacological research on *Z. decandra* suggest that these plants may be used to cure a wide range of ailments. Owing to growing interest in *Z. decandra*'s overall activity, it is crucial for understanding the link between antioxidants and inflammation and illnesses. The objective of this study is to determine the free radical scavenging, analgesic, and anti-inflammatory activities of ethanolic extracts of *Z. decandra* in an experimental animal model.

2. Materials and Methods

2.1 Animals

The experimental Wistar albino rats weighing 180-250 g were provided by the Nandha College of Pharmacy's Animal House of Pharmacology Department. A room temperature was maintained at 22 ± 2ºC. In addition, a 12 h light/dark cycle was maintained. Prior to the tests starting, the animals fasted for 12 h while still having access to water. The experimental protocol was authorised by the Institutional Animal Ethical Committee (688/2/CPCSEA).

2.2 Chemicals

Indomethacin, carrageenan, and pentazocin drugs were obtained from Sigma, and all other chemicals were obtained from local chemical suppliers, and all were of analytical grade.

2.3 Plant material

The plant *Z. decandra* was collected from Erode, Tamil Nadu, and authenticated by Dr. G. Jayanthi, Research Department of Botany, Vellalar College for Women, Erode. A voucher specimen (VCW/2/21BT-65) of the collected sample was deposited in the Institutional Herbarium for future reference.

2.4 Preparation of extract

The dry plant (500 g) made course powder and was subjected to extraction with ethanol by the Soxhlet apparatus. After extraction, the filtrate was concentrated in a water bath. The extract was stored until further use.

2.5 Evaluation of antioxidant activity

2.5.1 DPPH scavenging assay

The different concentrations of the plant extracts were prepared using analytical methanol (40, 80, 120, 160, and 200 g/ml), and ascorbic acid was used as an antioxidant standard. The reaction mixture consists of 1 ml of extract, 3 ml of methanol, and 0.5 ml of 1.0 mM DPPH in methanol and is allowed to react at room temperature for 30 min. The same amount of methanol and DPPH was mixed to prepare the blank solution. The samples were prepared in triplicate for each analysis, and the antioxidant activity of the plant extract against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV spectrophotometry at 517 nm (Stankovic *et al.*, 2016; Tijjani *et al.*, 2015). The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = Ab - Aa/Ab \times 100$$

where, "Ab" is the absorption of the blank sample and "Aa" is the absorption of the extract.

2.5.2 Hydrogen peroxide radical scavenging (·OH) assay

A 200 ml volumetric flask was filled with 50 ml of potassium dihydrogen phosphate solution and 39.1 ml of 0.2 M sodium hydroxide solution. Finally, distilled water was used to prepare 200 ml of phosphate buffer (pH 7.4). To create the free radicals, 50 ml of phosphate buffer solution was mixed with an equivalent volume of hydrogen peroxide (made in accordance with Indian Pharmacopoeia standards), and the mixture was then left at room temperature for 5 min to complete the reaction. The range of concentrations chosen for the EEZD extract was 40 to 200 mg/ml, with ascorbic acid serving as a benchmark. In order to quantify the absorbance at 230 nm in a UV spectrophotometer, extracts (1 ml) in distilled water were added to a 0.6 ml hydrogen peroxide solution. The absorbance was compared to a blank solution made up of phosphate buffer solution without hydrogen peroxide (Torey *et al.*, 2010). The tests were carried out three times. The following equation was used to calculate the extract's H_2O_2 scavenging percentage:

$$\% \text{ of } \cdot OH \text{ radical scavenging activity} = (A0 - A1)/A0 \times 100$$

where, "A0" is the absorbance of the control and "A1" is the absorbance of the sample.

2.6 Evaluation of analgesic activity

2.6.1 Tail immersion method

The rats were divided into four groups (n = 6). As a control, Group I animals were given 0.5% CMC (0.1 ml/10 gm) in normal saline intraperitoneally. Pentazocin (reference, 30 mg/kg) was given to Group II, whereas Groups III and IV received the extract in 0.5% w/v Na CMC in normal saline at doses of 200 and 400 mg/kg, respectively. The Wistar albino rats' tails were gently immersed in hot water maintained at 55°C to test their sensitivity and removed their tails from the hot water within 5 seconds. The reaction time was calculated at 0, 30, 60, 90, and 120 min after the samples were administered (Benni *et al.*, 2011; Reddy *et al.*, 2012).

2.7 Evaluation of anti-inflammatory activity

2.7.1 Carrageenan induced rat paw edema

The carrageenan-induced paw edoema method was used to assess the anti-inflammatory efficacy of the test compounds in Wistar albino rats. The animals were fasted overnight and placed into four groups. Animals in the standard group received indomethacin at a dose of 10 mg/kg by oral route. The EEZD extract was made into a suspension with 0.5% w/v carboxymethylcellulose and administered by oral route at doses of 200 and 400 mg/kg, respectively, to the test groups. The rats in the control group were given a vehicle solution that did not include any of the test substances. Rats in all groups were given 0.1 ml of 1% carrageenan in the subplantar region of the right hind paw one hour after receiving the test compounds. The thickness of the paws was measured with a Vernier caliber before and after the carrageenan challenge, at intervals of one hour up to four hours (Chouhan *et al.*, 2014; Usman *et al.*, 2012; Aruna *et al.*, 2010).

Group I (Control): 1% Carrageenan solution (0.1 ml/kg)

Group II (Standard): Carrageenan and Indomethacin (10 mg/kg)

Group III (Test): Carrageenan and EEZD (200 mg/kg)

Group IV (Test): Carrageenan and EEZD (400 mg/kg)

The per cent inhibition of paw thickness for treated groups was calculated by comparing it with the mean paw thickness of the control group:

% inhibition = 100 (1 – Vt/Vc)

Vc: control mean paw thickness; Vt: test mean paw thickness

2.7.2 Cotton pellet granuloma method

Group I (Control): Received vehicle

Group II (Standard): Indomethacin (10 mg/kg, p.o.)

Group III (Test): EEZD (200 mg/kg, p.o.)

Group IV (Test): EEZD (400 mg/kg, p.o.)

Cotton pellets weighing 10 ± 1 mg were autoclaved and implanted subcutaneously into both sides of the groin region of each rat. Animals in Groups III and IV received the EEZD orally for 7 days at doses of 200 mg/kg and 400 mg/kg. Animals in Group II received indomethacin orally for the same time period at a dose of 10 mg/kg. The animals were euthanized on the eighth day, and the pellets, as well as the granuloma tissues, were carefully removed, dried in a 60°C oven, weighed, and compared to the control and standard groups (Suresha *et al*., 2012; Kumbhare *et al*., 2011; Tijjani *et al*., 2015). The percentage inhibition was calculated by using a formula:

% inhibition = $W_C – W_T / W_C$ x 100

where, W_C = weight of granuloma in control group; W_T = weight of granuloma in the test and standard group.

2.8 Statistical analysis

Results are expressed as mean ± SEM. The statistical analysis employed one-way analysis of variance (ANOVA), which was followed by Dunnett's multiple comparison test. $p<0.05$ was considered as statistically significant.

3. Results

The DPPH assay could be considered an appropriate method for evaluating the potential of samples to scavenge free radicals. The radical scavenging activity of the standards ascorbic acid and EEZD tested, summarised in Table 1, demonstrated potent free-radical scavenging activity has been observed at the dose of 200 mg/kg with 97.13 ± 0.14% and 88.18 ± 0.24% DPPH radicals scavenging activity, respectively. The findings of the hydrogen peroxide scavenging activity were displayed in Table 2. It shows that the maximal hydrogen peroxide scavenging activity of ethanolic leaf extract of *Z. decandra* was 84.80 ± 0.24%, which is equivalent to the 95.13 ± 0.43% standard treatment.

Table 1: Antioxidant effect of EEZD by DPPH scavenging assay

S.No.	Concentration (µg/ml)	% Inhibition by ascorbic acid	% Inhibition by test sample
1.	40	90.52 ± 0.28	24.10 ± 0.32
2.	80	93.83 ± 0.15	64.72 ± 0.09
3.	120	94.73 ± 0.24	73.70 ± 0.62
4.	160	96.33 ± 0.48	85.49 ± 0.12
5.	200	97.13 ± 0.14	88.18 ± 0.24

Values expressed as *Mean of three readings ± SEM

Table 2: Antioxidant effect of EEZD by hydrogen peroxide radical scavenging assay

S. No.	Concentration (µg/ml)	% Inhibition by ascorbic acid	% Inhibition by test sample
1.	40	89.62 ± 0.71	31.43 ± 1.12
2.	80	91.65 ± 0.34	55.32 ± 0.97
3.	120	92.80 ± 0.92	68.80 ± 0.70
4.	160	93.40 ± 0.72	72.49 ± 1.00
5.	200	95.13 ± 0.43	84.80 ± 0.24

Values expressed as *Mean of three readings ± SEM

The analgesic activity of an ethanolic leaf extract of *Z. decandra* was studied using the tail immersion method, and the results are shown in Table 3. Pentazocine (30 mg/kg) was used as a reference control. In the tail immersion method, mean latency and percentage analgesic activity were observed after drug administration. Pentazocine showed a significant ($p<0.01$) increase in mean latency from 30 min to 120 min, and the percentage of analgesic activity was 52.14%. Ethanolic leaf extract of *Z. decandra* at 200 mg/kg significantly ($p<0.01$) enhanced the latency in an effective manner at 90 min, and the percentage analgesic activity was 20.29%. Ethanolic leaf extract of *Z. decandra* (400 mg/kg) revealed a significantly ($p<0.01$) enhancement of the latency in an effective manner at 30, 90, and 120 min of observation, and the percentage analgesic activity was 38.57% at 90 min. Ethanolic leaf extract of *Z. decandra* showed analgesic activity in a dose-dependent manner.

Table 4 shows the anti-inflammatory activity of an ethanolic leaf extract of *Z. decandra on a* carrageenan-induced paw edoema model in rats. Indomethacin was used as a reference standard in the study. In Group 1, carrageenan increased the paw thickness due to increased production of prostaglandin. Indomethacin significantly ($p<0.01$) reduced the paw thickness compared to control, and the percentage inhibition at the 3rd h was 55.07%. Both doses of ethanolic leaf extract of *Z. decandra* significantly ($p<0.01$) reduced the paw thickness compared to the control, and they had the equipotent activity as that of the reference control, indomethacin. The percentage inhibition of 200 and 400 mg/ kg of ethanolic leaf extract of *Z. decandra* showed 42.03% and 46.38%, respectively.

Table 3: Analgesic effect of EEZD on tail immersion method in rats

Groups	Mean latency to tail immersion in min					% Analgesic activity
	0	30	60	90	120	
Group-I: Control	1.7 ± 0.06	1.6 ± 0.04	1.5 ± 0.02	1.4 ± 0.03	1.6 ± 0.04	-
Group-II: Pentazocine (30 mg/kg)	1.6 ± 0.04	2.9 ± 0.06**	5.2 ± 0.04**	8.7 ± 0.06**	6.4 ± 0.02**	52.14
Group-III: (200 mg/kg)	1.4 ± 0.08	1.9 ± 0.04*	2.1 ± 0.06*	4.8 ± 0.02**	3.6 ± 0.04**	20.29
Group-IV: (400 mg/kg)	1.8 ± 0.04	2.1 ± 0.06*	3.6 ± 0.01**	6.8 ± 0.04**	5.2 ± 0.01**	38.57

All values are presented as mean ± SEM, n=6. One way ANOVA followed by Dunnett's 't' test was performed as the test of significance at *p<0.05, **p< 0.01.

Table 4: Anti-inflammatory activity of EEZD in rats by carrageenan induced paw edema

Groups	Paw thickness in mm					% Inhibition
	0 h	1 h	2 h	3 h	4 h	
Group-I Carrageenan (control)	1.4 ± 0.01	3.8 ± 0.03	0.02	6.9 ± 0.01	5.5 ± 0.03	-
Group-II: Indomethacin (10 mg/kg)	1.7 ± 0.02	2.4 ± 0.03**	2.6 ± 0.01**	3.1 ± 0.02**	2.4 ± 0.01**	55.07
Group-III (200 mg/kg)	1.5 ± 0.01	3.2 ± 0.02**	3.8 ± 0.01**	0.03**	3.8 ± 0.02**	42.03
Group-IV: (400 mg/kg)	1.8 ± 0.01	3.0 ± 0.03**	3.3 ± 0.02**	3.7 ± 0.03**	3.0 ± 0.01**	46.38

Values were mean ± SEM, n=6, **p<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

Table 5: Anti-inflammatory activity of EEZD on cotton pellet induced granuloma method

Groups	Wet cotton pellet		Dry cotton pellet	
	Weight (mg)	% Inhibition	Weight (mg)	% Inhibition
Control	216.12 ± 2.08	-	56.12 ± 2.42	-
Indomethacin (10 mg/kg)	80.44 ± 4.09**	54.56%	20.22 ± 1.72**	55.33
EEZD (200 mg/kg)	99.32 ± 4.52**	38.67%	26.53 ± 1.43**	43.76
EEZD (400 mg/kg)	89.62 ± 3.06**	50.44%	24.15 ± 1.46**	51.06

All values are presented as mean ± SEM, n=6. One way ANOVA followed by Dunnett's 't' test was performed as the test of significance at **p<0.01.

Table 5 shows the anti-inflammatory activity of an ethanolic leaf extract of *Z. decandra* on cotton pellet granulomas in rats. Indomethacin was used as a reference standard in the study. The extracts significantly inhibited cotton pellet granuloma. The percent inhibition for the indomethacin standard was found to be 55.33%. The per cent inhibition for the extract was 43.76% and 51.06% at doses of 200 and 400 mg/kg, respectively.

4. Discussion

The highly reactive oxygen species known as free radicals is present in our bodies and is responsible for damaging some of the substrates in our biological system. The antioxidants are hypothesised to have an impact on DPPH because of their capacity to donate hydrogen. It's crucial to engage in radical scavenging activities to stop free radicals from playing a harmful role in a variety of disorders, including cancer. In the DPPH assay, the addition of the EEZD extract reduces the violet-coloured DPPH solution to diphenylpicryl hydrazine, a yellow-coloured result, in a concentration-dependent way. The findings of this investigation indicate that the EEZD extract demonstrated radical scavenging activity through its ability to donate hydrogen or transfer electrons. There is a strong correlation between phytoconstituents and antioxidant activity that scavenges free radicals (Rahman *et al*., 2015).

In the presence of DMSO, ascorbic acid and iron EDTA were responsible for the formation of formaldehyde, which then transformed into hydroxyl radicals. Because hydroxyl radicals directly interact with DNA, breaking down DNA as a result, free radicals have the ability to cause mutations, vital part in the development of cancer. When Fe+3-EDTA premixture, ascorbic acid, and H_2O_2 are incubated at pH 7.4, hydroxyl radicals are created. This results in the breakdown of 2-deoxyd-ribose and the production of a substance that resembles malondialdehyde (MDA). The reaction mixture is treated with the ethanolic extract of EEZD to get removal of hydroxyl radicals and stop further damage. By blocking the interaction of hydroxyl radicals with DNA, the extract could be used as antioxidant medicines. They demonstrated significant hydroxyl radical scavenging activity when compared to the conventional antioxidant, ascorbic acid (Rahman *et al*., 2015).

Carrageenan-induced paw edoema is a well-established animal model for assessing the anti-inflammatory properties of natural and synthetic chemical substances. The production of edoema in the paw is a biphasic event that lasts 1-4 h. The initial phase (1 h or 1.5 h) is predominantly non-phagocytic edoema, which is followed by a second phase (2-4 h). The early phase was triggered by the influence of mediators such as histamine, serotonin, and bradykinin on vascular permeability (Prakash *et al*., 2011). Prostaglandin overproduction

has been linked to late-stage or second-stage edoema (Muhammad *et al*., 2012). The pretreatment of Z. *decandra* ethanolic extract demonstrated that the extract (200 and 400 mg/kg) is beneficial in the early phases of inflammation, which is mostly produced by the production of histamine and serotonin. The extract's anti-inflammatory action lasts until the fourth hour of the experiment. The anti-inflammatory effect of the extract lasts until the fourth hour of the experiment. The ethanolic extract of Z. *decandra* showed significant activity against carrageenan -induced edoema in both phases.

Cotton pellet-induced granuloma development is regarded as an applicable experimental model for assessing macrophage dysfunction and granuloma formation, maintenance, and progression in several disease conditions. Implantation of cotton pellets subcutaneously induces inflammation in three phases. The transductive phase (first 3 h), in which the gain in wet weight of the pellet was observed, was followed by an exudative phase lasting 3-72 h in which around the granuloma cells were leaked from the bloodstream, followed by the proliferative phase (3-6 days), in which the gain in dry weight of the pellet happened (Suresha *et al*., 2012). Administration of EEZD at doses of 400 mg/kg significantly reduced granulomatous tissue formation when compared to control. The fact that the EEZD was effective in suppressing granuloma formation in chronic inflammatory conditions.

5. Conclusion

Antioxidants are massively important substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress. The antioxidant potential of an ethanolic extract of *Z. decandra* was investigated, and it became clear that it presented the highest antioxidant activity compared with reference antioxidant ascorbic acid for DPPH scavenging activity. These *in vitro* assays indicate that *Z. decandra* is a significant source of natural antioxidants, which could help prevent the progression of various diseases caused by free radicals. The ethanolic extract of *Z. decandra* displayed analgesic and anti-inflammatory qualities that were comparable to those of non-steroidal anti-inflammatory medications like indomethacin. It has also been proposed that *Z. decandra*'s mode of action involves inhibiting the production of histamine and prostaglandins. Hence, future research will require the isolation and characterization of active chemical constituents to find the antioxidant, analgesic, and anti-inflammatory effectiveness of ethanolic extracts of the plant.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article

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