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PHYTOCHEMICAL & PHARMACOLOGICAL EVALUATION OF POLYHERBAL FORMULATION FOR ANALGESIC ACTIVITY

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Abstract:
In human society pain is the primary and fundamental difficulties connected with various illnesses. Despite the availability of adequate medications, synthetic analgesic agents have various side effects. Screening medicinal plants with claimed analgesic effects is one of the study topics that may lead to the discovery of novel molecules with greater safety and efficacy. Thus this study aims at deciphering the synergistic analgesic activity of Calotropis procera , Ricinus communis & Curcuma longa. Qualitative & quantitative estimation of phytochemicals were performed & polyherbal formulation was made by standard protocol. The In vivo analgesic effect of Polyherbal formulation was checked by Tail flick test and hot plate test albino rats. Results revealed that % yield for Calotropis procera, Ricinus communis and Curcuma longa as 9.44, 17.48 and 17.13 % respectively. All the three plant were found to be laden with phytochemical that are responsible for analgesic activity. Total phenol content of Calotropis procera, Ricinus communis and Curcuma longa was found to be 0.863, 0.525 and 0.752 mg/ 100 mg respectively. Total flavonoids content of Calotropis procera, Ricinus communis and Curcuma longa was found to be 0.571, 0.325 and 0.457mg/ 100 mg respectively. The pH of polyherbal churna was found to be 7.5 while moisture content was observed to be 1.15%. In the tail flick test, the polyherbal formulation exhibited a dose-dependent increase in basal reaction time, indicative of potential analgesic activity. Similarly, in the hot plate test, the formulation demonstrated an increase in latency time and percentage protection, reinforcing its analgesic potential. The observed analgesic effects of the polyherbal formulation, as evidenced by delayed pain responses, suggest its potential as a natural source of analgesic agents. However, its efficacy, safety profile, and mechanism of action warrant further investigation. This study contributes to the understanding of natural remedies for pain management and underscores the significance of plant-derived compounds in drug discovery and development.

Keywords: *Analgesic, Pain, Medicinal plants, herbal medicines, Calotropis procera, Ricinus communis and Curcuma longa, Tail flick test, Polyherbal formulation (PHF)*

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INTRODUCTION:

Pain is an unpleasant sensory experience induced by acute or potential tissue injury. Pain is also a defensive mechanism of the body that allows it to appropriately respond to pain-causing agents and an index for illness detection. In human society, pain and inflammation are the primary and fundamental difficulties connected with various illnesses. Because of difficulties and adverse effects, currently available medications such as opioids, nonsteroidal anti-inflammatory drugs, and some other agents may not be beneficial to all people. As a result, appropriate analgesics are still required, and researchers are still investigating this. Pain is caused by a number of stimuli, including tissue mechanical pinching, low temperature, chemical compounds (such as neuronal activator of pH produced in accidents), and hyperosmotic solutions, all of which have no commonality (Parkitny *et al*., 2013; Bagdas *et al*., 2020).

Given the variety of pain-causing stimuli, the complexity of pain development, leading, and sensation mechanisms, and the fact that pain is the most common clinical complaint of patients, biological sciences researchers around the world are still looking for ways to overcome pain; obviously, resolving even a minor question in this field could be an ointment for suffering patients. Severe pain causes dread and anxiety in patients, as well as heightened stress reactions in the hypothalamus via cortical activation. Furthermore, worry and related stress responses increase blood viscosity and platelet buildup (Woolf *et al*.1998).

Despite the availability of adequate medications, analgesic and anti-inflammatory agent side effects such as gastrointestinal distress, gastric ulcer, bleeding, and liver damage remain a major issue in clinical use. As a result, the search for better, safer, and more effective agents is intensifying. Screening medicinal plants with claimed analgesic and anti-inflammatory effects is one of the study topics that may lead to the discovery of novel molecules with greater safety and efficacy. diverse practises have traditionally been employed to control pain and inflammation in diverse regions. Because of their low cost, ease of use, and environmental friendliness, herbal treatments are frequently used in underdeveloped nations to treat pain and inflammation. Thus this study aims at deciphering the synergistic analgesic activity of *Calotropis procera* , *Ricinus communis* & *Curcuma longa (*Boger et al., 1982; Patida *et al.*, 2014; Malairajan *et al*., 2006).

Calotropis procera is a drought-resistant medicinal perennial plant that grows primarily in dry to semi-arid environments. Secondary metabolites found in it include phenols, flavonoids, terpenoids, sugars, alkaloids, tannins, cardenolides, glycosides, saponins, and steroids. It has hepatoprotective, antioxidant, inflammatory, antibacterial, and antimalarial effects. It is often used to treat common ailments such as fever, leprosy, eczema, diarrhoea, dysentery, and jaundice. This is a brief overview of the phytochemistry of Calotropis procera and its traditional use (Kaur *et al*., 2021).

Ricinus communis, or castor bean, contains phytochemical components such as saponins, flavonoids, alkaloids, steroids, and glucosides. The presence of significant phenolic compounds was discovered in the plant's leaves. Whereas the roots test revealed the Indole-3-acetic. The studies show that the plant has numerous medicinal properties that can help with ailments such as cancer, diabetes, ulcers, and anthelmintics, among others. Furthermore, the plant's anti-inflammatory characteristic allows medicinal application. As a result, all elements of the medicinal plant R. communis are thought to be quite valuable in the medical sector (Scarpa and Guerci, 1982).

Turmeric (Curcuma longa) and its component, curcumin, have long been used for their medicinal benefits. The majority of turmeric's and curcumin's medical effects may be related to their anti-inflammatory, antinociceptive, and antioxidant properties. Curcumin's medicinal effectiveness has been limited due to its quick plasma clearance and conjugation, prompting researchers to study the benefits of complexing curcumin with other compounds to boost systemic bioavailability. Numerous ongoing clinical trials should provide a better knowledge of curcumin's processes and therapeutic potential (Roth *et al*., 1998).

MATERIALS & METHODS:

Collection of plant

Leaves of *Calotropis procera, Ricinus communis* & rhizome of *Curcuma longa* were collected from diffrents parts of Bhopal. The plant material was cleaned, Shed dried & subjected for Defatting.

Defatting of plant material

The dried plant material was coarsely powdered and kept in bottle with petroleum ether as solvent for 24 hrs. This process removes fats & oils present in plant sample.

Extraction by Soxhlet Apparatus Process
The dried plant material was coarsely powdered & filled in extraction bottle with hydroalcoholic (Methanol: water; 70:30) solvent.

Phytochemical analysis
In order to detect the various constituents present in the Hydroalcoholic extract of *Calotropis procera*, *Ricinus communis* and *Curcuma longa*, were subjected to the phytochemical tests as per standard methods.

Estimation of Total phenol content
Total phenolic content Total phenolic content of all the extracts was evaluated with Folin-Ciocalteu method.

Estimation of Total flavonoid content
Determination of total Flavonoid content was based on aluminum chloride (Hossain *et al*., 2013).

Preparation of Polyherbal churna
The extract of *Calotropis procera*, *Ricinus communis* and *Curcuma longa* dried and finely powdered. The finely powdered extract were passed through sieve number 40 and 1g of each of the individual drugs were weighed and mixed in appropriate ratio (1:1:1 w/w/w). The prepared churna was packed in airtight container.

Standardization of Polyherbal Churna
Determination of pH
The pH of 1% solution of formulated polyherbal churna was identified by pH meter.

Moisture Content (Loss on Drying)
The churna was placed in a weighing bottle. It was dried at 105 degree in hot air oven and weighed after 15 minutes. When the weight of the formulation became constant, then percentage of water loss on drying was calculated.

Swelling Index
1g of formulation was placed in a stoppered measuring cylinder containing 9 ml water and kept aside for 24 hours. The swelling in the formulation was noticed and swelling index was calculated.

***In-vivo* analgesic activity**
The Albino rats (100 - 200 g) were kept in polypropylene cages (three in each cage) at an ambient temperature of 25 ± 20°C and 55-65% relative humidity 12 ± 1 h light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions and were fed with standard food pellets and water ad libitum. The animals were deprived of food 24h before experimentation and had free access to water. The animals were maintained under standard environmental conditions throughout the period of experimentation.

Grouping of animals
The animals were divided into four groups of 5 animals each.
Group I - Control received 2ml of 1% Gum acacia suspension
Group II - 1% Gum acacia suspension of PHF 50 mg/kg.
Group III - 1% Gum acacia suspension of PHF 100 mg/kg body.
Group-IV - 1% Gum acacia suspension of Indomethacin 20mg/kg

Acute toxicity study
Acute toxicity study was performed in accordance with OECD guidelines 425.No adverse effect or mortality was detected in albino rats up to 3 gm/kg, p. oof polyherbal formulation during the 24 to 72 hrs observation periods. For this period the rats were continuously observed for 5 hrs for any gross behavioral, neurological or autonomic toxic effect and lethally after 24 to 72 hrs.

Tail immersion method
Tail immersion test was used to assess the analgesic activity of polyherbal combination which involved immersing the extreme 5 cm of rat's tail in a water bath at a temperature of (55±0.50C). After immersing within a few minutes, the rat reacted by withdrawing the tail. The initial reaction time for withdrawing the tail was recorded by immersing the marked part of tail at hot water at 0 min and animals were pretreated with 0.5 ml of distilled water (control) or 20 mg/kg of reference drug (Indomethacin) or the PHF (50 and 100 mg/kg) orally. The reaction time was recorded in 0.01s units by a stopwatch at 30, 60, 90 and &120 min. The cut off time was 15 sec to avoid the damage to the rats (Ramabadran *et al*., 1989).

Hot plate method
The method originally developed by Woolfe G, MacDonald, (1944). The paws of rats are very sensitive to heat at optimum temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. The animals were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5 ◦C. A cut off period of 15 s, was observed to avoid damage to the paw. Reaction time and the type of response were noted

using a stopwatch. Control rats were treated with vehicle (12% Tween 80, 1 ml/kg).

Indomethacin was used as positive control (20 mg/kg) and Polyherbal Formulation (50 and 100 mg/kg, o.s.).The latency was recorded before and after 15, 30, 60 and 120 min following orally of 50 and 100 mg/kg of each of the extract to different groups of five animals each. Average reaction times were then calculated and the percentage variation calculated using the following ratio:

$$\text{Percentage protection} = \frac{\text{Drug latency} - \text{base line latency}}{\text{base line latency}} x100$$

Statistical analysis

All data were calculated statistically by standard error mean (n=6) and statistically significant were verified by applying one way ANOVA.

RESULTS & DISCUSSION:

The yields were found to be 13.58, 12.74, 10.25 % w/w of *Calotropis procera*, *Ricinus communis* and *Curcuma longa* respectively in petroleum ether extract. While 19.44, 17.48 and 17.13 % w/w of *Calotropis procera*, *Ricinus communis* and *Curcuma longa* respectively for Hydroalcoholic extract. The analysis of *Calotropis procera* extracts revealed the presence of flavonoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antioxidant activity. Alkaloids, carbohydrates and resins were absent in the selected plant extracts studied. The result of *Ricinus communis* extracts shows the presence of flavonoids, glycosides, phenols, saponins, alkaloids and tannins. Total phenol content of *Calotropis procera*, *Ricinus communis* and *Curcuma longa* was found to be 0.863, 0.525 and 0.752 mg/ 100 mg respectively.

Total flavonoids content of *Calotropis procera*, *Ricinus communis* and *Curcuma longa* was found to be 0.571, 0.325 and 0.457mg/ 100 mg respectively. The pH of polyherbal churna was found to be 7.5 while moisture content was observed to be 1.15%.

The tail flick test is a common method used to evaluate the analgesic activity of substances. It involves measuring the time taken by the rat to withdraw its tail from a noxious heat source. The control group treated with a 1% gum acacia suspension exhibited relatively consistent basal reaction times at various time points (0-120 min). This suggests that the experimental conditions were stable over the duration of the test.

Indomethacin, a known analgesic drug, demonstrated a gradual increase in reaction time over the 120-minute observation period. This delayed reaction is indicative of the analgesic effect of indomethacin, as it blunted the pain response to the noxious stimulus. The groups treated with the polyherbal formulation (PHF) also exhibited increased basal reaction times, albeit to a lesser extent than indomethacin. This suggests that the PHF may possess some degree of analgesic activity, as evidenced by the delayed tail flick response.

The hot plate test is another method used to assess analgesic activity. It involves measuring the time taken by a rat to exhibit a pain-related response on a heated surface. Indomethacin showed a significant increase in mean latency time compared to the control group. This increase in latency time indicates the analgesic effect of indomethacin, as it delayed the pain response on the hot plate. The groups treated with the polyherbal formulation also exhibited increased latency times compared to the control group, suggesting potential analgesic activity. The percentage protection values further support this observation, indicating a degree of pain relief. The data from both the tail flick test and hot plate test indicate that the polyherbal formulation (PHF) may possess analgesic properties. The observed increases in reaction times and latency times, as well as the percentage protection values, suggest that the PHF has the potential to alleviate pain responses in rats.

Table 1: Extractive values obtained from *Calotropis procera*, *Ricinus communis* and *Curcuma longa* using different solvents

S. No.	Plants	Petroleum ether extract	Hydrolcoholic extract
		%Yield	
1.	*Calotropis procera*	13.5%	19.4%
2.	*Ricinus communis*	12.7%	17.4%
3.	*Curcuma longa*	10.2%	17.1%

Table 2: Preliminary phytochemical screening of *Calotropis procera*

S. No.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	+ve
		Dragendorff's Test	-ve
2	Glycosides	Raymond's Test	+ve
		Killer Killani Test	-ve
3	Carbohydrates	Molisch's Test	+ve
		Fehling's Test	-ve
4	Tannins	Vanillin- HCl Test	+ve
		Gelatin Test	-ve
5	Flavonoids	Lead acetate	+ve
		Shinoda Test	+ve
6	Resins	Color detection with ferric chloride	-ve
		Turbidity Test	-ve
7	Steroids	Libermann- Bur chard Test	+ve
		Salkowski Reaction	+ve
8	Proteins & Amino acids	Biuret Test	+ve
		Precipitation test	-ve
		Ninhydrin Test	+ve
9.	Phenols	Ellagic Acid Test	+ve

Table 3: Preliminary phytochemical screening of *Ricinus communis*

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1.	Alkaloids	Mayer's Test	+ve
		Dragendorff's Test	-ve
2.	Glycosides	Raymond's Test	+ve
		Killer Killani Test	+ve
3	Carbohydrates	Molisch's Test	-ve
		Fehling's Test	-ve
4.	Tannins	Vanillin- HCl Test	+ve
		Gelatin Test	-ve
5.	Flavonoids	Lead acetate	+ve
		Shinoda Test	+ve
6.	Resins	Color detection with ferric chloride	-ve
		Turbidity Test	-ve
7.	Steroids	Libermann- Bur chard Test	+ve
		Salkowski Reaction	+ve
8.	Proteins & Amino acids	Biuret Test	+ve
		Precipitation test	-ve
		Ninhydrin Test	+ve
9.	Phenols	Ellagic Acid Test	+ve

Table 4: Preliminary phytochemical screening of *Curcuma longa*

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1.	Alkaloids	Mayer's Test	+ve
		Dragendorff's Test	-ve
2.	Glycosides	Raymond's Test	-ve
		Killer Killani Test	-ve
3.	Carbohydrates	Molisch's Test	-ve
		Fehling's Test	-ve
4.	Tannins	Vanillin- HCl Test	+ve
		Gelatin Test	-ve
5.	Flavonoids	Lead acetate	+ve
		Shinoda Test	+ve
6.	Resins	Color detection with ferric chloride	-ve
		Turbidity Test	-ve
7.	Steroids	Libermann- Bur chard Test	+ve
		Salkowski Reaction	+ve
8.	Proteins & Amino acids	Biuret Test	-ve
		Precipitation test	-ve
		Ninhydrin Test	+ve
9.	Phenols	Ellagic Acid Test	+ve

Table 5: Estimation of total phenol and flavonoids content of *Calotropis procera, Ricinus communis* and *Curcuma longa*

S. No.	Hydroalcoholic extract	Total phenol content	Total Flavonoids content
		mg/ 100 mg of dried extract	
1.	*Calotropis procera*	0.863	0.571
2.	*Ricinus communis*	0.525	0.325
3.	*Curcuma longa*	0.752	0.457

Table 6: Results of Standardization of polyherbal churna

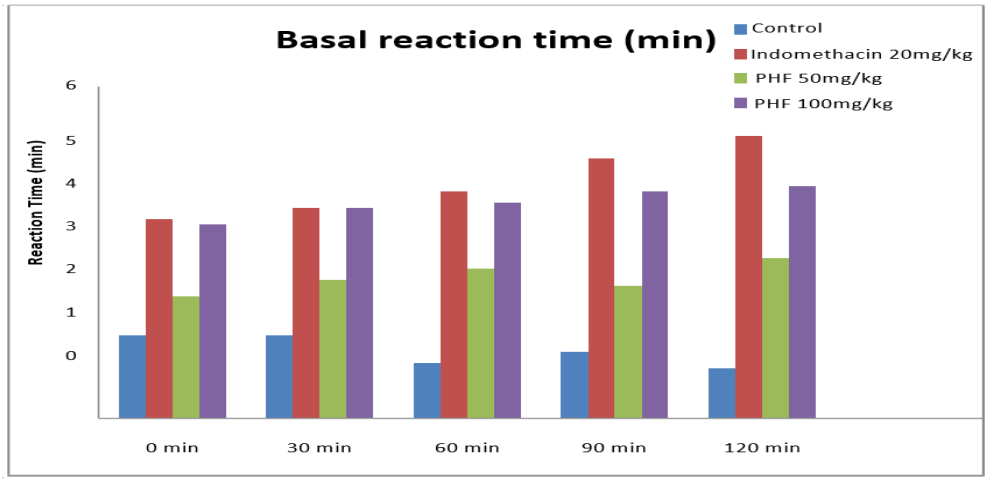
S. No.	Parameters	Results
1.	pH	7.5
2.	Moisture content	1.15%

Table 7: Effect of PHF on tail flick response in rats after immersion in 55° water baths

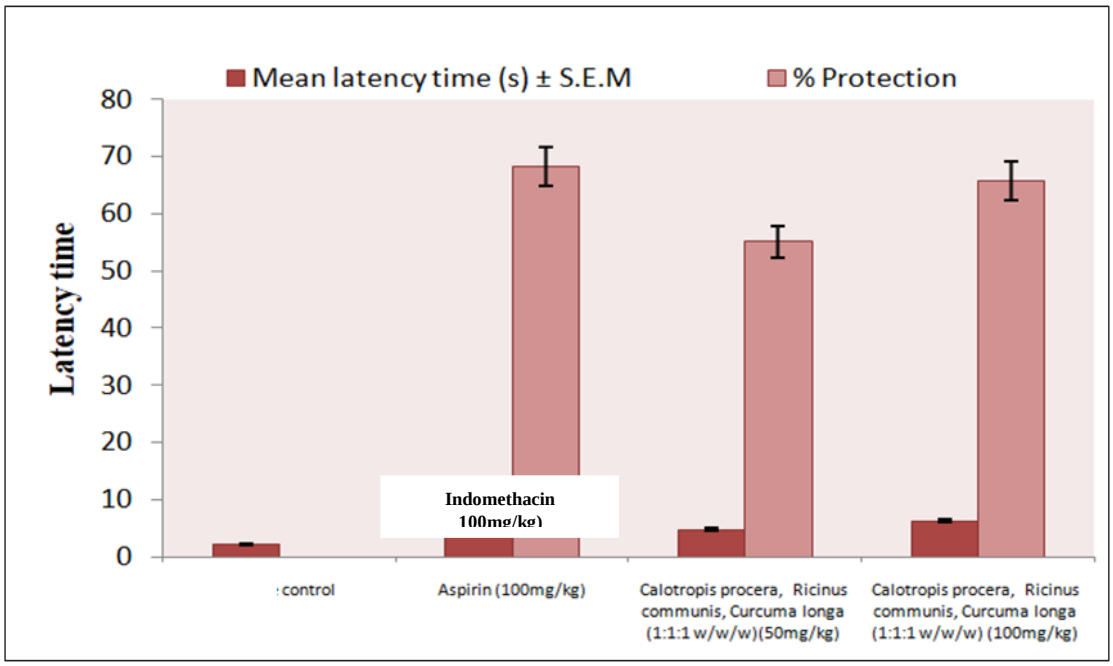
Treatment (mg/kg)	Dose	Basal reaction time (min)				
		0min	30min	60min	90min	120min
Control	(2ml of 1% Gum acacia suspension)	1.5±0.16	1.5±0.18	1 ± 0.23	1.2±0.19	0.9±0.20
Indomethacin	20 mg/kg o.s.	3.6±0.35	3.8±0.27	4.1±0.85	4.7±0.74	5.1±0.27
Calotropis procera, Ricinus communis, Curcuma longa (1:1:1 w/w/w)	50 mg/kg o.s.	2.2±0.61	2.5±0.22	2.7±0.22	2.4±0.01	2.9±0.82
Calotropis procera, Ricinus communis, Curcuma longa (1:1:1 w/w/w)	100 mg/kg o.s.	3.5±0.56	3.8±0.64	3.9±0.85	4.1±0.69	4.2±0.71

Table 8: Effects of PHF and Indomethacin oral suspension on the latency of rats exposed to the hot plate

Treatment	Mean latency time (s) ± S.E.M	% Protection
Control	2.16 ± 0.166	--
Indomethacin (20mg/kg o.s)	6.83 ± 0.401**	68.3
Calotropis procera, Ricinus communis, Curcuma longa (1:1:1 w/w/w) (50mg/kg o.s.)	4.83 ± 0.477**	55.2
Calotropis procera, Ricinus communis, Curcuma longa (1:1:1 w/w/w) (100mg/kg o.s.)	6.33 ± 0.614**	65.8



Graph 1: Effect of PHF on tail flick response in rats after immersion in 55° water baths, the treated groups (Standard and PHF) were compared with control group



Graph 2: Effects of polyherbal formulation on the latency of rat exposed to the hot plate

CONCLUSION:

The current investigation shows that polyherbal formulation made by *Calotropis procera* , *Ricinus communis* & *Curcuma longa*. Extract is an effective analgesic medication. Its capacity to activate opioid receptors in the central nervous system may account for its analgesic action. Endogenous pain molecules, which are implicated in peripheral analgesia, may also be inhibited. The presence of alkaloids and other polyphenolic chemicals in these plants may explain its analgesic action. The agonistic activity of PHF, together with its unique profile of gradual onset and prolonged persistence, suggests that it could be used to treat opioid misuse and withdrawal symptoms.

REFERENCES:

1. Parkitny L, McAuley JH, Di Pietro F, Stanton TR, O'Connell NE, Marinus J, van Hilten JJ, Moseley GL. Inflammation in complex regional pain syndrome: a systematic review and meta-analysis. Neurology. 2013 Jan 1;80(1):106-17.
2. Bagdas D, Gul Z, Meade JA, Cam B, Cinkilic N, Gurun MS. Pharmacologic overview of chlorogenic acid and its metabolites in chronic pain and inflammation. Current Neuropharmacology. 2020 Mar 1;18(3):216-28.
3. Woolf CJ, Bennett GJ, Doherty M, Dubner R, Kidd B, Koltzenburg M, Lipton R, Loeser JD,

Payne R, Torebjork E. Towards a mechanism-based classification of pain?. Pain. 1998 Sep 1;77(3):227-9.
4. Boger DL, Patel M, Mullican MD. Synthetic analgesics: Preparation of racemic 6, 7-benzomorphans. Tetrahedron Letters. 1982 Jan 1;23(44):4559-62.
5. Patidar A, Birla D, Patel V, Chaturvedi M, Manocha N. A Review on advantages of Natural Analgesics over Conventional Synthetic Analgesics. International Journal of Pharmacy & Life Sciences. 2014 May 1;5(5).
6. Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJ. Analgesic activity of some Indian medicinal plants. Journal of ethnopharmacology. 2006 Jul 19;106(3):425-8.
7. Kaur A, Batish DR, Kaur S, Chauhan BS. An overview of the characteristics and potential of Calotropis procera from botanical, ecological, and economic perspectives. Frontiers in Plant Science. 2021 Jun 17;12:690806.
8. Scarpa A, Guerci A. Various uses of the castor oil plant (Ricinus communis L.) a review. Journal of ethnopharmacology. 1982 Mar 1;5(2):117-37.
9. Roth GN, Chandra A, Nair MG. Novel bioactivities of Curcuma longa constituents. Journal of natural products. 1998 Apr 24; 61(4):542-5.
10. Hossain MA, AL-Raqmi KA, Al-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pacific journal of tropical biomedicine. 2013 Sep 1;3(9):705-10.
11. Ramabadran K, Bansinath M, Turndorf H, Puig MM. Tail immersion test for the evaluation of a nociceptive reaction in mice: Methodological considerations. Journal of pharmacological methods. 1989 Mar 1;21(1):21-31.
12. Woolfe G, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride (DEMEROL). Journal of Pharmacology and Experimental Therapeutics. 1944; 80: 300–30.