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Research Article

Evaluating the Analgesic Activity of Leaf, Stem Bark Extract, and Isolated Nutraceuticals of *Caesalpinia bonducella L* (*Roxb.*)

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ABSTRACT

This study aimed to evaluate the analgesic potential of *Caesalpinia bonducella L (Roxb.)* by evaluating the efficacy of leaf and stem bark extracts and isolated nutraceuticals. Nutraceuticals were isolated *via* column chromatography and characterized using IR and NMR. Analgesic activities were assessed *in-vivo* using the hot plate, capsaicin-induced pain, and tail flick tests on 36 albino mice across nine groups. Phytochemical screening revealed flavonoids and polyphenols. Pure nutraceuticals displayed superior analgesic properties compared to the control. Ethanol extracts of *C. bonducella* leaf and bark exhibited higher analgesic efficacy than other extracts and the control ($p < 0.05$). The study suggests the plant's potential in the nutraceutical field, highlighting its therapeutic value and positioning it as a valuable resource for India's nutraceutical sector.

Introduction

Since the beginning of time, plants have made a substantial contribution to maintaining human health and improving the quality of human life by being necessary components of medicines, beverages, flavors, cosmetics, and colors. The foundation of herbal medicine is the notion that certain organic substances found in plants have the potential to both cure and enhance health. The study of plants has attracted more attention in recent years. Consequently, a large body of evidence has been gathered demonstrating the potential benefits of medicinal plants used in many traditional systems. The use of herbal remedies has been receiving significant public interest.[1]

Additionally, plant extracts served as the basis for several Western medications. Several herbs are mostly used to treat digestive (*Apium graveolens L.*), metabolic (*Carica papaya*), and liver (*Berberis vulgaris*) ailments as well as cardiovascular (*Argania spinosa (L.)* Skeels), respiratory (*Urtica dioica L.*), and central nervous system issues (*Peganum harmala L.*). They may be utilized as medications or supplements in managing or treating a range of illnesses due to their potential for significant therapeutic effects.[2,3] The extracted constituent(s), extracts, and herbal medicines/medicinal plants have shown a variety of biological effects. These have been used and still are used as folk medicine or as a dietary supplement for a variety of diseases.

Researchers from all around the globe are still interested in ethnopharmacological research on clinically significant plants and herbs. However, the absence of standardized quality control profiles is one of the barriers to the use of the Ayurvedic or Siddha formulation. Medicinal plants are those that have qualities or substances that may be used

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therapeutically or synthesize metabolites to generate valuable medications, according to the World Health Organization (WHO).[4,5]

The ancient Indian medical system of Ayurveda mentions the plant *Caesalpinia bonducella*. The Caesalpiniaceae family, of which *C. bonducella* is a member, is widely distributed worldwide, with special emphasis on India, Sri Lanka, and the Andaman and Nicobar Islands. Several species—like *C. nuga* and *C. jayoba*—are mistakenly identified as synonyms for *C. crista*. To form *C. jayoba*, *C. crista* must hybridise.[6–10] The name "Bonducella" originates from the Arabic word "Bonduce," which signifies "a small ball" and describes the globular form of the seed.[7] Bioactive homo iso-flavonoids like bonducellin, which have been demonstrated to have a number of positive properties, including antibacterial, antioxidant, anticancer, anti-estrogenic, anti-inflammatory, and anti-androgenic activities, are abundant in the seeds and kernels of this plant, according to Billah *et al.* (2013).[10-12] Nithiyanandam *et al*. (2023) suggested that the aqueous solution has better *in-vitro* antioxidant properties than alternate solvents. Furthermore, the extract derived from this solution demonstrated a noteworthy enhancement in antioxidant activity, coupled with a reduction in lipid peroxidation. This effect was particularly evident in the context of acetaminophen (APAP)-induced spleen and cardiotoxicity, where the extract played a pivotal role in restoring tissue morphology.[13] Although *C. bonducella* is often used in conventional medicine, relatively less documented systematic pharmacological research has evaluated its analgesic effects.

Subbiah *et al*. (2019) highlighted the medicinal characteristics of several components of *C. bonducella L*, specifically emphasizing the seed and shell. The plant's shells, seeds, and twigs contain many alkaloids, including Natin.[14] The seed includes bonducin, a powerful glycoside, saponins, and terpenoids. Srinivasan *et al.* (2023) highlighted the nutritional composition of the shell, which includes a variety of fatty acids, including palmitic, oleic, stearic, linoceric, and linolenic, as well as fatty oil, starch, phytosterols, and sucrose.[15] The shell includes protein and amino acids, with a quantity ranging from 7.430 to 25.346%. Sasidharan *et al*. (2022) have shown that seeds exhibit anti-diabetic characteristics, which are especially significant in the setting of type 2 diabetes. Elevated blood glucose levels define this metabolic disorder due to decreased insulin production or responsiveness.[16]

In the contemporary pharmaceutical landscape, evaluating leaf and stem bark extracts for their analgesic properties, along with the isolated (Roxb.), holds immense significance. Chronic pain management is a prevailing challenge, and the search for natural compounds with analgesic properties is paramount. This study's findings could contribute valuable insights into the development of novel analgesic agents derived from *C. bonducella*, potentially addressing the ongoing need for effective and safer pain management options in the present pharmaceutical scenario. The exploration of plant-derived nutraceuticals aligns with the growing trend toward harnessing the therapeutic potential of natural compounds, offering promising avenues for the development of pharmaceutical interventions with enhanced efficacy and reduced side effects.

C. bonducella, a significant plant in Ayurvedic medicine, possesses a wide range of pharmacological actions, making it a good candidate for many therapeutic uses. The plant has several medicinal advantages, particularly in its seed kernel, which shows substantial antidiabetic qualities.[17] It assists in regulating blood sugar levels and displays possible antihyperlipidemic actions that are essential for diabetes management.[18] Furthermore, *C. bonducella* has conventional abortifacient properties, which have been traditionally used for female fertility control, suggesting its potential in the field of reproductive health. In addition, the extracts of this substance have strong antioxidant activities, assisting in mitigating oxidative stress and reducing cellular damage induced by free radicals. Furthermore, its analgesic and anti-inflammatory actions indicate its potential usefulness in treating many pain and inflammation-related disorders.[19] Furthermore, the herb's ability to reduce fever and its efficacy against certain filarial parasites highlights its promise in managing fevers and combating filarial infections. In addition, *C. bonducella* has antibacterial, antidiarrheal, antimalarial, and antifungal properties, underscoring its function as a natural antimicrobial and antifungal agent with potential uses in gastrointestinal disorders and malaria treatment. The seed extracts dissolved in water additionally demonstrate action in preventing sperm production, showing potential as a contraceptive. Similarly, the extracts obtained from the leaves using methanol display activity against tumor growth, suggesting potential for cancer therapy. Ultimately, its ability to prevent ulcers provides promising advantages in the treatment of gastrointestinal disorders. The various pharmacological effects of *C. bonducella* highlight its potential in traditional as well as contemporary medicinal applications.[20] Hence, in this study, we evaluated the analgesic activity of ethanol, petroleum chloroform, and ether extract of leaf and stem bark of *C. bonducella* and also assessed the analgesic activity of its major secondary metabolite, bonducellin, and caesalpinianone for their analgesic activity.

Materials and Methods

Collection and Identification of Plant Material

C. bonducella's leaf and stem bark were collected from the local area surrounding the university and the identity of the plant specimen was confirmed by a botanist from a recognized university (Fig. 1).

Fig. 1: Stembark and the leaf of *C. bonducella*

Preparation of Plant Extract

The plant's leaves and stem bark were collected and thoroughly cleaned with distilled water, and the bark was then diced into small pieces. The samples were then air-dried in a shaded environment. The mechanical grinder was constantly used to crush dried components into fine powder. A sequential extraction method was used to extract powdered samples of *C. bonducella*, weighing 100 g, using petroleum ether, chloroform, and ethanol. The extraction procedure was conducted using soxhlet equipment, with solvents used in ascending order of polarity. Extraction was continued until the solvents exhibited no coloration. After being concentrated in a rotary evaporator, the samples were stored at -70°C in temperature for later use.

Phytochemical Screening

The extracts underwent a first phytochemical screening using the methods and analyses outlined by Dey and Raman.

Isolation of Pure Compounds

Crude ethanol extracts from the aerial portions of *Ceasalpinia bonducella* were separated using column chromatography on silica gel to isolate two main compounds: bonducellin and caesalpinianone.

Characterization of Isolated Pure Compound

LC-MS

An LC-DAD-MSn system was used to examine the samples. Weighted extracts of stem bark and leaf (20 mg/mL ethanol) were extracted, followed by a 10-minute sonication. The materials underwent centrifuged at 13,000 revolutions per minute for 15 minutes before being used in LC analysis. The LC-MS system consists of an Agilent 1260 quaternary pump, an Agilent MS 500 mass spectrometer, and an Agilent 1260 diode array detector. The system operated between the 100 to 2000 m/z range. The following MS settings were implemented: The needle voltage is set at 4.9 kilovolts, the shield voltage is set at 600 volts, and the capillary voltage is set at 80 volts. The RF loading is set at 80%. The nitrogen nebulizing gas pressure is 25 pounds per square inch (psi), while the drying gas pressure is 15 psi. The drying gas temperature is set at 300°C.

The compounds were identified using a combination of information from literature and reference compounds. After being centrifuged for 10 minutes, the sample solutions were placed into vials for examination. The stationary phase was a 3.0 by 150 mm by 3.5 m Agilent Eclipse XDB-C18 column.

NMR and IR spectra

The bioactive chemicals were identified using a 300 JEOL NMR spectrometer operated at a frequency of 75 MHz, with TMS used as the internal standard. The Jasco-IRA1 IR spectrophotometer has been utilized to acquire the IR spectra. The bonducellin and caesalpinianone compounds underwent extensive purification, resulting in an initial purity level of 99.5%.

Critical toxicity study

The LC-MS system included an Agilent 1260 diode array detector and a Varian MS 500 mass spectrometer, functioning in the 100 to 2000 m/z range. The MS parameters used were: nitrogen nebulizing gas pressure at 25 psi, drying gas pressure at 15 psi, drying gas temperature at 300°C, shield voltage at 600 V, capillary voltage at 80 V, and RF loading at 80%.

Analgesic Activity

Animals

Male and female mice weighing 25 to 30 grams were obtained from the Institute's Laboratory Animal Resource Section and acclimated to the lab environment for at least a week before any experiments were performed (Fig. 2). 9 groups were formed for the experiment (each group consisted of 4 animals, a total of 36 mice) as follows:

- Group 1: Healthy control (0.9% saline)
- Group 2: Treated with ethanolic stem-bark extract (200 mg/kg)
- Group 3: Pet ether stem-bark extract (200 mg/kg) was used as a treatment.
- Group 4: 200 mg/kg of chloroform stem-bark extract was used as a treatment.
- Group 5: 200 mg/kg of ethanol leaf extract was used as a treatment.
- Group 6: Pet ether leaf extract (200 mg/kg) was used as a treatment.

Fig. 2: Laboratory mice

- Group 7: 200 mg/kg of chloroform leaf extract was used as a treatment.
- Group 8: Pure bonducellin (200 mg/kg) was used as a treatment.
- Group 9: Pure caesalpinianone (200 mg/kg) was used as a treatment (Fig. 2).

Study design

The mice were divided into nine groups, each with four animals, after being weighed and given numbered ID tags. Oral gavage was used to deliver each sample. Group 1 received a saline solution with a concentration of 0.9% and served as the negative control. Groups 2 to 7 were administered various extracts at a dosage of 200 mg/kg, whereas groups 8 and 9 were given the extract in its pure form.

Capsaicin-induced pain

The mice's right hind paw was injected intraplantar (1.6 g/paw) with capsaicin (20 L) prepared in PBS. Following injection with capsaicin, each animal was watched for 5 minutes. The duration of licking the paw that had been shot was seen as a sign of discomfort. One hour before the injection of capsaicin, animals were administered a vehicle, a test drug, or an intraperitoneal injection of morphine.

Hot plate test

The hot plate test has been adapted with minor changes to evaluate the analgesic response. The animals were chosen 24 hours earlier based on their responsiveness by removing any mice that persisted on the device kept at 55 ± 0.50°C for more than 10 seconds. Complete antinociception was deemed to occur with a delay of 30 scounds. Mice that had been given a vehicle, test drug, or morphine one hour before the test had their response times measured (Fig. 3).

Tail flick test

Analgesiometer with radiant heat tail-flick (Fig. 4) was used to measure the delay. The creatures were kept in cages with a radiant heat source attached to the base of their tails. The animals were chosen for the test 24 hours in advance based on their response in the model. Mice that stayed on the device for over 10 seconds were eliminated. The total analgesia lasted for around 20 seconds. The reaction time was assessed in animals that were treated with either morphine or the experimental medication before the experiment, as well as in control mice (Fig. 4).

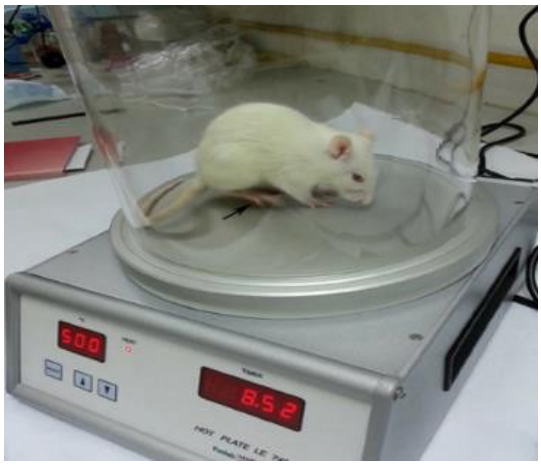

Fig. 3: Hot plate test

Fig. 4: Tail flick test

Statistical Analysis

The data were shown as the mean value ± the standard error of the mean (SEM). The study compared groups using One-way ANOVA and then conducted the Post Hoc Dunnett test to analyze the differences. The results were considered statistically significant with a significance level of $p < 0.05$.

RESULTS

Phytochemical Screening and Crude Extract Yield

The yield of the extracts of stem bark were 27.5, 25.8 and 24 g for ethanol, pet ether, and chloroform extract, respectively while for leaf ethanol, pet ether, and chloroform extract, the yield of crude extract was 26, 23.15 and 20.1 g, respectively. The qualitative phytochemical examination revealed that the extract contained triterpenoid and flavonoid but was devoid of volatile oils, tannin, alkaloid, and saponin (Table 1).

Characterization of Isolated Pure Compound

A 12 g yellow crystalline solid obtained was Bonducelllin, while a 10.5 g colorless amorphous solid was identified as caesalpinianone (Fig. 5).

Liquid chromatography–mass spectrometry graph

The isoflavonoid components of *C. bonducella* extract were successfully isolated and identified in the current investigation. We were able to discover 10 flavonoid

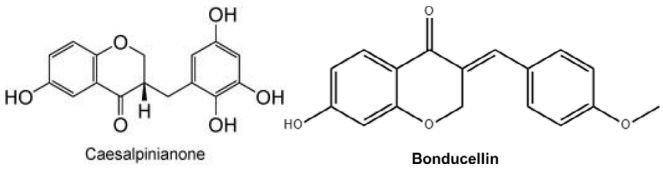

Fig. 5: Structure of bonducellin and caesalpinianon

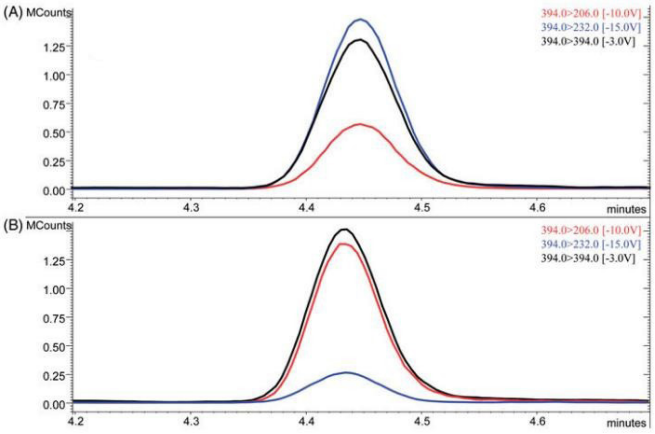

Fig. 6: LC-MS graph of a) Bonducellin and b) Caesalpinianone

components in the leaf and stem bark extracts of *C. bonducella* using liquid chromatography/mass spectrometry analysis, including two significant compounds: Bonducellin and ceasalpinianone. Stem bark and leaf extract of *C. bonducella* were shown to have remarkably comparable phytochemical properties. However, the concentrations of these chemicals in stem bark and leaves are not the same. The flavonoids in both sections were identical, according to the LC/MS study (Fig. 6).

IR and ^{13}C-NMR

Caesalpinianone's infrared spectra revealed strong absorption bands at 3394 (OH), 1641 (CO), and 1612 (CC)

Table 1: The quantitative investigation of phytochemical components in various extracts of caesalpinianone bonducella's leaves and stembark

S. No.	*Constituents*	*Stem bark extract*			*Leaf extract*		
		Ethanol	*Pet. ether*	*Chloroform*	*Ethanol*	*Pet. ether*	*Chloroform*
1	Steroids	+	+	-	+	+	-
2	Flavonoids	+	+	+	+	-	+
3	Saponins	+	+	-	+	+	-
4	Glycosides	+	+	+	+	+	+
5	Phenols	+	+	+	+	+	+
6	Tannins	+	+	+	+	+	+
7	Alkaloids	+	+	+	+	+	+
8	Terpenes	+	+	+	+	+	+
9	Volatile oil	+	-	+	+	-	+
10	Reducing sugar	+	+	+	+	+	+

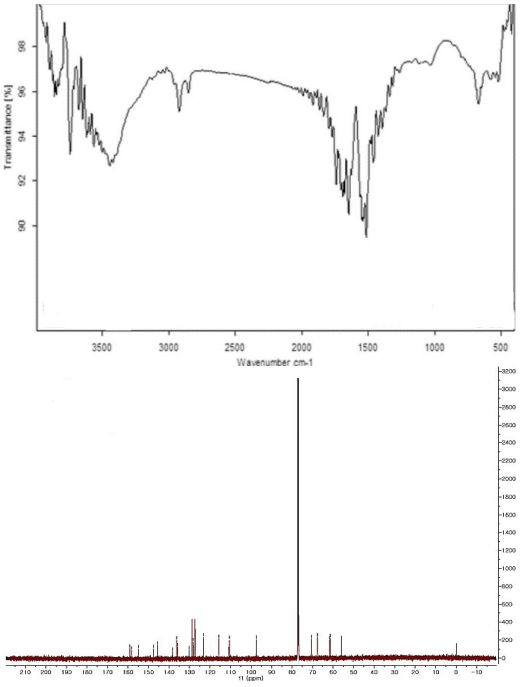

Fig. 7: IR and ^{13}C-NMR graph of caesalpinianone

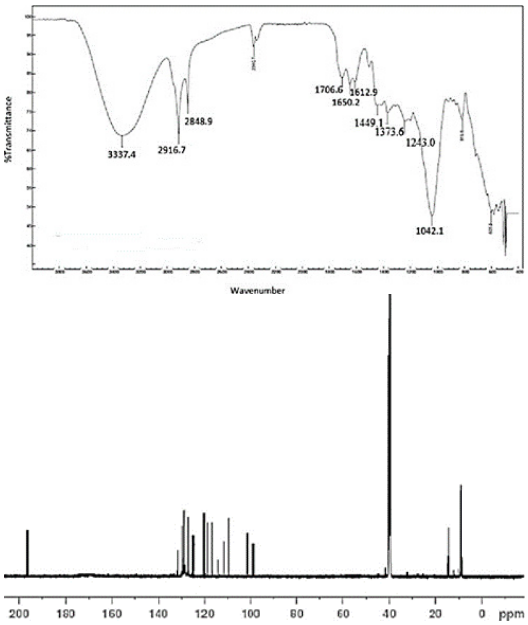

Fig. 8: IR and ^{13}C NMR graph of bonducellin

cm ^{1}. All 16 carbon resonances may be seen in the 13C NMR spectrum (CD3OD, 75 MHz) of carbon number 1. Six methine and two methylene carbons were found in its DEPT spectra. Additionally, it was found that one methine carbon underwent sp^3 hybridization, while five methine carbons underwent sp^2 hybridization (Fig. 7).

IR spectrum of bonducellin showed the presence of a hydroxyl group (3441 cm^{-1}), aromatic ring (1600, 1500 cm^{-1} and a carbonyl group (1615 cm^{-1}).

^{13}C NMR showed "67.7 (C-2), 129.0, (C-3), 179.6(C-4), 129.5 (C-5), 111.2 (C-6), 164.7 (C-7), 102.6 (C-8), 135.3 (C-9), 162.6 (C-10), 114.4 (C-11), 126.7 (C-1ǵ), 132.3 (C-2ǵ), 114.4 (C-3ǵ), 160.4 (C-4ǵ), 114.4 (C-5ǵ), 132.3, (C-6ǵ), 55.5 (OCH3)" (Fig. 8).

Acute oral toxicity study

The 7-day experiment showed no mortality in the rats treated with 2000 mg/kg of orally supplied saffron ethanolic extracts. No significant behavioural alterations were seen in mice over the testing period. Henceforth, a dose of 200 mg/kg of all extract was chosen for analgesic activity.

Hot plate test

In comparison to the control, the leaf and stem extract of *C. bonducella* exhibited considerable analgesic effect ($p < 0.05$) at all tested dosages (Table 2; Fig. 9). However, as compared to other extracts, ethanol extract (200 mg/kg) of stem bark extract significantly increased response time, while mice treated with chloroform showed the lowest reaction time for both leaf and stem bark extract. In pure compounds, both the bonducellin and caesalpinianone showed highly significant differences in response time as compared to both the control and leaf and stem-bark extract-treated groups ($p < 0.05$) (Table 2) (Fig. 9).

Fig. 9: Hot plate test between control and the experimental groups at different time intervals

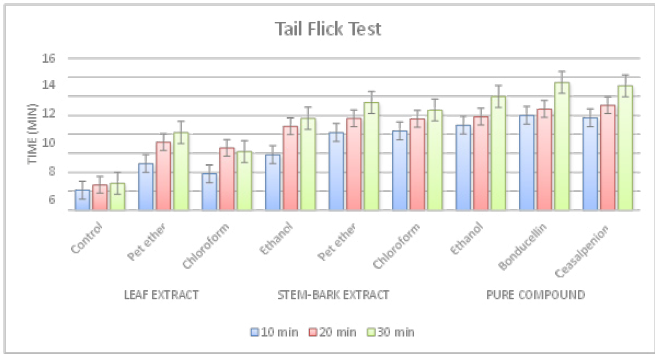

Fig. 10: Mice's reaction times in the tail-flick test between the control and experimental groups at different time intervals

Table 2: Mean response time in hot plate test at 10, 20, and 30 minutes

Groups	*Time (in minutes)*		
	10 minutes	*20 minutes*	*30 minutes*
Control	3.66 ± 0.33	3.83 ± 0.47	3.66 ± 0.33
Leaf extract			
Pet ether	7.16 ± 0.30*	8.16 ± 0.30*	8.83 ± 0.47*
Chloroform	6.16 ± 0.30	6.66 ± 0.33	7.16 ± 0.47
Ethanol	8.83 ± 1.04*	9.66 ± 0.33*	10.50 ± 0.60**
Stem bark extract			
Pet ether	9.66 ± 0.21**	11.33 ± 0.95**	12.00 ± 0.36**
Chloroform	8.33 ± 0.25*	9.83 ± 0.47*	10.33 ± 0.55**
Ethanol	10.83 ± 0.60**	11.50 ± 0.34**	12.16 ± 0.87**
Pure Compound			
Bonducellin	12.64 ± 0.91**	14.25 ± 0.53**	15.71 ± 0.95**
Caesalpinianone	11.96 ± 0.62**	14.08 ± 1.01**	15.64 ± 1.27**

*Significant at P<0.05; ** Significant at P<0.01

Table 3: Reaction times of mice in tail-flick test

Groups	*10 min*	*20 min*	*30 min*
Control	2.09 ± 0.47	2.66 ± 0.33	2.83 ± 0.47
Leaf extract			
Pet ether	4.91 ± 1.20	7.16 ± 0.30*	8.16 ± 0.30**
Chloroform	3.82 ± 0.48	6.55 ± 0.22*	6.16 ± 0.70*
Ethanol	5.84 ± 1.35*	8.83 ± 0.47**	9.66 ± 0.33**
Stem extraction			
Pet ether	8.16 ± 0.40*	9.66 ± 0.42*	11.33 ± 0.34**
Chloroform	8.33 ± 0.47*	9.60 ± 0.21**	10.53 ± 0.35**
Ethanol	8.95 ± 0.68**	9.83 ± 0.60**	11.96 ± 0.30**
Pure Compound			
Bonducellin	9.98 ± 0.79**	10.64 ± 0.51**	13.44 ± 1.13**
Caesalpinianone	9.72 ± 1.06**	11.04 ± 1.35**	13.08 ± 0.76**

*Significant at P<0.05; ** Significant at P<0.01

Tail flick test

Compared to pet ether and chloroform, the results demonstrated that the tail-flick response of the ethanol extract-treated group of leaf and stem bark was high at 20 and 30 minutes (Table 3; Fig. 10). Statistical analysis showed a significant difference between the control and experimental groups at a significance level of $p < 0.05$. On the other hand, bonducellin and caesalpinianone pure extract treated groups showed a statistically significant difference in tail flick response compared to the control ($p < 0.05$) (Table 3) (Fig. 10).

Capsaicin-induced pain

In the control group, the average time taken by mice was 56.84 ± 4.31 sec to lick the capsaicin-injected paw whereas

Table 4: Effects of capsaicin-induced pain in control and experimental groups

Groups	*Time (s)*
Control	56.84 ± 4.31
Leaf extract	
Pet ether	57.40 ± 3.30
Chloroform	49.72 ± 5.75
Ethanol	58.68 ± 3.39
Stem bark extract	
Pet ether	59.36 ± 4.50
Chloroform	48.51 ± 7.06
Ethanol	61.36 ± 4.50*
Pure compound	
Bonducellin	63.15 ± 3.31**
Caesalpinianone	66.12 ± 4.21**

*Significant at $p < 0.05$; ** Significant at $p < 0.01$

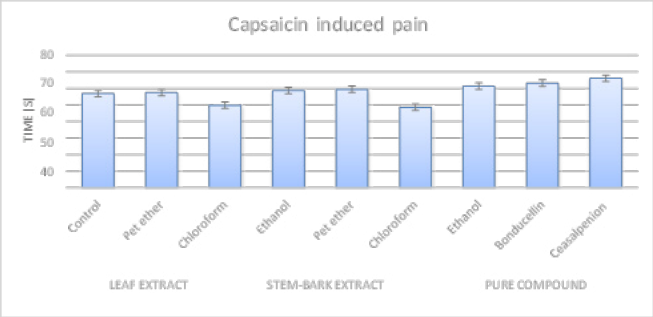

Fig. 11: Capsaicin-induced pain control and the experimental groups at different time intervals

various extracts of both leaf and stem extract showed a higher time to lick the capsaicin-injected paw as compared to the control group (Table 4, Fig. 11). Among the treated groups, mice administered ethanol stem bark extract of *C. bonducella* took more time to react than pet ether and chloroform extract of both leaf and stem bark. When compared to the control and other experimental groups, the capsaicin-induced pain responses of animals treated with bonducellin and caesalpinianone pure extract were significantly higher ($p < 0.05$). Table 3 depicts the impact of pain brought on by capsaicin in the experimental and control groups (Table 4) (Fig. 11).

Discussion

C. bonducella, an Indian plant identified by Ayurveda, the ancient medical system of India, attracts attention from researchers globally in ethnopharmacological investigations.[21] However, the absence of established quality control guidelines poses a serious barrier to the acceptance of Siddha or Ayurvedic medicines. The World Health Organisation (WHO) defines medicinal plants as those that synthesize metabolites for medications that are useful or that contain compounds that are used for therapeutic purposes.[22] The increasing emphasis on natural alternatives originates from the adverse effects linked to commercially available pharmaceuticals, such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs).[23]

In a recent study, various solvent extracts from *C. bonducella's* leaf and stem bark were prepared and evaluated for their analgesic efficacy.[24] The ethanol extracts from both portions were processed using column chromatography to separate and purify the phytochemicals. [25] These compounds were then analyzed and identified using NMR, IR, and LC-MS methods. Two chemicals, bonducellin and caesalpinianone, were isolated and identified.[26] Heat plate, tail immersion, and capsaicin-induced pain tests evaluated their analgesic effectiveness in mice. Although *C. bonducella* has been widely used in traditional medicine, there is a lack of comprehensive pharmacological investigations evaluating its therapeutic qualities. The plant, known to be a member of the Caesalpiniaceae family, has a broad distribution, particularly in India, Sri Lanka, and the Andaman and Nicobar Islands. There is ongoing confusion through similar species such as *C. bonduc* and *C. nuga*, and sometimes *C. jayoba* is inaccurately referred to as a synonym for *C. crista*. Although *C. bonducella* has been historically used, extensive pharmacological research on its medicinal potential is dearth.[27]

Our study utilized tail flick and hot plate tests to assess brain-mediated antinociception, differentiating responses through distinct neural pathways. The hot plate test, indicative of central-acting analgesics, revealed bonducellin's maximum reaction time. The sequence of inhibitory impact among fractions highlighted ethanol's predominant presence, implicating higher brain regions in mediating analgesic effects.[28]

In contrast to previous studies focused on *C. bonducella* seed kernel, our research extended to other plant parts. Isolated compounds exhibited superior activity compared to solvent extracts, indicating their crucial role in analgesic efficacy. Notably, bonducellin and caesalpinianone, two flavonoids isolated in this study, significantly contributed to analgesic potential.[29-31] In previous studies, the oil derived from *C. bonducella* seeds demonstrated anti-inflammatory, antipyretic, and analgesic properties.[32]

The present study revealed that pure compounds exhibited maximum activity compared to solvent leaf and stem bark extracts, suggesting their pivotal role in analgesic action. Flavonoids present in *C. bonducella* contribute to its potential analgesic activity. This aligns with previous findings on related compounds from close relatives of *C. bonducella.* [33]

C. bonducella exhibits various pharmacological activities, making it valuable in India's nutraceuticals sector. Our

experiment involved testing different solvent extracts of leaf and stem bark for analgesic efficacy. The isolated bioactive nutraceuticals, identified through LC-MS, IR, and NMR, displayed promising analgesic activity in various *in-vivo* models.

While the ethanol extract of stem bark demonstrated maximum analgesic activity, pure nutraceuticals surpassed this, indicating their potential as therapeutic agents for pain management. Continued comprehensive investigations are warranted to explore the plant's efficacy and adaptability in nutraceuticals. Studies utilizing MS and NMR spectroscopic techniques further demonstrated the cytotoxic potential of *C. bonducella* compounds.

Conclusion

In conclusion, *C. bonducella* holds significant potential in contemporary medicine and nutraceutical development. Further research is imperative to fully understand and harness its wide-ranging therapeutic benefits. By delving into its pharmacological characteristics and modes of action, scientists can unlock novel insights and develop innovative therapeutic approaches. Continuous inquiry into *C. bonducella* will enhance our understanding of its medicinal properties and lead to the development of safer and more effective remedies for various health conditions, ultimately improving human well-being. Additionally, efforts to standardize quality control measures for Ayurvedic medications could enhance the acceptance and utilization of *C. bonducella* in traditional and modern medicine.

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References

1. Welz AN, Emberger-Klein A, Menrad K. Why people use herbal medicine: insights from a focus-group study in Germany. BMC Complement Altern Med. 2018;18(1):92. doi:10.1186/s12906-018-2160-6.
2. Bagul MS, Rajani M. Phytochemical evaluation of classical formulation–A case study. Indian Drugs. 2005;42(1):15-19. doi:10.1234/IDR.2005.42.1.15
3. Jayakrishnan BM, Perumal N, Hashim KM. In vitro antioxidant studies and phytochemical screening on the seeds of Caesalpinia bonduc. Eur J Exp Biol. 2014;4(4):47-51. doi:10.9734/ejab/2014/8651
4. Wadkar GH. Phytochemical Studies and Hepatoprotective Activity of the Leaves of Caesalpinia Bonducella (Linn.) Flem [Dissertation]. KLE University; 2009.
5. Sagar MK, Ashok PK, Chopra H, Singh M, Upadhyaya K. Analgesic, and anti-inflammatory properties of Caesalpinia (BONDUC) Seeds. Pharm Res. 2009;1:54-59. doi:10.9734/BJPR/2012/1228
6. Kapoor LD. Hand of Ayurvedic Medicinal Plants CRC Press 88 p; vol 10; 2000.
7. Konan AB, Bleyere MN, Amonkan AK, Bouafou MKG, Datte JY. Why African traditional birth attendants used ceasalpinia bonduc leaves to facilitate childbirth in parturient women. Int J Pharm Sci Rev Res. 2014;4(1):11-16.
8. Jabbar A, Zaman MA, Iqbal Z, Yaseen M, Shamim A. Anthelmintic activity of chenopodium album (L.) and Caesalpinia crista (L.) against trichostrongylid nematodes of sheep. J Ethnopharmacol. 2007;114(1):86-91. doi:10.1016/j.jep.2007.07.027
9. Asolkar LV, Kakkar KK, Chakre OJ. To glossary of Indian medicinal plants with active principles. Ethnobotanical Database Bangladesh. 1992;1:150.
10. Kirtikar KR, Basu BD 1975. Indian medicinal plants. 2nd Edt, New Delhi. International Book Distributors.
11. Billah MM, Islam R, Khatun H, Parvin S, Islam E, Islam SA, Mia AA. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of Caesalpinia bonducella (L.) Roxb leaves. BMC complementary and alternative medicine. 2013 Dec;13:1-7. doi:10.1186/1472-6882-13-101
12. Karthikeyan M, Baskar B, Kandasamy V, Balasundaram U. Glutathione elicits enhanced biosynthesis of bonducellin, a homoisoflavonoid, in Caesalpinia bonducella leaf callus. Plant Cell Tissue Organ Cult. 2023:1-9. doi:10.1007/s11240-023-02551-1
13. Nithiyanandam S, Jaisankar V, Parthasarathy M, Katturajan R, Evan Prince S. Antioxidant mediated defensive potency of Caesalpinia bonducella nut on Acetaminophen-inebriated spleen and cardiotoxicity: Implications on oxidative stress and tissue morphology in an In vivo model.
14. Subbiah V, Nagaraja P, Narayan P, Nagendra HG. Evaluation of pharmacological properties of Caesalpinia bonducella seed and shell extract. Pharmacognosy Journal. 2019;11(1). doi:10.5530/pj.2019.11.1
15. Srinivasan P, Karunanithi K, Muniappan A, Singamoorthy A, Kadaikunnan S, Narayanan SP, Thiruvengadam M, Nagamuthu P. Botany, traditional usages, phytochemistry, pharmacology, and toxicology of Guilandina bonduc L.: a systematic review. Naunyn-Schmiedeberg's Archives of Pharmacology. 2023 Nov 21:1-29. doi:10.1007/s00210-023-02376-3
16. Sasidharan S, Kp S, Bhaumik A, Kanti Das S, Nair J H. Administration of Caesalpinia bonduc seed extracts ameliorates testosterone-induced benign prostatic hyperplasia (BPH) in male Wistar rats. Research and Reports in Urology. 2022 May 26:225-39. doi:10.2147/RRU.S120994
17. Ramadurai S, Balasundaram U. Rhizomicrobiomics of Caesalpinia bonducella, a wonder plant for PCOS treatment. Physiology and Molecular Biology of Plants. 2020 Dec;26(12):2453-63. doi:10.1007/s12298-020-00922-4
18. Kashtoh H, Baek KH. New insights into the latest advancement in α-amylase inhibitors of plant origin with anti-diabetic effects. Plants. 2023 Aug 14;12(16):2944. doi:10.3390/plants12092944
19. Rao L, Lamba AK, Kaur J, Jalwal P. AN OVERVIEW ON HERBS USED IN TREATMENT OF DIABETES. NeuroQuantology. 2022;20(19):2456. doi:10.14704/nq.2022.20.19.2456
20. Benelli G, Maggi F, Petrelli R, Canale A, Nicoletti M, Rakotosaona R, Rasoanaivo P. Not ordinary antimalarial drugs: Madagascar plant decoctions potentiating the chloroquine action against Plasmodium parasites. Industrial Crops and Products. 2017 Sep 1;103:19-38. doi:10.1016/j.indcrop.2017.03.022
21. Ahmad K, Quamari MA, Ahmad H, Hafiz KA. Phytochemical Profile and Pharmacological Activities of Karanjawa (Caesalpinia bonducella L.): An Important Botanical Origin Drug of Unani System of Medicine.
22. Hossain MA, Sharfaraz A, Hasan MI, Somadder PD, Haque MA, Sarker MR, Alam MM, Hasan AM, Sohel M, Rahman MH. Molecular docking and pharmacology study to explore bio-active compounds and underlying mechanisms of Caesalpinia bonducella on polycystic

ovarian syndrome. Informatics in Medicine Unlocked. 2022 Jan 1;33:101073. doi:10.1016/j.imu.2022.101073
23. Singh V, Raghav PK. Review on pharmacological properties of Caesalpinia bonduc L. Int J Med Arom Plants. 2012;2(3):514-30. doi:10.1016/S2221-1691(13)60091-3
24. Alamgir AN. Therapeutic use of medicinal plants and their extracts: Volume 1. Springer International Publishing AG.; 2017. doi:10.1007/978-3-319-58311-7
25. Meyer-Glitza PS, Langer T, Wieder O. Non-Steroidal Anti-Inflammatory Drug Use in Endurance Sports: Balancing Performance and Health Implications-a Literature Review.
26. Billah MM, Islam R, Khatun H, Parvin S, Islam E, Islam SA, Mia AA. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of Caesalpinia bonducella (L.) Roxb leaves. BMC complementary and alternative medicine. 2013 Dec;13:1-7. doi:10.1186/1472-6882-13-101
27. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017 Sep 22;6(4):42. doi:10.3390/plants6040042
28. Suffness M, Douros J. Drugs of plant origin. In: Methods Cancer Res. Academic Press. 1979;26:73-126.
29. Dey B, Sita Raman MV. Laboratory Manual of Organic Chemistry. S. Viswanathan Publication; 1957.
30. Del-Vechio-Vieira G, Sousa OVd, Miranda MA, Senna-Valle L, Kaplan MAC. Analgesic and anti-inflammatory properties of essential oil from Ageratum fastigiatum. Braz Arch Biol Technol. 2009;52(5):1115-1121. doi:10.1590/S1516-89132009000500008
31. Lima V, Silva CB, Mafezoli J, et al. Antinociceptive activity of the furanocoumarin seen in mice. Fitoterapia. 2006;77(7-8):574-578. doi:10.1016/j.fitote.2006.09.005
32. Shukla S, Mehta A, Mehta P, Vyas SP, Shukla S, Bajpai VK. Studies on anti-inflammatory, antipyretic, and analgesic properties of Caesalpinia bonducella F. seed oil in experimental animal models. Food and Chemical Toxicology. 2010 Jan 1;48(1):61-4. doi:10.1016/j.fct.2009.09.018
33. Olubanke Olujoke O, Wen Jun H, Jun Ting F, Guang Zhi Z, Chang Jiu J, Yu Quing Z, Joseph Abayomi O, Afolabi Akintunde A, Ning Hua T. Cytotoxic flavonoids from the young twigs and leaves of Caesalpinia bonduc [Linn] Roxb. doi:10.1155/2017/8654789

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