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Preclinical Evidence for *Peltophorum Pterocarpum* Extracts And Pure Compound In Pain And Inflammation Models Along With In-Vitro Antioxidant And Acute Toxicity Assessment To Validate Its Traditional Uses.

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Abstract:
Peltophorum pterocarpum, a medicinal plant known for its analgesic, anti-inflammatory and antioxidant properties, was studied for the pharmacological effects and toxicity of its ethanol and hexane leaves extracts, focusing on the isolated compound bergenin. Bergenin was extracted using methanol and assessed for antioxidant activity using the FRAP assay. *In vivo* tests in rodents showed significant analgesic and anti-inflammatory effects without toxicity at tested doses. The hexane extract and bergenin exhibited strong dose-dependent responses. Findings confirm the plant's traditional uses and identify bergenin as a safe, promising candidate for development as a natural analgesic and anti-inflammatory agent.

Key Words: *Peltophorum pterocarpum,* bergenin, analgesic, anti-inflammatory, antioxidant.

INTRODUCTION:

The pharmacological characteristics and modes of action of botanical medicines have been the subject of numerous investigations [1]. These investigations have revealed a number of bioactive components that give these plants characteristic therapeutic qualities for the benefit of human and livestock ailments leading to its massive usage [2]. The World Health Organization (WHO) reports that more than 80% of people worldwide get their primary medical treatment from traditional medicines [3][4]. The drawback including the dire need to standardize herbal remedies, identifying the specific active ingredient responsible for the disease, the possibility of potential side effects and their interactions with conventional prescribed drugs and challenges faced with the variations in the strength and caliber of plant extracts [5]. Nevertheless, in order to uncover new compounds with minimum pathogenic resistant pharmaceutical corporations have updated their approach in the area of natural product research as their viable source. As this Ethnopharmacological knowledge is also beneficial for the development of new, safe, potent and reasonably priced medications [6]. For this purpose, ethnopharmacological features of *Peltophorum pterocarpum* are highlighted in this study, along with its potential application as therapeutic agent.

The phytological description of this deciduous tree *Peltophorum pterocarpum* (DC.) Backer Ex .K. Heyne is affiliated to the legume family, *Fabaceae* [7]. There are over 20,000 distinct species and more than 1,450 genera in the family. Because of distinctive yellowish flowering, *Peltophorum pterocarpum* is frequently referred to as "yellow flamboyant" or "yellow flame tree." Tropical region of Southeast Asia is where the plant is indigenous. It is found in Brazil, Southern Nigeria, the state of Florida, Hawaiian Islands (USA), the province of West Bengal (India) and Northern Australia where it is often grown as a plant for decorations and widely cultivated as a roadside tree in greater part of West Pakistan [8, 9].

Literature survey reveal the therapeutic spectrum of each part of plant, ranges from its positive effect as antibacterial, antihyperglycemic, cardiac ionotropic effect, antioxidative, inflammation reducing, hypolipidemic, antiarthritic to its effect as anti-cancerous agent [10]. Among other medical applications, African ethnomedicine utilizes the aerial portion of *Peltophorum pterocarpum*, which comprises its leaves and flowers, as a treatment for memory loss. There have also been reports of the leaf, stem and root bark extracts' ability to improve cognition [11]. Bergenin, a cholinesterase inhibitor, was isolated and identified as a result of earlier phytochemical analyses of the leaves extract. Other substances found in the plant include quercetin 3-O-beta-D-glucopyranoside via its stem-bark, stigmasterol, β-sitosterol, campesterol, lupenone and lupeol. Additional reports include quercetin, kaempferol, hentriacontanol, myomositol, bergenin and nor bergenin from the flowers, peltopterin A and B from the leaves and (-) epicatechin and leucocyanidin from the bark [12].
The orbit of its folk uses includes in sleep deprivation, dermatitis, ringworm, GI disorders of loose bowels or hardened feces, oral mucositis, as a mouthwash and eye wash, as well as a lotion for sores and muscle aches. This is because of its strong

medicinal properties of antibacterial, antiphlogistic activities. In addition to this it has dominant antioxidant, hypoglycemic and cardio protective effect [13].
The objective of this study design was to validate the analgesic, anti-inflammatory and antioxidant potential claims and investigate its acute toxicity effect.

Material and method:
Plant collection and Identification:
Peltophorum pterocarpum leaves collected from University of Karachi Department of Pharmacognosy. The identified sample specimen is available in herbarium at the Faculty of Pharmacy and pharmaceutical sciences, university of Karachi, Pakistan.

Procedure of Extraction and isolation of bergenin:
Three kg of dried *Peltophorum pterocarpum* leaves were soaked in hexane for 15 days. Hexane extract then underwent filtration and evaporation after fifteen days, residuals were then soaked in ethanol. Later which the ethanol extract strained and the filtrate then evaporated via a rotary evaporator at a temperature of 45 ºC. The extracts (with the appropriate labeling) were then kept in air-sealed glass bottles. Bergenin was isolated from the methanol leaves extract of *Peltophorum pterocarpum* by earlier reported procedure [11].

Phytochemistry:
The Preliminary phytochemical screening of ethanol extract of *Peltophorum pterocarpum* leaves were executed by employing the standard procedures described by Harborne (1973), Sofowara (1993) and Trease and Evans (1989) [14] [15] [16].

Biological assays
Board of Advanced Studies and Research University of Karachi (ASRB), provided confirmation and approval for biological research, Resol. No. 07 (09) File No.0265/Pharm.

Animals:
Both males and females of Swiss albino rats weigh around 150–300 g and Swiss albino mice weigh around 25–30 g were used in this investigation. Before conducting a biological and toxicological evaluation, the animals were acclimatized and housing was maintained in a laboratory setting with a temperature around 23±3ºC and 12-hour light / 12-hour dark photoperiod. They were also given unrestricted access to food and water.

Drugs:
The test groups were administered the pure compound bergenin as well as ethanol and hexane extract of the plant *Peltophorum petrocarpum* orally and intraperitoneally (i.p.) at varying doses based on body weight after being dissolved in DMSO (10%).

Acute toxicity assessment:
Paradigm for the evaluation of acute toxicity in mice and rats:
The treatments groups were divided into four major groups of rats and mice, six animals (n=6) in each group was allotted. Group-1 was used as control and given normal saline. Group 2,3 and 4 subdivided into 4 treatment groups. Group-2 (2A, 2B, 2C, 2D) and Group-3 (3A, 3B, 3C, 3D) treated with 150, 250, 300 and 500 mg/kg of body weight of hexane and ethanol extracts respectively. Whereas Group 4 (4A, 4B, 4C, 4D) were exposed with bergenin at strength of 50, 75, 100 and 150 mg/kg body weight three days prior to the toxicity evaluation using intraperitoneal route of administration to mice. Same paradigm will be repeat for rats with doses of 150, 300 ,500 and 600 mg/kg of body weight of hexane and ethanol extracts respectively and for bergenin 50, 100, 200 and 300 mg/kg body weight three days prior to the toxicity evaluation using oral route of administration to rats.

Acute toxicity assessment methodology
Compound and extracts were tested for acute systemic toxicity using previously published procedures [17]. Different doses specified in the above paradigm were administered to groups of six mice and rats. After the administration of the drug, the subjects were continuously monitored for changes in their general behavior, physiological responses, number and the rate of mortality for half an hour, 1 hr., 2hr, 3hr and for up to 3 days. The results were recorded and Median lethal dose or LD50 was determined using the formula listed below.

$$`LD50 = \sqrt{\text{Highest nonlethal dose} \times \text{least lethal dose}}$$

The subsequent formula was used to calculate the Therapeutic index

$$\text{Therapeutic Index (TI)} = \frac{\text{Median-lethal-dose (LD50)}}{\text{Median-effective-dose (ED50)}}$$

Here, ED50 stands for effective strength in 50 percent of the population [17]

Anti-oxidant assay:
A 50 ml volumetric flask containing 100 mg of ascorbic acid as standard drug along with the extracts and bergenin, was dissolved in enough solvent (ethanol + water, 1: 1 ratio) to assess the *in-vitro* antioxidant activity by ferric reducing power assay. A stock solution containing 2000 µg/ml was used. From the stock solution, four distinct concentration of hexane, ethanol extracts, bergenin and ascorbic acid (standard drug) were prepared.

a. 1000 µg/ml
b. 500 µg/ml
c. 250 µg/ml
d. 100 µg/ml

A test tube was filled with 1ml of each i.e. of the pure compound, both extracts, and standard drug. 5ml of 1% potassium ferric cyanide solution and phosphate buffer of 0.2 M, 6.6 pH were added to the test tube and thoroughly mixed. For 20 minutes, this mixture was then incubated 50°C. Following incubation, addition of 5 ml of trichloroacetic acid was done to halt the reaction, and later was centrifuged for a time period of approx. 10 minutes at 3000 rpm. 5ml of the supernatant were collected into an additional test tube following centrifugation. 5ml of distilled water along with 1ml of ferric chloride (0.1%) were incorporated to this supernatant. After 10 minutes, a spectrophotometer was used to detect absorbance at 700 nm. The reducing power and sample concentration are linearly correlated. The entire experiment was carried out three times. Whole experiment was conducted in triplicate. Control sample was prepared by adding all reagents but without test sample [17]. The percentage of reduction power of test samples compared to standard (Ascorbic acid) was calculated by following formula:

$$\textit{(\%) of reduction power} = [1- (1- A_S / A_C)] \times 100$$

A_S = absorbance of sample
A_C = absorbance of standard at maximum concentration tested

Analgesic Assay
Using the following techniques for [18] evaluation of analgesic potential was performed on the pure compounds along with extracts.

a. Tail immersion technique, which uses thermal radiation as a pain source.
b. Analgesia meter, which uses physical pressure as a pain source
c. The hot plate technique, which involves leaping off a hot plate at temp of 55°C.
d. Writhing induced via acetic acid working on the principle of chemical radiant as a pain source.

Paradigm for the evaluation of analgesic & anti-inflammatory activity
Rats and mice were divided into five main treatment groups A, B, C, D and E. Additionally, Group B, C and D were then subdivided into nine treatment groups, each of which had five animals (n=05). From Main Grp B the subgroup B1, B2 and B3 and Group C the subgroup of C1, C2, C3 were given hexane and ethanol extracts at doses of 100, 125 and 150 mg/kg of body weight, respectively. While bergenin at doses of 15, 25 and 50 mg/kg of body weight were given to Group D the subgroup D1, D2, D3. Normal saline was administered to Group A served as a control. Group E served as a standard group and received standard drugs.
This research paradigm was modified for anti-inflammatory activity. As carrageenan (0.1 ml/ 10g of 1% solution in 0.9% saline solution) which was given to control Group A for anti-inflammatory analysis [17, 19].

Tail immersion technique:
Thermal pain was induced by immersing the mice's tail tip (1–2 cm) in water at 45±1°C. Reaction time (latency) was recorded before and after treatment at 30, 60, and 120 min using a stopwatch. Diclofenac sodium served as the standard, with a 180-second cutoff to prevent tissue damage. Results were compared between control, standard, and test groups.

Hot plate method
This test was conducted using Eddy's hot plate that scrutinizes pain response (nociception) and analgesic (pain-relieving) effects. Acetylsalicylic acid, was taken as a standard comparative drug and the temp was retained at 55 ± 1°C. Using a stopwatch, the latent initial reaction of paw lick was recorded in seconds and was calculated before as well as after using control, standard and the tested drugs individually for 30, 60 and 120 minutes, as specified in above reference procedure. To prevent paw damage or burn, a 15-second cutoff time was allotted.

Analgesy meter method
The analgesy-meter measured mechanical pain thresholds by applying increasing pressure to a rat's hind paw until withdrawal, recorded in grams (g). Baseline and post-treatment responses (30, 60, 120 min) were compared after administering pure compound, extracts and standard drug acetyl salicylic acid. The nociceptive response (paw withdrawal) indicated pain sensitivity, with results compared across control, standard and test groups. The test was halted immediately upon withdrawal to record the force applied.

Writhing response technique using acetic acid:
The writhing test involved injecting mice with 1% acetic acid (0.1 mL/10g) to induce abdominal contractions (writhes). Test compounds (hexane, ethanol extracts and pure compound) were administered 30 min prior, and writhes were counted after 5

min post-injection for 20 minutes. Acetyl salicylic acid served as the standard, with results compared to controls. The subsequent formula evaluated and assessed the degree of analgesia.
The calculation narrates the proportion of writhing inhibition.

$$\textit{Inhibition (\%)} = \frac{\textit{Mean No. of writhes (Control)} - \textit{Mean No. of writhes (Test)}}{\textit{Mean No. of writhes (Control)}} \times 100$$

Anti-inflammatory assessment:
The test listed below was selected to evaluate the anti-inflammatory properties of extracts and pure substances [17].

Carrageenan induced paw edema method
Carrageenan-induced paw edema was assessed by injecting 0.1 mL/10g carrageenan (incorporated in 0.9% sodium chloride solution) into the sub plantar surface of left hind paw 1 hour after administering control, bergenin (15, 25, 50 mg/kg of body weight) and both extracts (100, 125, 150 mg/kg of body weight). Paw volume was measured via plethysmometer at 30, 60, and 120 min post-injection, with edema inhibition (%) calculated versus baseline. Results were compared to the standard Diclofenac sodium (10 mg/kg of body weight) and control groups. Inflammation was quantified as the increase in paw volume relative to carrageenan-induced swelling.

$$\textit{Percentage Inhibition (\%)} = [(Vc-Vt)/Vc] * 100$$

Where,
Vc= Arithmetic Mean edema of control treatment/group
Vt= Arithmetic Mean edema of test treatment/group

Results:
Pharmacognostic Evaluation
Upon phytochemical screening it was found that alkaloids, saponins, flavonoids, steroids, glycosides and stigma sterols were all present in ethanol extract of *Peltophorum pterocarpum* leaves [20,21,22].

Characterization of Bergenin from *Peltophorum pterocarpum* Leave Extract
Bergenin was purified from the methanol leave extract of *Peltophorum pterocarpum* as a white crystalline solid. The isolated compound exhibited a melting point of 235°C, aligning with previously reported data for authentic bergenin [11] Thin-layer chromatographic analysis further confirmed its identity, with the compound migrating to a Rf of 0.57, closely equivalent to the reported Rf value 0.58 [23].

Acute Toxicity Assessment of Bergenin and Plant Extracts in Mice
Following intraperitoneal administration at the doses specified in **Table 1**, mice were monitored for behavioral changes, signs of toxicity and mortality over a 72-hour period. As summarized in Table 1, bergenin (50 mg/kg of body weight.) as well as hexane and ethanol extracts (150 mg/kg of body weight) were well tolerated, with no adverse effects or mortality observed. All treated animals exhibited normal locomotor activity and maintained stable body weight throughout the observation period, indicating an absence of acute toxicity at the tested doses.
Mice administered higher doses of bergenin (75, 100 and 150 mg/kg of body weight) exhibited immediate adverse effects, including hyperactivity, shivering and discomfort, followed by writhing within 30 minutes. Within 1–3 hours, the animals became drowsy, refused food and water and ultimately died within 72 hours.
Similarly, hexane and ethanol extracts at 250, 300 and 500 mg/kg of body weight induced acute toxicity, with abnormal behaviors (writhing, shivering, flattened posture, tail erection) appearing immediately post-administration. By 30 minutes, mice displayed drowsiness, unconsciousness and closed eyes. All animals received 250 mg/kg of body weight died within 72 hours, while those treated with 300 and 500 mg/kg of body weight dead by 48 and 24 hours, respectively.

Table 1-: Acute Toxicity assessment of *Peltophorum pterocarpum* in mice

Compound and Extracts	Doses mg/kg	Quantal incidence of symptoms	Quantal incidence of mortality	% age of mortality
Bergenin	50	0/6	0/6	0
	75	6/6	6/6	100
	100	6/6	6/6	100
	150	6/6	6/6	100
Peltophorum pterocarpum (Hexane Extract)	150	0/6	0/6	0
	250	6/6	6/6	100
	300	6/6	6/6	100
	500	6/6	6/6	100
Peltophorum pterocarpum (Ethanol Extract)	150	0/6	0/6	0
	250	6/6	6/6	100
	300	6/6	6/6	100
	500	6/6	6/6	100

Control	-	0/6	0/6	0

Determination of Median Lethal Dose (LD50) and Therapeutic Index

The median lethal dose (LD50) results, determined by intraperitoneal administration in mice, were established at 61.23 mg/kg for bergenin and 193.64 mg/kg for both hexane and ethanol extracts **(Table 2)**. Subsequent therapeutic index calculations yielded values of 1.2246 for bergenin and 1.2909 for the both extracts (**Table 2**), indicating their relative safety margins. These findings shown that pure compound bergenin, hexane and ethanol extracts can be administered intraperitoneally (i.p.) at doses of less than 50 and 150 mg/kg of total body weight found safe respectively, and to be lethal at doses greater than 75 and 250 mg/kg of total body weight, respectively.

Table 2: LD50 and therapeutic index for *Peltophorum pterocarpum* in mice

Compound and Extracts	LD50 mg/kg of body weight	Therapeutic index
Bergenin	61.23	1.2246
Hexane Extract	193.64	1.2909
Ethanol Extract	193.64	1.2909

Acute Toxicity Assessment of Bergenin and Plant Extracts in Rats

Oral toxicity assessment demonstrated an excellent safety profile for both bergenin and plant extracts. Rats administered bergenin at doses ranging from 50-300 mg/kg (5×ED50) while hexane and ethanol extracts from 150-600 mg/kg (5×ED50) showed no signs of toxicity or mortality during the 72-hour observation period (**Table 3**). The dose selection was guided by previously determined ED50 values of 50 mg/kg for bergenin and 150 mg/kg for the extracts in mice. Remarkably, even at doses five times higher than the effective dose, neither treatment group exhibited any adverse effects, suggesting a wide therapeutic window for both preparations.

Table 3: Acute Toxicity assessment of *Peltophorum pterocarpum* in rats

Compound and Extracts	Doses mg/kg	Quantal incidence of symptoms	Quantal incidence of mortality	% age of mortality
Bergenin	50	0/6	0/6	0
	100	0/6	0/6	0
	200	0/6	0/6	0
	300	0/6	0/6	0
Peltophorum pterocarpum (Hexane Extract)	150	0/6	0/6	0
	300	0/6	0/6	0
	500	0/6	0/6	0
	600	0/6	0/6	0
Peltophorum pterocarpum (Ethanol Extract)	150	0/6	0/6	0
	300	0/6	0/6	0
	500	0/6	0/6	0
	600	0/6	0/6	0
Control	-	0/6	0/6	0

Antioxidant Activity

The reducing power capacity of bergenin, along with hexane and ethanol extracts, were evaluated. A concentration-dependent increase in reducing power capacity was observed across all tested samples, though their activity remained lower than that of the standard drug (ascorbic acid). Among the tested samples, pure bergenin exhibited the highest activity (0.333 ± 0.014 absorbance units, 23.10%), followed by the ethanol extract (0.219 ± 0.0007, 15.10%) and the hexane extract (0.134 ± 0.001, 9.20%) at 1000 µg/ml. These findings suggest that bergenin possesses a strong ability to neutralize free radicals by stabilizing them more effectively than the crude extracts. Statistical analysis of antioxidant capacity (**Figure 1 and Table 4**) revealed a non-significant correlation ($P < 0.05$) between the test samples and ascorbic acid, with coefficients of determination (r^2) as follows: hexane extract (0.982), bergenin (0.878), and ethanol extract (0.977). These results indicate that while the test compounds exhibit reducing potential, their efficacy does not parallel that of the standard antioxidant.

Table 4: Percentage of reducing power capacity of *Peltophorum pterocarpum*

Concentration µg/ml	Percentage of reducing power capacity			
	Standard Drug (Ascorbic acid)	Bergenin	Hexane Extract	Ethanol Extract
100	7.30	1.2	2.7	5.6
250	35.60	1.4	4.0	6.9
500	71.30	3.1	6.4	8.5
1000	-	23.1	9.2	15.1

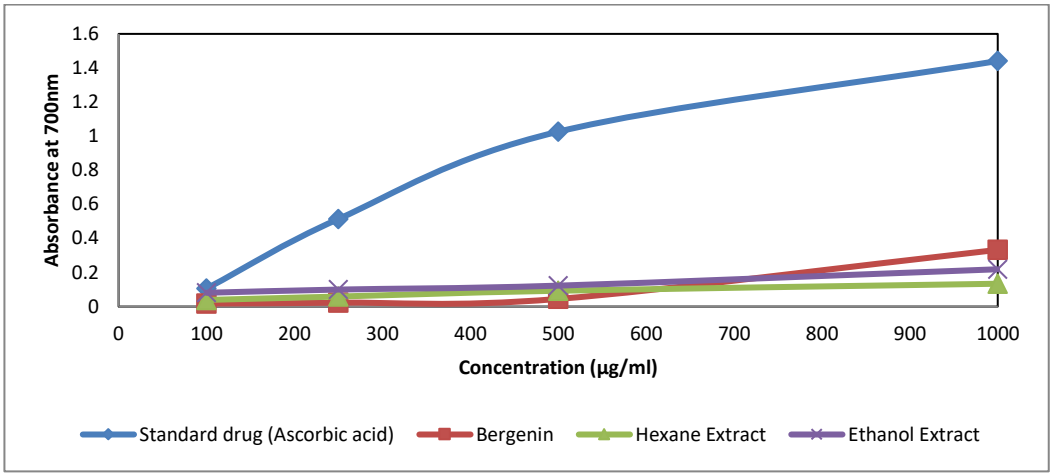

Figure 1: *In vitro* anti-oxidant activity of *Peltophorum pterocarpum* by reducing power method

Analgesic Efficacy of Bergenin and *Peltophorum pterocarpum* Leaves Extracts

The analgesic potential of bergenin, along with hexane and ethanol extracts derived from *Peltophorum pterocarpum* leaves, was evaluated using the tail immersion assay and compared against a standard analgesic and control. Intraperitoneal administration of bergenin and the ethanol extract demonstrated lower analgesic activity across all tested doses compared to the reference drug (**Table 5**). Notably, maximal analgesia was observed at 60 minutes post-administration, with the bergenin (50 mg/kg of body weight), hexane and ethanol extracts (150 mg/kg of body weight) exhibiting the highest efficacy relative to the standard. A time-dependent increase in pain resistance was evident for all test samples, peaking at 60 minutes before gradually declining. While all tested formulations showed limited efficacy against thermal stimuli compared to the standard.

The hot plate analgesic assay revealed that baseline reaction times in rats prior to drug administration ranged between 1 and 4 seconds (**Table 6**). Post-treatment evaluations demonstrated that the maximal reduction in paw-licking time occurred at 60 minutes following administration of bergenin (50 mg/kg of body weight), hexane extract (150 mg/kg of body weight) and ethanol extract (125 mg/kg of body weight). In addition, all tested strengths of bergenin, hexane and ethanol extract prolonged latency periods at 60 minutes, a decline in analgesic efficacy was observed by 120 minutes. Notably, the observed effects did not reach statistical significance when compared to the reference drug.

Basal pain responses were measured using an analgesy meter, as detailed in the methodology. The pain-relieving property of pure substance bergenin, along with hexane and ethanol extracts, was presented in **Table 7**. Results indicated a time-dependent increase in analgesic activity for all tested doses, peaking at 120 minutes post-administration. Pure bergenin exhibited significant ($P < 0.05$) analgesia at 50 mg/kg of body weight after 120 minutes, while doses of 15 and 25 mg/kg of body weight demonstrated notable efficacy comparable to the reference drug. Similarly, the hexane and ethanol extracts displayed maximal analgesic potency at 150 mg/kg of body weight, with doses of 100 and 125 mg/kg of body weight also showing robust activity relative to the standard.

The acetic acid-induced writhing assay (**Table 8**) revealed significant analgesic activity for pure bergenin, as well as hexane and ethanol extracts. Pure bergenin abolished abdominal constrictions (100% inhibition) at all tested doses. Similarly, the hexane extract (150 mg/kg of body weight) and ethanol extract (100, 125, and 150 mg/kg of body weight) completely suppressed writhing reflexes, exhibiting efficacy comparable to the reference drug.

Table 5: Analgesic effect of *Peltophorum pterocarpum* by radiant heat induced tail immersion response in mice

Compound and Extracts	Dose mg/kg	Reaction Time (seconds)				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	15	1.072±0.001	1.794±0.029*	4.656±0.148	1.702±0.004**	2.306±0.045*
	25	1.038±0.001	3.698±0.037*	4.434±0.022*	2.664±0.005**	2.958±0.065*
	50	1.172±0.004	1.950±0.007**	10.286±0.142	1.980±0.004**	3.847±0.039*
Peltophorum pterocarpum (Hexane Extract)	100	2.426±0.009	4.850±0.069	8.384±0.052	2.636±0.014*	4.574±0.036*
	125	2.896±0.009	2.742±0.013*	6.158±0.321	4.862±0.193	4.164±0.134
	150	2.838±0.010	11.582±0.021*	11.782±0.082	7.576±0.101	8.444±0.053
Peltophorum pterocarpum (Ethanol Extract)	100	1.400±0.014	2.778±0.002**	4.620±0.020*	2.340±0.007**	2.780±0.010**
	125	1.880±0.003	2.636±0.014*	4.240±0.002**	3.140±0.003**	2.974±0.005**
	150	2.280±0.040	3.440±0.001*.	8.460±0.113	3.420±0.001**	4.400±0.038*
Standard Drug (Diclofenac sodium)	5	3.620±0.170	6.180±0.070	7.140±0.060	7.700±0.130	6.160±0.107
Control	-	1.6206±0.001	1.708±0.008	1.722±0.001	1.798±0.004	1.712±0.003

Table 6: Analgesic effect of *Peltophorum pterocarpum* against hot plate induced pain in rats

Compound and Extracts	Dose mg/kg	Reaction Latency (seconds)				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	15	0.714±0.045	0.940±0.019*	1.282±0.045*	0.958±0.062	0.973±0.042*
	25	0.956±0.059	1.430±0.020*	1.588±0.016*	1.326±0.039*	1.325±0.033*
	50	1.192±0.069	1.522±0.035*	2.478±0.002**	1.726±0.015*	1.729±0.030*
Peltophorum pterocarpum (Hexane Extract)	100	0.810±0.064	0.968±0.061	1.164±0.003**	1.118±0.003**	1.015±0.032*
	125	1.240±0.023	1.386±0.013*	1.928±0.042*	1.610±0.006**	1.541±0.021*
	150	1.118±0.005	1.538±0.182	2.110±0.009*	1.696±0.001**	1.615±0.049
Peltophorum pterocarpum (Ethanol Extract)	100	0.568±0.019	0.604±0.006**	1.184±0.017*	0.942±0.069	0.824±0.027*
	125	0.764±0.121	0.966±0.015*	2.334±0.009*	1.660±0.037*	1.431±0.045*
	150	1.448±0.028	1.658±0.001**	2.018±0.073	1.822±0.091	1.736±0.048*
Standard Drug (Acetyl salicylic acid)	20	4.330±0.030	7.890±0.050	7.250±0.040	7.020±0.015	6.622±0.033
Control	-	4.330±0.030	6.412±0.013	5.130±0.011	4.840±0.009	5.178±0.0157

Table 7: Evaluation of analgesic effect of *Peltophorum pterocarpum* by pressure caused pain in rats

Compound and Extracts	Dose mg/kg	Applied pressure causing pain (g)				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	15	6.000±0.002	8.840±0.082	9.140±0.009*	9.100±0.018*	8.270±0.027*
	25	4.240±0.001	12.200±0.026*	7.800±0.029*	10.500±0.009**	8.685±0.016*
	50	0.500±0.004	9.580±0.033*	9.700±0.014*	15.800±0.004**	10.395±0.013*
Peltophorum pterocarpum (Hexane Extract)	100	5.400±0.008	3.500±0.018*	6.400±0.003**	8.200±0.027*	5.875±0.014*
	125	8.900±0.045	9.000±0.009**	8.100±0.114	8.800±0.028*	8.700±0.049*
	150	5.600±0.002	6.400±0.075	10.900±0.030*	15.200±0.018*	9.525±0.031*
Peltophorum pterocarpum (Ethanol Extract)	100	0.500±0.002	6.100±0.001**	6.700±0.005**	10.200±0.003**	7.000±0.002**
	125	6.700±0.001	7.300±0.001**	8.100±0.021*	8.700±0.001**	7.700±0.006**
	150	6.900±0.007	6.800±0.013*	9.900±0.013*	12.200±0.013*	8.950±0.010**
Standard Drug (Acetyl salicylic acid)	10	2.480±0.001	8.572±0.002**	8.660±0.002**	9.294±0.001**	7.250±0.001**
Control	-	2.240±0.005	3.800±0.003	4.080±0.006	3.400±0.005	3.630±0.004

Table 8: Effect of *Peltophorum pterocarpum* on writhing induced by acetic acid in mice

Compound and Extracts	Dose mg/kg	No. of writhes (Mean ± SD)	% Protection
Bergenin	15	0±0.00	100
	25	0±0.00	100
	50	0±0.00	100
Peltophorum pterocarpum (Hexane Extract)	100	10.0±0.029	85.83
	125	0.6±0.374	99.15
	150	0±0.00	100
Peltophorum pterocarpum (Ethanol Extract)	100	0±0.00	100
	125	0±0.00	100
	150	0±0.00	100
Standard Drug (Acetyl salicylic acid)	5	23±2.48	67.42
Control (Acetic Acid)	0.1ml	70.60±0.00	-

Anti-inflammatory effects in the Carrageenan-Inflicted Edema Model

Sub plantar injection of carrageenan induced acute inflammation, with edema peaking within 30 minutes post-injection presented in **Table 9**. Pure bergenin exhibited significant anti-inflammatory effects, suppressing serotonin and histamine release in the early phase. At 50 mg/kg body weight, bergenin demonstrated maximal inhibition (93.333%, $P < 0.01$), outperforming both standard and control groups. This inhibitory effect persisted across all tested doses throughout the experiment. Similarly, the extract of hexane showed potent activity ($P < 0.01$) at 125 and 150 mg/kg of body weight, reducing edema by 93.790% and 94.379%, independently. The carrageenan-induced edema model in rats typically exhibits a biphasic response. During the initial phase (≤1 h post-injection), edema formation is mediated primarily by serotonin release. The ethanol extract (125 and 150 mg/kg of body weight) demonstrated significant suppression of serotonin-mediated inflammation (92.810% and 90.196% inhibition, respectively; P<0.01) compared to both standard drug and control groups. In the subsequent phase (2-3 h), the extract exhibited dose-dependent inhibition of cyclooxygenase activity, resulting in marked suppression of prostaglandin synthesis (85.97%, 91.716%, and 91.492%) inhibition at tested doses. By comparison, diclofenac sodium (10 mg/kg of body weight) showed 84.836% inhibition of carrageenan-induced edema throughout the experimental period.

Table 9: Anti-inflammatory effect of *Peltophorum pterocarpum* by carrageenan induced paw edema in rats

Compound and Extracts	Dose mg/kg	Change in paw volume (ml)				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	15	0.104±0.001	0.424±0.001** (54.929%)	0.376±0.002** (73.846%)	0.376±0.002** (79.701%)	0.32±0.001** (88.882%)
	25	0.048±0.001	0.484±0.001** (38.591%)	0.344±0.013* (71.538%)	0.050±0.001** (99.000%)	0.231±0.004** (88.039%)
	50	0.270±0.003	0.520±0.001** (64.788%)	0.408±0.001** (86.730%)	0.290±0.001** (98.507%)	0.372±0.001** (93.333%)
Peltophorum pterocarpum (Hexane Extract)	100	0.286±0.056	0.462±0.002** (75.211%)	0.506±0.001** (78.846%)	0.570±0.003** (78.800%)	0.456±0.016* (88.888%)
	125	0.328±0.013	0.540±0.001** (70.140%)	0.430±0.001** (90.192%)	0.392±0.007** (95.223%)	0.423±0.006** (93.790%)
	150	0.286±0.005	0.462±0.001** (75.211%)	0.392±0.001** (89.807%)	0.348±0.008** (95.522%)	0.372±0.004** (94.379%)
Peltophorum pterocarpum (Ethanol Extract)	100	0.236±0.013	0.528±0.008** (58.873%)	0.492±0.032* (75.384%)	0.424±0.010** (85.970%)	0.420±0.015* (87.973%)
	125	0.330±0.014	0.538±0.012* (70.704%)	0.448±0.075 (88.653%)	0.444±0.016* (91.716%)	0.440±0.003** (92.810%)
	150	0.250±0.017	0.538±0.001** (59.436%)	0.448±0.003** (80.961%)	0.364±0.012* (91.492%)	0.400±0.008** (90.196%)
Standard Drug (Diclofenac sodium)	10	0.97±0.030	1.22±0.060 (64.788%)	1.33±0.060 (65.384%)	1.29±0.060 (75.373%)	1.202±0.052 (84.836%)
Control	0.1*	1.200±0.005	1.91±0.035	2.24±0.021	2.54±0.100	2.73±0.028

Discussion:

Different parts of *Peltophorum pterocarpum* are utilized to treat a range of illnesses, including skin conditions, stomatitis and constipation. As Flower extracts is beneficial in insomnia, its bark as stated earlier has also been used for Giardiasis medicinally. The leaves' decoction of *Peltophorum pterocarpum* has been a prominent historic remedy employed for generations to alleviate various skin disorders with its potent healing properties. The infusion derived from the stems of *Peltophorum pterocarpum* is highly valued for its efficacy in addressing diarrheal infection as well as being utilized for therapeutic gargles, tooth powder applications and the alleviation of muscular soreness [24]. Furthermore, the extract obtained from the vibrant flowers of this plant is deemed effective in the treatment of digestive disorders and for relieving myalgia, showcasing the diverse range of medicinal uses it attributes [25]. Bark infusion has demonstrated germicidal properties and to be effective in treating cough and gastroenteritis in medical applications. Its roots are beneficial for throat inflammation, toothaches, cancer and injuries [25, 26].

In regards to its analgesic or anti-inflammatory activity no scientific study has come into light. Therefore, the goal of this investigation was to assess the analgesic, anti-inflammatory and antioxidant qualities of this plant in a scientific manner. Hexane and ethanol were selected as the preferred solvents in this study to extract the greatest number of chemical constituents from *Peltophorum pterocarpum* leaves since their solubility properties make them ideal for primary extraction whereas methanol is a preferred solvent for isolating the pure compound bergenin [27, 28]. The preliminary phytochemical analysis of *Peltophorum pterocarpum* leaves ethanol extract demonstrated appearance of alkaloids, flavonoids, glycosides, saponin, steroids, stigma sterol and the outcomes were similar to those of earlier phytoconstituents analysis performed in earlier research [7].

Safety profile assessment of bergenin, hexane and ethanol extracts did not cause any toxicity nor death at lower doses in mice by intraperitoneal route neither in rats by oral route of administration; even with an increase in the dose up to 300 mg/kg of body weight by pure substance and 600 mg/kg of body weight by the ethanol and hexane extract. This implies that the doses employed for biological activities were safe and that the mice tolerated the research medications well. In addition, acute safety assessment in rodents determines the maximum tolerated dose and no-observed-adverse-effect level guiding the selection of safe yet pharmacologically effective doses for therapeutic use. This ensures a balance between efficacy and toxicity in preclinical-to-clinical studies.

The Ferric Reducing Antioxidant Power (FRAP) test is a popular technique for assessing the antioxidant potential of herbal extracts, which is closely linked to their analgesic, anti-inflammatory and neuroprotective effects. The FRAP assay measures the ability of herbal compounds to reduce Fe^{3+} to Fe^{2+}, indicating their electron-donating (antioxidant) capacity. Herbal extracts work through antioxidant mechanisms, including scouting ROS-reactive oxygen species and RNS-reactive nitrogen species which neutralize superoxide (O_2^-) and hydroxyl (OH^-) and peroxynitrite ($ONOO^-$) radicals due to the presences of herbal polyphenols, preventing oxidative damage linked to pain and inflammation [29]. Upregulation of Endogenous Antioxidant Enzymes (SOD, CAT, GPx) herbal extracts enhance SOD-superoxide dismutase, CAT-catalase and GPx-glutathione peroxidase alleviating pain brought on by oxidative stress [30, 31].

Additionally, natural therapeutics may be involved in the inhibition of lipid peroxidation, or by modulation of NF-ϰB and Nrf2 Pathways by activating Nrf2, promoting antioxidant gene expression, while inhibiting NF-ϰB, a key pro-inflammatory mediator [32]. Next, chelation of pro-Oxidant metal ions (Fe^{2+}, Cu^{2+}): Flavonoids chelate transition metals, preventing Fenton reaction-driven ROS generation, which contributes to chronic pain conditions [33]. The results of the antioxidant activity assessment demonstrated significant reducing power across the tested compounds, attributable to the presence of hydroxyl

(–OH) groups in their phytochemical constituents. Among the evaluated samples, the pure compound bergenin exhibited the most potent reducing activity, surpassing that of the crude extracts. This enhanced efficacy can be attributed to chemical structural specificity of bergenin contained flavonoid content and polyphenolic structure. The –OH groups in bergenin enhance its reducing power compared to crude extracts, which facilitate superior electron donation and free radical scavenging capacity [34].
Four anti-nociception investigation of *Peltophorum pterocarpum* study designs; , tail flick test, analgesy meter, hot plate and acetic acid instigated writhing response paradigm were employed to assess the analgesic property considering nociception, or an animal's response to painful stimuli, is frequently recorded in analgesic research investigation [35].The stimuli could be Mechanical (as witnessed in tail or paw pressure tests), chemical (commonly employed acetic acid-inflicted writhing response or formalin tests), or/and thermal (as evident in the experiment modality of tail immersion or hot plate testing) [35].

Analgesic effect of pure compound bergenin, hexane and ethanol extracts of *Peltophorum pterocarpum* leaves was determined by tail immersion method and compared to standard drug and control, after i.p. administration of pure compound bergenin and ethanol extract, all doses presented less analgesic activity compared to standard drug. Highest analgesic activity was observed after 60 minutes in pure compound at a dose of 50 mg/kg of body weight and hexane extract at a dose of 150 mg/kg of body weight as compared to standard drug. Results of present study revealed that pure compound bergenin, hexane and ethanol extracts were found less effective against thermal stimulus of pain which is specific central anti-nociceptive test.

The hot plate model, which measures pain threshold in response to heat, is frequently used to investigate medications that exhibit central mechanism analgesia. Since a 15-second cutoff time is frequently employed to protect rodent from harm, this test was selected since it is sensitive to potent analgesics and has minimal tissue damage [36]. Hexane and ethanol extracts demonstrated a significant central analgesic property after an hour at all tested dosages of the pure component bergenin, according to the results (Table 6) of the hot plate technique's analgesic effect. Prime speculations revolve around, alkaloids, that have demonstrated analgesic property in various researches by counteracting with pain-modulating neurotransmitters in the central nervous system or they may interact with other receptors located in supra-spinal regions.
It was discovered that the conventional medication and the tested medications had different onsets of action. The hexane and ethanol extracts at doses of 150 and 125 mg/kg of body weight. while, bergenin at a dose of 50 mg/kg of body weight took 60 minutes to start functioning whereas standard drug required half an hour although comparing the mean analgesic activity to the standard treatment/group, there was no discernible decrease in latency reaction. This indicates a delayed activity in the extracts and bergenin. It may have to do with active analgesic metabolites or the amount of time it requires for the medication to penetrate the central compartment and reach the intended location [37].

The net pain relieving results of pure substance bergenin, hexane and ethanol extract use in this research, for basal reaction time using analgesy meter. It was found that the dose dependent analgesic magnitude of pure compound bergenin, hexane and ethanol extracts were noted. However, the degree of anti-nociceptive action of bergenin at 50 mg/kg body weight was found significant against applied physical pressure as compared to the standard drug and extracts. The mechanism of action of tested drugs as analgesia to reduce pain sensitivity in animal models, typically rodents can be attributed to various pathways, including: Modulation of Opioid Pathways act on μ, δ, and κ opioid receptors inhibiting pain signal transmission in the central nervous system, inhibition of Prostaglandin Synthesis (COX-1 and/or COX-2 enzymes inhibition) reducing prostaglandin-mediated inflammation, swelling and pain , antioxidant and anti-inflammation through the mitigation of oxidative stress and NF-κB-mediated inflammation, indirectly alleviating pain [38].

The peripheral pain-relieving properties of the extracts and pure compound was measured using the acetic acid-instigated writhing response, which is used for a rapid and precise evaluation of the peripheral sedative/pain alleviating effect of herbs [39]. The anti-nociceptive properties of pure compound and extracts at therapeutic levels that are passive to identify in other techniques, like the hot plate test, can be detected by this highly sensitive test, despite the fact that it is a non-selective pain test that may yield false-positive results with tranquilizers, muscle paralyzers/relaxants, and other pharmaceutical [40, 41]. Since it suggests several hypotheses of how nociception works, including the release of cationic monoamines (such as serotonin and/or histamine), neuropeptide substance P (SP), inflammatory peptide bradykinins [40-44], and pro-inflammatory cytokines (such as interleukin IL-1β, and tumor necrosis factor TNF-α) [44]. Bergenin and ethanol extract at all tested doses, while, hexane at 150 mg/kg of body weight presented 100% suppression of writhes, indicating a considerable peripheral analgesic activity in comparison to the standard medication used. The impediment of the writhing reaction is a sign of the plant's auxiliary analgesic trait. In line with the aforementioned findings, the extract presents dose proportionality analgesic trait, defining that when the dosage is raised, so does the analgesia, suggesting that raise in the concentration of phytochemicals increases analgesic/sedative pain-relieving property [45]. Comparatively, conventional drug acetylsalicylic acid, a prominent Nonsteroidal Anti-Inflammatory Drug that works on the principle of removal of the inflammatory mediators of pain in periphery by the inhibition of acetic acid was less effective than the extract [46]. As the bergenin, hexane and ethanol extracts inhibit the writhing in mice, it is possible that the tested drugs act through the same mechanism of action as that of acetylsalicylic acid.

Furthermore, Carrageenan-induced paw edema was investigated using a carrageenan-inflicted acute anti-inflammatory model. Considering it to incorporates several mediators, the carrageenan paw edema test is frequently utilized to examine anti-inflammatory potential of both steroidal and nonsteroidal drugs [47]. Carrageenan being the non-antigenic phlogistic substance is often employed in the testing of anti-inflammatory medications that do not require systemic results [48]. Acute

Inflammation is characterized by two distinct phases, the first one initiated by histamine and serotonin causing inflammation following 1% carrageenan that was injected sub planetary into the rat hind paw, while prostaglandin synthesis mostly causes the second phase, which is where the edema peaked. Furthermore, the generation of free radicals derived from oxygen and the local invasion and triggering of neutrophils are also linked to the inflammatory response. The hexane and ethanol extracts, at 125 and 150 mg/kg of body weight respectively, as well as bergenin at a strength of 50mg/kg of body weight. exhibit a larger percentage of edema inhibition than the conventional medication comparatively. The paradigm employed in this assessment is crucial as it evaluates medications with possible anti-inflammatory effects, making the positive findings from the extracts all the more compelling, thereby reinforcing the initial hypothesis. This assertion seamlessly fits into the broader understanding that plants possess a diverse range of medicinal attributes, each with unique methods of action that frequently result in synergistic outcomes when combined. It is widely acknowledged within the researchers that the effectiveness of plant-derived compounds often lies in their ability to target multiple biological pathways simultaneously, leading to enhanced therapeutic benefits that are greater than the sum of their individual parts [49]. The validation of this concept through empirical evidence further underscores the relevance of exploring natural remedies for their multifaceted properties, as well as highlights the potential for creating novel drug combinations that leverage the inherent synergies found within plant-based sources. By acknowledging the intrinsic complexity and adaptability of plant compounds, researchers can capitalize on the diverse array of therapeutic possibilities that exist within nature, paving the way for a more holistic approach to drug discovery and development [50]. In addition to this, the existence of secondary metabolites is hypothesized to be the cause of the therapeutic efficacy of plant extract [51, 52]. Since it has been discovered that secondary metabolites, such as saponins; inhibit the synthesis of nitric oxide. Therefore, the extract may be beneficial in treating sub-chronic inflammation based on its effectiveness in this paradigm.

Conclusion:
This study provides comprehensive evidence supporting the therapeutic potential of *Peltophorum pterocarpum* leaves and its major bioactive constituent, bergenin. Both ethanol and hexane extracts, along with the isolated bergenin, demonstrated significant analgesic, anti-inflammatory, and antioxidant activities across using *In vivo* models. The extracts and pure compound were well-tolerated at therapeutic doses, with no signs of acute toxicity observed in mice or rats. Bergenin exhibited the highest pharmacological efficacy, particularly in the inhibition of pain and edema, as well as in reducing oxidative stress. These findings not only reinforce the ethnomedicinal relevance of *Peltophorum pterocarpum* but also highlight bergenin as a promising candidate for further development as a plant-based analgesic and anti-inflammatory agent.

Ethical approval
Board of Advanced Studies and Research University of Karachi (ASRB), provided confirmation and approval for biological research, Resol. No. 07 (09) File No.0265/Pharm.

Conflict of Interest
There is no conflict of interest.

REFERENCES:
1. Moshidi PM, Mupangwa J, Muya CM, Washaya S. Phytochemical content, antibacterial, antioxidant and cytotoxic activities, of leaves extracts of Eucalyptus globulus, Peltophorum africanum and Vangueria infausta. Discover Agriculture. 2024;2(1):133.
2. Pancholi B, Gupta S, Verma Bk. Bioactive Constituents From Bark Of Peltophorum Pterocarpum Baker Ex K. Heyne. Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 24, No. (2) : 2022 : 427-433
3. Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB. Chapter 7-Preparation, Standardization, and Quality Control of Medicinal Plants in Africa, doi: 10.1016. B978-0-12-809286-6.00007-8; 2017.
4. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines-A review. International journal of biodiversity and conservation. 2012 Mar 20;4(3):101-12.
5. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide research trends on medicinal plants. International journal of environmental research and public health. 2020;17(10):3376.
6. Süntar I. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochemistry Reviews. 2020;19(5):1199-209.
7. Di Ciaccio LS, Catalano AV, López PG, Fortunato RH, Salvat AE. Peltophorum (Caesalpinioideae, Fabaceae): A review on ethnobotanical, pharmacological and phytochemical profiles. Journal of Herbal Medicine. 2024:100883.
8. Monehin JA, Oriola AO, Olawuni IJ, Odediran SA, Ige OO, Idowu TO, Ogundaini AO. Cholinesterase Inhibitory Compounds from Peltophorum Pterocarpum Flowers. Chemistry Africa. 2024;7(5):2899-906.
9. CABI Compendium. Peltophorum pterocarpum (copperpod). Wallingford (UK): CABI International; 2022. doi:10.1079/cabicompendium.39510.
10. Ansari P, Reberio AD, Ansari NJ, Kumar S, Khan JT, Chowdhury S, Abd El-Mordy FM, Hannan JM, Flatt PR, Abdel-Wahab YH, Seidel V. Therapeutic Potential of Medicinal Plants and Their Phytoconstituents in Diabetes, Cancer, Infections, Cardiovascular Diseases, Inflammation and Gastrointestinal Disorders. Biomedicines. 2025 Feb 12;13(2):454.
11. Elufioye TO, Obuotor EM, Agbedahunsi JM, Adesanya SA. Isolation and characterizations of Bergenin from Peltophorum pterocarpum leaves and its cholinesterase inhibitory activities. EJMP. 2016;11(2):1-7.

12. Jerline M, Manikandan R, Saradhadevi M, Balamuralikrishnan B, Younis M, Anand AV. Influences of Ethanolic Extract of Peltophorum pterocarpum Leaves and Bark on Lipid Profile, Liver and Carbohydrate Metabolizing Enzymes on Toxin-induced Wistar Rats. Free Radicals and Antioxidants. 2023;13(1):34-40.
13. Htwe MM, Kyaw ZTA, Ngwe H, editors. A Study on Antioxidant Activity of Bergenin and its Derivative from the Bark of Peltophorum pterocarpum (DC.) K. Heyne (Pan-mèzali). 3rd Myanmar Korea Conf Res J; 2020.
14. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis1987.
15. Sofowora A. Phytochemical screening of medicinal plants and traditional medicine in Africa edition. Spectrum Book Ltd., Nigeria. 1993;150:156.
16. Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences; 2009 May 27.
17. Tasleem F, Azhar I, Ali SN, Perveen S, Mahmood ZA. Analgesic and anti-inflammatory activities of Piper nigrum L. Asian Pacific journal of tropical medicine. 2014;7:S461-S8.
18. Muhammad A. Assays in analgesic studies. 2024. Journal of Science Technology and Research (JSTAR), Volume No.5, Issue No.1 (2023): 57-75
19. Semis HS, Gur C, Ileriturk M, Kaynar O, Kandemir FM. Investigation of the anti-inflammatory effects of caffeic acid phenethyl ester in a model of λ-Carrageenan–induced paw edema in rats. Human & experimental toxicology. 2021;40(12_suppl):S721-S38.
20. Jash SK, Gangopadhyay A, Sarkar A, Gorai D. Phytochemical investigation of the hexane extract of stem bark of Peltophorum pterocarpum (DC.). Der Pharma Chemica. 2013;5(5):49-53.
21. Jash SK, Singh RK, Majhi S, Sarkar A, Gorai D. Peltophorum pterocarpum: chemical and pharmacological aspects. Int J Pharm Sci Res. 2014;5(1):26-36.
22. Biju AK, Nisha B, Shanmugam R, Nisha B. A thorough examination of Peltophorum pterocarpum phytochemicals in network pharmacology-based management of acne vulgaris. Cureus. 2024 Aug 29;16(8).
23. Pushpalatha HB, Pramod K, Devanathan R, Sundaram R. Use of bergenin as an analytical marker for standardization of the polyherbal formulation containing Saxifraga ligulata. Pharmacognosy magazine. 2015 May;11(Suppl 1):S60.
24. Taiwo OE, Efere MO, Joseph MA, Saburi AA. Acetyl and buteryl cholinesterase inhibitory effect of Peltophorum pterocarpum (DC) Backer ex K. Heyne (family Leguminosae). Journal of Pharmacognosy and Phytotherapy. 2013;5(5):77-82.
25. Bhatia P, Sharma A, George AJ, Anvitha D, Kumar P, Dwivedi VP, Chandra NS. Antibacterial activity of medicinal plants against ESKAPE: An update. Heliyon. 2021 Feb 1;7(2).
26. Yosri N, Alsharif SM, Xiao J, Musharraf SG, Zhao C, Saeed A, Gao R, Said NS, Di Minno A, Daglia M, Guo Z. Arctium lappa (Burdock): Insights from ethnopharmacology potential, chemical constituents, clinical studies, pharmacological utility and nanomedicine. Biomedicine & Pharmacotherapy. 2023 Feb 1;158:114104.
27. Sbihi HM, Nehdi IA, Mokbli S, Romdhani-Younes M, Al-Resayes SI. Hexane and ethanol extracted seed oils and leaf essential compositions from two castor plant (Ricinus communis L.) varieties. Industrial Crops and Products. 2018 Oct 15;122:174-81.
28. Zafar R, Ullah H, Zahoor M, Sadiq A. Isolation of bioactive compounds from Bergenia ciliata (haw.) Sternb rhizome and their antioxidant and anticholinesterase activities. BMC Complementary and Alternative Medicine. 2019 Dec;19:1-3.
29. Halliwell B, Gutteridge JM. Free radicals in biology and medicine: Oxford university press; 2015.
30. Tena N, Martín J, Asuero AG. State of the art of anthocyanins: Antioxidant activity, sources, bioavailability, and therapeutic effect in human health. Antioxidants. 2020;9(5):451.
31. Makena MR, Ko M, Mekile AX, Senoo N, Dang DK, Warrington J, et al. Secretory pathway Ca2+-ATPase SPCA2 regulates mitochondrial respiration and DNA damage response through store-independent calcium entry. Redox biology. 2022;50:102240.
32. Sivandzade F, Prasad S, Bhalerao A, Cucullo L. NRF2 and NF-κB interplay in cerebrovascular and neurodegenerative disorders: molecular mechanisms and possible therapeutic approaches. Redox biology. 2019 Feb 1;21:101059.
33. Chandimali N, Bak SG, Park EH, Lim HJ, Won YS, Kim EK, Park SI, Lee SJ. Free radicals and their impact on health and antioxidant defenses: a review. Cell Death Discovery. 2025 Jan 24;11(1):19.
34. Chandimali N, Bak SG, Park EH, Lim HJ, Won YS, Kim EK, Park SI, Lee SJ. Free radicals and their impact on health and antioxidant defenses: a review. Cell Death Discovery. 2025 Jan 24;11(1):19.
35. Ezeja M, Omeh Y, Ezeigbo I, Ekechukwu A. Evaluation of the analgesic activity of the methanolic stem bark extract of Dialium guineense (Wild). Annals of medical and health sciences research. 2011;1(1):55-62.
36. Vijayasanthi M, Doss A. ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF Delonix elata Linn. InEthnomedicinal Plants with Therapeutic Properties 2019 Feb 11 (pp. 203-212). Apple Academic Press.
37. Holford N. Pharmacodynamic principles and the time course of delayed and cumulative drug effects. Translational and Clinical Pharmacology. 2018 Jun 1;26(2):56-9.
38. Karcz M, Abd-Elsayed A, Chakravarthy K, Aman MM, Strand N, Malinowski MN, Latif U, Dickerson D, Suvar T, Lubenow T, Peskin E. Pathophysiology of pain and mechanisms of neuromodulation: a narrative review (A Neuron Project). Journal of pain research. 2024 Dec 31:3757-90.
39. Gawade SP. Acetic acid induced painful endogenous infliction in writhing test on mice. Journal of pharmacology & pharmacotherapeutics. 2012 Oct;3(4):348.
40. Belay R, Makonnen E. Anti-inflammatory activities of ethanol leaves extract and solvent fractions of Zehneria scabra (Cucurbitaceae) in rodents. Asian Journal of Natural Product Biochemistry. 2020;18(1).
41. Das SC, Bhadra S, Roy S, Saha SK, Islam MS, Bachar SC. Analgesic and anti-inflammatory activities of ethanolic root extract of Swertia chirata (Gentianaceae). Biological. 2012;5(1):131.

42. Collier H, Dinneen L, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. British journal of pharmacology and chemotherapy. 1968;32(2):295.
43. Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. International journal of applied and natural sciences. 2012;1(1):8-26.
44. Ayanaw MA, Yesuf JS, Birru EM. Evaluation of analgesic and anti-inflammatory activities of methanolic leaf and root extracts of Gomphocarpus purpurascens A. Rich (Asclepiadaceae) in mice. Journal of Experimental Pharmacology. 2023:1-11.
45. Hao X, Zhou Y, Ling Y, Miyoshi H, Sumitani M, Chan KY, Park HJ, Feng Z, Rao Y. Effects of high-dose opioid analgesia on survival, pain relief, quality of life and adverse drug reactions in cancer and neuropathic pain patients: a retrospective cohort study in real-world clinical practice. Annals of Translational Medicine. 2022 Sep;10(18):998.
46. Enechi OC, Okeke ES, Nwankwo NE, Nweze JE, Obilor CP, Okoye CI, Awoh OE. Membrane Stabilization, Albumin Denaturation, Protease Inhibition, and Antioxidant Activity as Possible Mechanisms for the Anti-Inflammatory Effects of Flavonoid-Rich Extract of Peltophorum pterocarpum (DC.) K. Heyne (FREPP) Stem Bark: doi. org/10. 26538/tjnpr/v4i10. 25. Tropical Journal of Natural Product Research (TJNPR). 2020;4(10):812-6.
47. Mansouri MT, Hemmati AA, Naghizadeh B, Mard SA, Rezaie A, Ghorbanzadeh B. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian journal of pharmacology. 2015 May 1;47(3):292-8.
48. Musa YM, Haruna AK, Ilyas M, Yaro AH, Ahmadu AA, Usman H. Phytochemical, analgesic and anti-inflammatory effects of the ethylacetate extract of the leaves of Pseudocedrella kotschyii. African Journal of Traditional, Complementary and Alternative Medicines. 2008 Oct 16;5(1):92-6.
49. Choudhury A. Potential role of bioactive phytochemicals in combination therapies against antimicrobial activity. Journal of Pharmacopuncture. 2022 Jun 30;25(2):79.
50. Chaachouay N, Zidane L. Plant-derived natural products: a source for drug discovery and development. Drugs and Drug Candidates. 2024 Feb 19;3(1):184-207.
51. Schaible H-G, Ebersberger A, Natura G. Update on peripheral mechanisms of pain: beyond prostaglandins and cytokines. Arthritis research & therapy. 2011;13:1-8.
52. Twaij BM, Hasan MN. Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses. International Journal of Plant Biology. 2022 Feb 21;13(1):4-14.