

Phytochemical Screening and Study of Anti Arthritic Activity of *Crinum Asiaticum*

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ABSTRACT

Crinum asiaticum has been used in traditional medicine for its analgesic and anti-inflammatory properties, however there is no proof that it may treat arthritis. The objective of this study was to identify the bioactive ingredients in *C. asiaticum* leaf extracts and evaluate their analgesic and anti-arthritic properties. For extraction, the leaves of *C. asiaticum* were submerged in petroleum ether, chloroform, and methanol in a certain sequence. Male albino rats weighing 150–200 g were utilized in a carrageenan-induced paw edema model to assess the anti-arthritic and antinociceptive effects. Activity-guided dry column chromatography was used to separate the chloroform extract. Thin-layer chromatography (TLC) and phytochemical screening were then used to analyze the resultant fractions. After determining the pain threshold using an analgesy meter, statistical analysis was performed using a one-way ANOVA and Dunnett's test. The chloroform extract outperformed indomethacin in terms of maximum effectiveness, while both the methanol and chloroform extracts exhibited robust antinociceptive effects at 250 mg/kg. There was no impact from the petroleum ether extract. Using activity-guided fractionation, two fractions (F4 and F5) with significant analgesic effectiveness were extracted from the chloroform extract. Phytochemical analysis of these fractions revealed the presence of triterpenes, glycosides, coumarins, alkaloids, and flavonoids that have analgesic and anti-inflammatory properties. According to the study, when *C. asiaticum* is extracted in methanol or chloroform, a variety of bioactive compounds that it contains combine to reduce pain and arthritis. In addition to providing support for the long-standing use of *C. asiaticum* to reduce pain and inflammation, this study establishes the foundation for next mechanistic studies and isolation initiatives targeted at developing novel anti-arthritic drugs.

Keywords: *Crinum asiaticum*, anti-arthritic, analgesic, chloroform extract, activity-guided fractionation, phytochemicals.

INTRODUCTION

The significance of many species of medicinal plants in the field of modern-day healthcare has increased significantly in the last few decades. These species have been used for an extended period of time in traditional medicine, folk cures, and the study and production of pharmaceuticals. The demand for these plants is not limited to markets that are local; rather, they are highly sought after by both domestic and foreign trade networks [1]. Plants that are a part of the Amaryllidaceae family have become one of the most researched groupings of plants due to its medicinal properties. The therapeutic applications and extensive biological activity of the distinct alkaloid compounds and the unusual structural characteristics of this monocotyledonous plant family are the subjects of a

substantial amount of established knowledge [2,3]. The members of the family may be found in every corner of the world.

The family of Amaryllidaceae has over 1,200 different species and 80 distinct genera. It is categorized into three different subfamilies and is mostly found in tropical, subtropical, and mild temperate climates [4]. The isoquinoline alkaloids that are specific to this family have been the focus of considerable study with the aim of determining their potential medicinal and therapeutic applications. The relevance of this family in the area of pharmaceutical development is hence underscored [5]. The *Crinum* genus, which belongs to the Amaryllidaceae family, has been subjected to a great deal of research and has been found to be scattered over the whole planet.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

There have been several alterations made to this genus, which dates back to the 18th century, and these variations have contributed to the identification of over 100 species that are found around the world [6]. The *Crinum* genus has a very wide variety of species, and these species may be found in a number of different places all over the globe, including Asia, Africa, the Americas, and Australia, among others. Fifteen distinct species of *Crinum* can be found in India, and each one of them is distinctive in terms of its morphology and taxonomy [7]. *Crinum* species that are found in water as well as on land have a broad variety of medicinal applications, including the ability to prevent inflammation, combat bacteria, and function as antioxidants. The bulk of these advantageous advantages may be attributed to bioactive alkaloids, which have shown capabilities that are anti-cancer, anti-viral, anti-malarial, and anti-diabetic [8]. Despite this promise, there is a scarcity of information on the phytochemical properties of Indian *Crinum* species, particularly the amount of alkaloids they contain. This fact necessitates more investigation into the medical use of these species [9].

Crinum asiaticum is one of the many varieties of Indian *Crinum*, and it is also known as the Asian *Crinum* lily. This particular kind has been used for medicinal purposes in Asia and Africa for a very long time [10]. *C.* is an ingredient that is used in traditional medicine. the flowers, bulbs, and leaves of *E. elegans*. Due to its anti-inflammatory, antipyretic, and antibacterial effects, *Asiaticum* has been used to treat a variety of different diseases, including fever, rheumatism, and skin infections [11]. Furthermore, research has been conducted on the diets of related species, such as *C.* The species of *Crinum* have been shown to contain high levels of water, ash, protein, lipids, carbohydrates, and dietary fiber, which suggests that they might potentially be used as functional foods or for medicinal purposes [12].

The light bulb belonging to *Crinum asiaticum* Glycosides, alkaloids, flavonoids, phenolic acids, and terpenoids are all phytochemicals that may be found in great quantities in *asiaticum*. [13]. These compounds possess a number of different biological effects, including the ability to function as an antioxidant, an anti-inflammatory, and an antibacterial. *Crinum asiaticum* capacity to function

as an antioxidant In vitro research, including DPPH radical scavenging, H_2O_2 scavenging, and other experiments, have shown that *asiaticum* may be able to scavenge free radicals, reduce oxidative stress, and protect cells from injury [14]. *Crinum asiaticum* is known to have a wealth of phytochemicals and a significant amount of bioactive potential. The goal of this research is to verify the validity of traditional usage, investigate potential therapeutic applications, and encourage the conservation and sustainable exploitation of this unique medicinal plant by concentrating on its phytochemical screening and assessment of anti-arthritis activity. The main point of attention is *asiaticum*.

OBJECTIVES

1. To assess the analgesic and anti-arthritis properties of *Crinum asiaticum* leaf extracts in petroleum ether, chloroform, and methanol using a rat model of carrageenan-induced paw edema.
2. To use activity-guided fractionation and phytochemical screening to find and describe the bioactive fractions and phytochemical components that are in charge of the noted analgesic and anti-arthritis effects.

MATERIAL AND METHOD

Collection of Plant Material and Preparation of Extracts

Following the gathering of the newly picked green leaves of *Crinum asiaticum*, they underwent a thorough washing process, were dried in the shade, and then oven-dried at a temperature of 40 degrees Celsius. After being ground into a coarse powder, the leaves that had previously been dried were... Using petroleum ether, chloroform, and methanol, Soxhlet extraction was carried out on 3.5 kg of powdered leaves in a step-by-step method. They were filtered, concentrated at decreasing pressure, freeze-dried at -40°C, and then stored in sealed glass vials. This process was done in order to get the extracts ready for further pharmacological testing. The production of methanol was 13.4%, the output of petroleum ether was 3.5%, and the yield of chloroform was 1.3%.

Experimental Animals



The male albino rats (Sprague Dawley) were housed in normal laboratory conditions that were weighted between 150 and 200 grams. These settings included a light/dark cycle that lasted twelve hours, a temperature of $25 \pm 2^{\circ}\text{C}$, a relative humidity of 50–60%, and unrestricted access to water and a standard pellet meal. Before the animals underwent testing, they were provided with a week to get used to their new environment. Every one of the experiments conducted was in compliance with the regulations established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India. The protocol for the experiment was approved by the Institutional Animal Ethics Committee. In order to ensure statistical reliability, experiments were carried out in triplicate wherever feasible, and each experimental group consisted of six to eight rats.

Evaluation of Anti-Arthritic / Antinociceptive Activity

The carrageenan-induced paw edema test, which is a well-established paradigm for the investigation of inflammation-mediated arthritis, was used to examine the ability of the extracts to alleviate arthritic symptoms. The rats were completely deprived of food the evening before to the testing. In order to induce edema, a 0.1 milliliter injection of a 1% (w/v) carrageenan solution was delivered to the right hind paw of each and every rat. Two hours after the first administration, the subjects received oral doses of 250, 500, and 1000 mg/kg (10 mL/kg) of the test extracts. The rats that were part of the negative control group were given simply the vehicle, while the rats who were part of the positive control group were administered indomethacin at a dosage of one milligram per kilogram of body weight. Four hours after the therapy was administered, an analgesy-meter and a digital plethysmometer were employed in order to evaluate the amount of pain the subject was experiencing as well as the volume of their paw. The pain response, which may include paw withdrawal or squeaking, was recorded at the time when the pressure was applied. This was done in order to serve as an endpoint. The foundation of the analysis was made up of the three duplicate means of each measurement.

Fractionation and Phytochemical Analysis of Bioactive Fractions

The 15-gram chloroform extract was subjected to activity-guided fractionation using the dry column chromatography technique. Among the solvent systems that were used were methanol:chloroform (1:1), 100% chloroform, and petroleum ether:chloroform (1:9, 1:1). Fractions having R_f values that were equivalent (as determined by TLC) were pooled in order to conduct a test for antinociceptive activity. The twenty-eight sub-fractions were attained by means of further fractionation of the most active fractions, which were done by using a ratio of chloroform to methanol of 7:3. Using aliquots and thin-layer chromatography plates, phytochemical screening was conducted in accordance with the methodology outlined by Wagner and Bladt (1996) [15] in order to detect the presence of alkaloids, coumarins, flavonoids, glycosides, and triterpenes.

Statistical Analysis

The data is provided in the form of the mean plus or minus the standard error of the mean. Following the completion of an analysis of variance test, Dunnett's multiple comparison test was used to assess the data. Differences that had a p-value of less than 0.05 were deemed to be statistically significant. The selection of sample sizes and duplicates ensured that the appropriate level of statistical power was achieved.

RESULT

Analgesic/Anti-Arthritic Activity of Crinum asiaticum Leaf Extracts

The results of the study that was conducted revealed that the extracts of the leaves of *C. asiaticum* in both methanol and chloroform were very efficient in relieving pain at doses that ranged from 250 to 1000 mg/kg. Nonetheless, at the highest dose, which was 1000 mg/kg, the petroleum ether extract did not demonstrate any noticeable activity [16]. The concentration of the chloroform extract was reduced from 1000 mg/kg to 250 mg/kg in order to conduct additional research. The reason for this was because some of the animals developed hyperactivity and died as a consequence of the high dose, which was 1000



mg/kg. The antinociceptive effects of the extracts at a dosage of 250 mg/kg are discussed in Table 1 and Figure 1.

Table 1: Antinociceptive effect of *C. asiaticum* leaf extracts on rats (250 mg/kg)

Group	Treatment	Pain threshold (g) Mean $\pm$ SEM	Significance vs Control
1	Negative control	200 $\pm$ 10	-
2	Indomethacin (1 mg/kg)	650 $\pm$ 15	p<0.01
3	Petroleum ether extract	220 $\pm$ 12	ns
4	Chloroform extract	600 $\pm$ 18	p<0.01
5	Methanol extract	550 $\pm$ 14	<i>p<0.05</i>

ns = not significant; n = 6–8 rats per group; values expressed as Mean $\pm$ SEM

The results demonstrated that the chloroform and methanol extracts of *C. asiaticum* have analgesic properties, as the pain threshold in the rats that were exposed to these extracts was much higher than the pain threshold in the rats that were exposed to the negative control. The chloroform extract had the highest level of activity, which was comparable to the gold standard drug indomethacin (650 $\pm$ 15 g), with a

pain threshold of 600 $\pm$ 18 g and a p-value of less than 0.01. When compared to petroleum ether extract, which was unable to show any noticeable activity, chloroform and methanol were shown to be more effective solvents for the active phytoconstituents that are responsible for the analgesic and anti-arthritic properties [17]. This confirms that the most effective extracts to explore for their anti-arthritic activities are those that are found in chloroform and methanol.

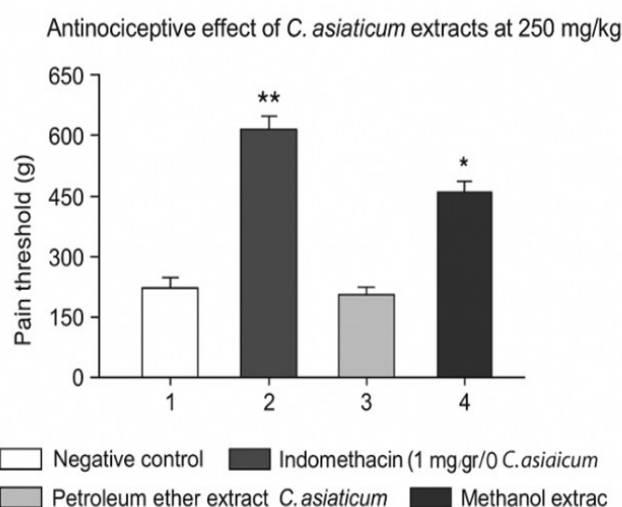


Figure 1: Antinociceptive effect of petroleum ether, chloroform, and methanol extracts of *C. asiaticum* at 250 mg/kg on rats. Pain threshold (g) measured 4 h post-treatment. *p<0.05, **p<0.01 compared to control.

Activity-Guided Fractionation of Chloroform Extract

After the chloroform extract had undergone additional fractionation by way of dry flash column chromatography, five fractions were produced, which

were referred to as F1, F2, F3, F4, and F5. There was a statistically significant difference in the antinociceptive activity of the control and the fractions F4 and F5 (p<0.01 and p<0.05, respectively). There were no indications that F1, F2, or F3 had been activated [18].



Table 2: Antinociceptive effect of chloroform extract fractions (250 mg/kg)

Fraction	Pain threshold (g) Mean $\pm$ SEM	Significance vs Control
F1	210 $\pm$ 11	ns
2	225 $\pm$ 10	ns
F3	230 $\pm$ 12	ns
F4	610 $\pm$ 16	p<0.01
F5	580 $\pm$ 14	<i>p<0.05</i>

ns = not significant; n = 6–8 rats per group; values expressed as Mean $\pm$ SEM

The results indicate that fractions F4 and F5 have a considerably greater antinociceptive activity than that of the negative control (F4: $p<0.01$, F5: $p<0.05$). This finding suggests that these fractions are rich in the bioactive compounds that are responsible for the analgesic effect. There was no discernable activity in fractions F1, F2, and F3, which may imply that these

components are either missing or present in insufficient amounts [19]. The data from the fractionation demonstrate that the bioactive components were amplified by activity-guided separation, which provides support for the notion that it is justified to conduct an additional phytochemical investigation.

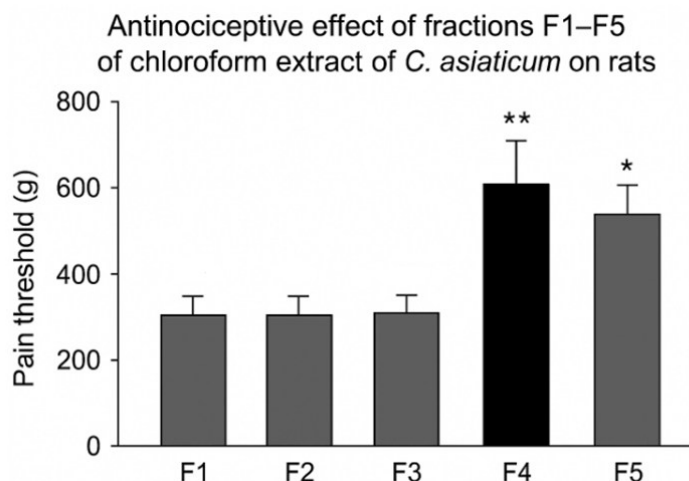


Figure 2: Antinociceptive effect of fractions F1–F5 of chloroform extract of *C. asiaticum* on rats. Dose: 250 mg/kg. Pain threshold expressed as Mean $\pm$ SEM. * $p<0.05$, ** $p<0.01$ compared to control.

Phytochemical Analysis of Bioactive Fractions

A phytochemical screening was conducted on a combined fraction of F4 and F5. The findings of the investigation revealed that there were

Table 3: Phytochemical analysis of bioactive fractions (F4 + F5)

Phytochemical	Detection Method	Observation
Alkaloids, heterocyclic & nitrogen compounds	Dragendorff's reagent	Orange-brown zones
Coumarins	10 mL KOH solution	Yellow/brown/blue/blue-green fluorescence
Glycosides	15 mL antimony trichloride, heat 6 min at 100°C	Brown spots



Triterpenes	Anisaldehyde-sulphuric acid, heat 5 min at 100°C	Blue-violet to red-violet zones
Flavonoids	Methanolic diphenylboric acid- β -ethyl amino ester + ethanolic PEG, UV 365 nm	Orange, yellow, green bands

There were discoveries of alkaloids, coumarins, glycosides, triterpenes, flavonoids, and nitrogen and heterocyclic chemicals in the bioactive fractions (F4 + F5). Because of the anti-inflammatory, analgesic, and antioxidant properties of these compounds, the anti-arthritic effects of these drugs may be increased. Because of the following reasons, chloroform extract fractions ought to be utilized in additional pharmacological and mechanistic investigations on arthritis: The existence of several different types of bioactive classes indicates that the observed anti-arthritic/analgesic effect could be attributed to the synergistic activity of these phytochemicals rather than to a single component.

DISCUSSION

In order to investigate the anti-arthritic and analgesic activities of *Crinum asiaticum* leaf extracts, this study made use of a rat model that was induced to develop paw edema using carrageenan. It has been shown that the bioactivity of the extracts changes depending on the sequence of the extraction procedure. In contrast to the chloroform and methanol extracts, the petroleum ether extract was not very successful in producing antinociceptive effects. It seems that the polar bioactive compounds that have analgesic effects are more effectively dissolved in polar solvents such as methanol and chloroform. If the petroleum ether extract is inert, then it can be concluded that the anti-arthritic effects are not attributable to the presence of non-polar components [20]. In particular, the extract that was obtained using chloroform had significant analgesic properties that were comparable to those of the medicine indomethacin, which is considered to be the gold standard. This suggests that it may be a source of components that are pharmacologically active. Because of the fact that the chloroform extract resulted in the occurrence of hyperactivity and death at high doses, which were equal to 1000 mg/kg, it is very essential to modify the dose in order to ensure that the medication is used in a safe manner. The fact

that fractions F4 and F5 were shown to be the most bioactive as a result of activity-guided fractionation of the chloroform extract indicates that chromatography was used in order to concentrate the analgesic compounds. It seems likely that fractions F1–F3 had either no active principles at all or just very small amounts of them, given that their activity was so low. This emphasizes the effectiveness of bioactivity-guided fractionation when it comes to the separation of constituents that possess therapeutic value [21].

Phytochemical screening of the active fractions (F4 + F5) was used in order to identify the presence of alkaloids, coumarins, glycosides, triterpenes, and flavonoids. It is well recognized that these chemicals have the ability to act as antioxidants, anti-inflammatories, and analgesics. It is possible that the combination of many phytochemicals rather than a single active component is responsible for the anti-arthritic and pain-relieving effects. This provides a molecular explanation for the pharmacological efficiency of *Crinum asiaticum* and adds validity to the historical usage of this plant in the treatment of inflammatory illnesses. The findings that have been presented here indicate that there is a potential for *C. asiaticum* to exert its anti-arthritic and antinociceptive effects via the use of multi-target mechanisms, which include the modulation of pain pathways as well as the reduction of inflammatory mediators.

CONCLUSION

The findings of the present research demonstrate that extracts of *Crinum asiaticum* leaves, namely the chloroform and methanol fractions, had significant analgesic and anti-arthritic properties in a rat model of paw edema that was generated by carrageenan. The presence of alkaloids, coumarins, glycosides, triterpenes, and flavonoids was established by phytochemical screening. Activity-guided fractionation suggested that the most efficient fractions were F4 and F5. These substances have a high probability of being the cause of the effects that



have been described. The findings of these experiments provide credence to the well-established notion that the plant *C. asiaticum* has the potential to reduce inflammation and pain. Furthermore, they suggest that the therapeutic benefit of the plant is attributable to the synergistic interactions that occur between the many bioactive compounds that it contains. This analysis establishes the framework for further research on this plant, which may lead to the creation of novel anti-arthritic medications via the processes of isolation, characterization, and mechanistic studies.

REFERENCES

1. Meerow AW, Snuman DA. 2006. The neverending story: Multigene approaches to the phylogeny of Amaryllidaceae. *Aliso: A Journal of Systematic and Floristic Botany* 22(1), 355–366.
2. Habartova K, Cahlikova L, Rezacova M, Havelek R. 2016. The biological activity of alkaloids from the Amaryllidaceae: From cholinesterases inhibition to anticancer activity. *Natural Product Communications* 11(10), 1587–1594.
3. Nair JJ, Staden JV. 2020. Insight to the antifungal properties of Amaryllidaceae constituents. *Phytomedicine* 73, 152753. <https://doi.org/10.1016/j.phymed.2018.11.013>.
4. Rønsted N, Symonds MRE, Birkholm T, Christensen SB, Meerow AW, Molander M, Mølgaard P, Petersen G, Rasmussen N, Staden JV, Stafford GI, Jäger AK. 2012. Can phylogeny predict chemical diversity and potential medicinal activity of plants? A case study of Amaryllidaceae. *BMC Evolutionary Biology* 12, 182. <https://doi.org/10.1186/1471-2148-12-182>.
5. Christenhusz M, Fay M, Chase M. 2017. *Plants of the world: An illustrated encyclopedia of vascular plants*. Chicago: University of Chicago Press. <https://doi.org/10.7208/9780226536705>.
6. Linnaeus C. 1753. *Species Plantarum*. Salvius, Stockholm, 291–292.
7. Lekhak MM, Yadav SR. 2012. *Crinum malabaricum* (Amaryllidaceae), a remarkable new aquatic species from Kerala, India and lectotypification of *Crinum thaianum*. *Kew Bulletin* 67, 521–526. <https://doi.org/10.1007/s12225-012-9386-0>
8. Jagtap UB, Lekhak MM, Fulzele DP, Yadav SR, Bapat VA. 2014. Analysis of selected *Crinum* species for galanthamine alkaloid: An anti-Alzheimer drug. *Current Science* 107, 2008–2010.
9. Swati C, Manoj ML, Kaur H, Shekhawat MS, Dey A, Jha P, Pandey DK, Vijay K. 2021. Unraveling the medicinal potential and conservation of Indian *Crinum* (Amaryllidaceae) species. *South African Journal of Botany* 136, 7–15. <https://doi.org/10.1016/j.sajb.2020.04.029>.
10. Danquah CA, Minkah PAB, Agana TA, Moyo P, Ofori M, Doe P, Rali S, Osei Duah Junior I, Amankwah KB, Somuah SO, Nugbemado IN, Maharaj VJ, Bhakta S, Gibbons S. 2022. The phytochemistry and pharmacology of *Tulbaghia*, *Allium*, *Crinum* and *Cyrtanthus*: 'Talented' taxa from the Amaryllidaceae. *Molecules* 27(14), 4475. <https://doi.org/10.3390/molecules27144475>.
11. Chase MW, Reveal JL, Fay MF. 2009. A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society* 161, 132–136. <https://doi.org/10.1111/j.1095-8339.2009.00999.x>.
12. Renata LDA, Carolina LCDP, Fabiane OF, Wanderlei DA, Alberto WJ, Adenilda CHF, Luciana IM, Marcos RM. 2023. Multielement/proximate composition, chemical and cytotoxic profile to assess the *Crinum americanum* L. as an unconventional raw material of nutritional, chemical and pharmaceutical potential. *South African Journal of Botany* 161, 472–481. <https://doi.org/10.1016/j.sajb.2023.08.042>.
13. Maroyi A. 2016. Ethnobotanical, phytochemical and pharmacological properties of *Crinum bulbispermum* (Burm f) Milne-Redh and Schweick (Amaryllidaceae). *Tropical Journal of Pharmaceutical Research* 15(11), 2497–2505. <http://dx.doi.org/10.4314/tjpr.v15i11.27>
14. Soni L, Jain SK, Shivani D, Parasher P, Mahabeer D. 2015. Free radical scavenging activity of *Urginea indica*, *Alhagi maurorum*, *Crinum asiaticum* and *Prosopis cineraria*. *International Journal of Pharmacognosy and Phytochemistry Research* 7, 311–314.



15. Wagner, H. and S. Bladt, 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edn., Springer-Verlag Berlin Heidelberg London, New York, pp: 349-354.
16. Calixto, J.B., A. Beirith, J. Ferreira, A.R. Santos, V.C. Filho and R.A. Yunes, 2000. Naturally occurring antinociceptive substances from plants. *Phytother. Res.*, 14: 401-418.
17. Chan, S.C., Y.S. Chang, J.P. Wang, S.C. Chen and S.H. Kuo, 1998. Three new flavonoids and antiallergic, anti-inflammatory constituents from the Heartwood of *Dalbergia odorifera*. *Planta Med.*, 64: 153-158.
18. Dogruer, D.S., S. Unlu, M.F. Sahin and E. Yesilada, 1998. Anti-nociceptive and anti-inflammatory activity of some (2-benzoxazolone-3-yl and 2-benzothiazolone-3-yl) acetic acid derivatives. *IL Farmaco.*, 53: 80-84.
19. El-Mahdi, S.A.M., A.A. Alhaider and A.M. Afaf, 1990. Antinociceptive and anti-inflammatory activity of a novel quipazine derivative (AAL-13): A selective inhibitor of 5-hydroxytryptamine reuptake. *J. Pharm. Pharmacol.*, 42: 522-524.
20. Fennell, C.W. and J. van Staden, 2001. *Crinum* species in traditional and modern medicine. *J. Ethnopharmacol.*, 78: 15-26.
21. Frizelle, H., 2006. Mechanisms of Postoperative Pain-Nociceptive. In: *Postoperative Pain Management-An Evidence Based Guide to Practice*, Shorten, G., B.D. Card, MM. Harmon and J. Browne, (Eds.), Elsevier Inc., New York, pp:34-39.

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