

Original Research Article

An Assessment of Analgesic and anti-inflammatory Activity of *Manilkara zapota* on Rat Model

Abstract:

Background: The word "herbal medicine" refers to the practice of using medicinal plants for the purpose of either preventing or treating using medicinal plants to prevent or treat sickness. This definition encompasses a broad range of practices, from the common and widespread usage of traditional medicines in every culture to the standardised and ~~titrated~~ titrated extracts of herbs.

Formatted: Font: Bold

Aims: In this study, an extract of *Manilkara zapota* was administered to rats in order to investigate the analgesic and anti-inflammatory properties of this plant to investigate this plant's analgesic and anti-inflammatory properties.

Formatted: Font: Bold

Methods: The carrageenan-induced acute inflammation technique was used to test the anti-inflammatory activity, and the acetic acid writhing and ~~tail flick~~ Tail-flick method was used to evaluate the analgesic efficacy.

Formatted: Font: Bold

Results: ~~When it came to~~ Regarding the anti-inflammatory action, the 1,000 mg/kg dosage extract was the only one that exhibited a highly significant ($p < 0.05$) result, ~~whereas. In contrast,~~ the other doses didn't show any statistically significant ~~findings~~ difference at all. None of the groups showed statistically significant results ~~in this test using the acetic acid writhing technique~~ using the acetic acid writhing technique in this test. The ~~tail flick~~ Tail-flick technique showed that a ~~dose of 1000 mg/kg~~ 1,000 mg/kg dose was statistically significant ($p < 0.05$) at both the 3 and 4 h time periods. ~~This was determined using the tail flick test. It took just 4 hours after~~

Formatted: Font: Bold

~~the 750 mg/kg dosage for the results~~The results took just 4 hours after the 750 mg/kg dosage to reach statistical significance ($p < 0.05$).

Conclusion: According to these findings, ~~M. zapota~~*M. zapota* exhibits both analgesic and anti-inflammatory efficacy when tested on rat models.

Keywords: ~~Manilkara zapota~~*Manilkara zapota*, Analgesic, Anti-inflammatory, Aspirin, Herbal medicine, Phytotherapy.

Formatted: Font: Bold

Introduction

~~Currently,~~ The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." [1]. Pain is an unpleasant sensation that is associated with sickness in humans. Acute and chronic pain, peripheral and central pain, nociceptive pain, and neuropathic pain all have inflammation and ~~the~~ inflammatory response as their root causes. White blood cells and the chemicals they release during inflammation work together to rid the body of invading germs and viruses. When there are no outside intruders present, the body's immune system may nonetheless trigger inflammation, as shown in conditions like arthritis [2]. Analgesics and anti-inflammatory pharmaceuticals include common over-the-counter medications like acetaminophen, diclofenac, ketorolac, opioids, etc. Aspirin, codeine, and morphine are the three mainstays of a standard analgesic regimen [3]. Common anti-inflammatory drugs include aspirin, ibuprofen, naproxen, and indomethacin. Inhibiting the enzyme cyclooxygenase (COX) that produces prostaglandins (PGs) is how NSAIDs work to alleviate pain and inflammation [4]. Unfortunately, these drugs also come with a host of potentially life-threatening adverse effects [5-7], including those affecting the digestive tract, the heart, the kidneys, the brain, and the neurological system. At least 100,000 people per year die from these poisons, and adverse reactions to synthetic medications account for 8% of all hospital admissions in the United States [8]. These synthetic drugs have serious adverse effects and may

be costly, so the patient may have trouble paying for the whole course of therapy. Therefore, there is a need for highly effective analgesics and NSAIDs with minimal adverse effects.

The use of plants for therapeutic purposes is as ancient as humanity. Documents, historical structures, and even the original plant remedies all attest to the long history of humankind's pursuit of medicinal substances in the natural world [9]. Phytotherapy refers to the scientific study of plants with ~~medicinal-therapeutic~~ characteristics, whereas herbalism refers to their practical use. For thousands of years, people have turned to plants as a source of medicine due to the wide variety of substances found in them. A wide variety of chemically active compounds, including phenols, glycosides, alkaloids, saponins, terpenoids, tannins, polysaccharides, flavonoids, plant lipids, resins, and essential oils, may be found in plants [10, 11]. Increasing or decreasing the concentration of the plant's chemical components by genetic modification may again give the desired medicinal effect. ~~The manufacture of secondary metabolites like alkaloids may be boosted by using reverse genetic~~ Using reverse genetics may boost the manufacture of secondary metabolites like alkaloids [12]. Traditional remedies for pain and inflammation include several herbs, including *Nigella sativa*, *Eucalyptus oil*, *Persea americana Mill*, and *Portulaca oleracea* L [13–16].

Sapodilla, sapote, chicozapote, chicoo, chicle, naseberry, and nispero are just a few of the names for the ~~Manilkara zapota~~ Manilkara zapota tree, ~~a long-lived evergreen that~~. This long-lived evergreen is native to the southwestern United States, southern Mexico, Central America, and the Caribbean. It is a member of the Sapotaceae family. The plant has been shown to have anti-inflammatory, anti-cancer, anti-tumor, and anti-arthritic properties [17–23]. Analysis of the phytochemicals in the leaves of ~~M. zapota~~ M. zapota from India showed that they had alkaloids, flavonoids, tannins, phlobatannins, triterpenes, saponins, and cardiac glycosides. Methanolic extracts of leaves, in particular, were shown to have a high total phenolic content (194.06 1.21 mg/g) and total flavonoid content (35.55 0.21 mg/g) [24, 25]. Flavonoids have been shown to have an antinociceptive effect [26, 27], with much of the research focusing on how they inhibit prostaglandin synthesis. Flavonoids, tannins, saponins, and phenolic components were found in the crude ethanol extract of ~~M. zapota~~ M. zapota leaves, which may account for the plant's demonstrated peripheral antinociceptive effect. A high concentration of flavonoids (169.37 mg quercetin equivalent per g of dry extract) suggests that the methanolic extract of ~~M. zapota~~ M.

Formatted: Font: (Default) Times New Roman, 12 pt, Italic

Formatted: Font: (Default) Times New Roman, 12 pt, Italic

zapota has anti-inflammatory properties. Bioflavonoids, also known as flavonoids, are a class of naturally occurring chemicals found in many types of plants. Both *in vitro* and *in vivo* studies have shown that these substances have anti-inflammatory effects [28].

Formatted: Font: (Default) Times New Roman, 12 pt, Italic

Formatted: Font: (Default) Times New Roman, 12 pt, Italic

Therefore, considering the ~~exploratory character of the prior work, it is worthwhile to further investigate the analgesic and anti-inflammatory activities of M. zapota leaves~~ prior work's exploratory character, further investigating the analgesic and anti-inflammatory activities of M. zapota leaves is worthwhile. This research set out to test the efficacy of ~~M. zapota~~ M. zapota leaf, in chloroform and ~~metahnolic~~ methanolic extracts, as analgesics and anti-inflammatory treatments in animal models.

Materials and Methods

Drugs, Chemicals, and Instruments

Sigma Aldrich (Germany) supplied ~~the~~ alloxan, carrageenan, acetic acid, and ethanol. Healthcare Pharmaceutical Limited (UK) provided the ibuprofen and aspirin as free samples. The anti-inflammatory and analgesic effects were measured using a plethysmometer and an analgesia ~~metre~~ meter, respectively.

Plant Collection and Extract Preparation.

The medicinal plant garden at the University of Dhaka's Faculty of Pharmacy provided the source for the ~~Manilkara zapota~~ Manilkara zapota leaf, which was subsequently authenticated and taxonomically identified. The plant specimens were stored ~~in accordance with~~ following the regulations of the Bangladesh National Herbarium. For future reference, the 7–10-~~d~~ day shade-dried and then roughly ~~pulverised~~ pulverized leaf was given the accession number 47380 by the herbarium authorities on 11-2-2019. The powdered leaves were soaked in 70% ethanol and ~~S~~ shaken violently for ~~the whole of the~~ 96 hours, ~~the powdered leaves were soaked in 70% ethanol. When the extract was done soaking, it was~~ The extract was soaked and filtered, and the resulting liquid was saved. The concentrated extract was then ~~filtered~~ desiccated using a rotary evaporator. ~~At last, the concentrated extract was dried~~ and put away for later use.

Formatted: Font: (Default) Times New Roman, 12 pt, Italic

Experimental Animal Handling

Male Wistar rats weighing between 125 and 200 g were obtained from the Jahangirnagar University Zoology Department in Bangladesh and housed at the University of Dhaka's Institute of Nutrition and Food Science on a 12:12 light:dark cycle with a constant temperature of 25 °C ~~degrees Celsius~~. Before beginning the experiment, the rats were maintained ~~here to acclimatise~~ acclimate; therefore, standard pellet food and fresh water were supplied ~~on a daily basis~~ daily. ~~In accordance with~~ All rat experiments were conducted following the recommendations of the Institutional Animal Ethics Committee (IEAC) ~~the recommendations of the Institutional Animal Ethics Committee (IEAC), all rat experiments were conducted~~. The Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT) provided rules for how animals should be cared for and used in scientific research.

Experimental Guidelines: All studies were conducted in conformity with the 2013 Declaration of Helsinki's ethical guidelines [29].

Experimental Design: Individual rats were weighed to determine their body weight, and then the animals were split into groups (Table 1) with an equal distribution of rodents according to their body weight and five rats in each group.

Evaluation of Anti-I-Inflammatory Activity:

Carrageenan was used to induce inflammation in the rats to examine the anti-i-inflammatory activity of the reference drug and the extract of ~~Manilkara zapota~~ Manilkara zapota.

Table 1: Group specification for anti-i-inflammatory activity

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Carrageenan Control	N/A	N/A	Car
2	Carrageenan + Ibuprofen	Ibuprofen	10	Car+Ib ₁₀
3	Carrageenan + Manilkara zapota <u>Manilkara zapota</u>	Manilkara zapota <u>Manilkara zapota</u>	500	Car+MZ ₅₀₀
4	Carrageenan + Manilkara <u>Manilkara</u>	Manilkara <u>Manilkara</u>	750	Car+MZ ₇₅₀

	zapota <u>Manilkara zapota</u>	zapota <u>Manilkara zapota</u>		
5	Carrageenan + <u>Manilkara zapota</u>	<u>Manilkara zapota</u>	1000	Car+MZ ₁₀₀₀

Carrageenan-Induced Acute Inflammatory Model:

Carrageenan-induced rodent paw edema testing is the standard method for determining an anti-inflammatory agent's efficacy. The anti-inflammatory test was conducted using a plethysmometer, ~~a specialised kind of device~~. The next step was to measure the size of each rodent's paw. Subplanar tissue of the left rear paw rat was injected with 1% of the newly manufactured carrageenan solution at ~~a dosage of~~ 0.1 mL per 100 g body weight to stimulate edema. After that, ~~onean~~ hour was allotted. Then, the test medication and extracts were administered to rats in ~~a variety of various~~ dosages. The ~~volume of the paw~~ paw volume was measured using a plethysmometer between 0 and 6 hours following Carrageenan infusion. The subsequent formula [30, 31] was then used to calculate the rate of edema obstruction.

$$\text{Percentage Inhibition} = \frac{V_{pc} - V_t}{V_{pc}} \times 100$$

Here,

VPC = volume of animals' paws in Positive Control rat

V₀ = volume of animals' paws in Treatment Group

Evaluation of analgesics activity:

The rodent is stimulated with pain through the acetic acid-induced writhing test and tail-flick method.

Table 2: Group specification for analgesic activity by acetic acid writhing method

Group	Group Specification	Treatment	Dose	Abbreviation	of
-------	---------------------	-----------	------	--------------	----

Number		species	Treatment species (mg/kg)	Groups
1	Acetic Acid Control	Physiological Saline	10ml/kg	Ace
2	Aspirin +Acetic Acid	Aspirin	100	As ₁₀₀ +Acetic Acid
3	<i>Manilkara zapota</i> + Acetic acid	<i>Manilkara zapota</i>	500	MZ ₅₀₀ +Acetic Acid
4	<i>Manilkara zapota</i> + Acetic acid	<i>Manilkara zapota</i>	750	MZ ₇₅₀ +Acetic Acid
5	<i>Manilkara zapota</i> + Acetic acid	<i>Manilkara zapota</i>	1000	MZ ₁₀₀₀ +Acetic Acid

Acetic acid-induced writhing test

The acetic acid-induced writhing technique was used to test for peripheral analgesic activity. In the 30 minutes before the intraperitoneal injection of acetic acid, several test samples were given. The rats were given an intraperitoneal injection of 0.9% acetic acid (10 ml/kg) while being exposed to unpleasant stimuli. Over the course of 20 minutes beginning immediately after acetic acid injection, the number of writhes (muscle contraction ~~ionstwists~~) was counted. The percentage of writhing inhibition was determined by counting the number of times an animal contracted its abdominal muscles, drew its hind limbs towards its abdominal walls, stretched its hind limbs, and periodically arched its back for twenty minutes. Equation [32] was used to figure out determine what percentage of writhes represented analgesic action.

$$\left\{ \frac{A. \text{ Control mean} - \text{ Treatment mean}}{A \text{ Control mean}} \right\} \times 100$$

Where T Control = the mean number of the writhing of each test group

A Control = The mean number of the writhing of the acetic acid control group

The analgesic activity of the extract is then also assessed via the "Tail Flick Tail-flick Method" on the same experiment rat model after giving a break for seven days. The effect of injected acetic was terminated by this time.

Table 3: Group specification for analgesic activity by tail flick Tail-flick method

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Tail Flick Tail-flick Stress (control)	Physiological Saline	10ml/kg	TFS
2	Aspirin + Tail Flick Tail-flick Stress	Aspirin	100	As ₁₀₀ +TFS
5	Manilkara zapota Manilkara zapota + Tail Flick Tail-flick Stress	Manilkara zapota Manilkara zapota	500	GP ₅₀₀ +TFS
6	Manilkara zapota Manilkara zapota + Tail Flick Tail-flick Stress	Manilkara zapota Manilkara zapota	750	GP ₇₅₀ +TFS
7	Manilkara zapota Manilkara zapota + Tail Flick Tail-flick Stress	Manilkara zapota Manilkara zapota	1000	GP ₁₀₀₀ +TFS

Tail flick Tail-flick method

The behavioural reaction of animals to painful stimuli is evaluated using a nociceptive test called the tail-flick experiment, which ~~was first outlined by Love and Smith~~ Love and Smith first outlined in 1941 [33]. A tail-flick analgesia ~~metre-meter~~ (UGO BASILE®, Germany) ~~programmed with radiant heat was used~~ was programmed with radiant heat to determine the lag time between ~~exposure to the stimuli and the onset of the avoidance reaction~~ stimuli exposure and avoidance reaction onset. A constant current of 4 ~~a~~ Amps was supplied through the exposed nichrome to get it up to the proper temperature, and the heat controls helped with this. By applying radiant heat to the rats' tails in the ~~centre~~ center, we may make them feel pain. ~~For both~~

~~untreated and treated rats, the time required to exhibit a tail flick reflex was recorded~~The time required to exhibit a tail-flick reflex was recorded for both untreated and treated rats. After administering test compounds to the animals, the experiments were conducted at 0, 15, 30, 45, and 60 minutes.

Statistical analysis:

All of our results (raw data) were recorded and ~~analysed~~analyzed on a spreadsheet in MS Excel, and they fall into multiple categories covering a wide range of study factors. Descriptive statistics were applied to the data, and the results are shown as a mean SD. The statistical significance of the observed variation between groups was assessed using the "One_Way ANOVA~~nova~~ Test" function in SPSS 1600. So long as the ~~'p'~~p-value ~~wasis~~ was less than 0.05 ($p < 0.05$), we classified the occurrences as statistically significant.

Results:

The data was expressed as time and percent inhibition. The higher dose extract only showed highly significant ($p < 0.05$) results at the 4 hours, and the other doses ~~didn't~~ didn't show statistically significant results.

Table 4: Anti-inflammatory activity of ~~M. zapota~~*M. zapota* extract and ~~Ibuprofen~~ibuprofen through paw edema test in a rat model (* presents the ~~level of significanees~~significance level of result).

Group	Time μ L				
	0 Minute (Just before carrageenan injection)	1 hour (just before treatment)	2 Hours	3 Hours	4 Hours
Car	118.26 \pm 3.94	113.34 \pm 5.86	140.22 \pm 5.59	146.37 \pm 6.10	152.49 \pm 5.45
Car+Ib₁₀	115.20 \pm 4.04	133.21 \pm 6.40	127.41 \pm 5.40	121.29 \pm 4.93	117.46 \pm 4.10
Car+MZ₅₀₀	119.29 \pm 5.43	135.20 \pm 5.99	139.37 \pm 6.61	135.73 \pm 4.19	131.97 \pm 5.21

			0.61%	7.27%	13.46%
Car+MZ₇₅₀	114.39±3.44	133.20±4.19	136.96±6.22	132.14±5.96	128.36±4.44
			2.32%	9.72%	15.82%
Car+MZ₁₀₀₀	116.30±4.16	134.69±5.71	134.08±5.10	128.31±5.19	121.90±6.14
			4.38%	12.33%	20.06%

Analgesic Activity of *Manilkara zapota* *Manilkara zapota*:

Writhing test: The result of the acetic acid writhing test is shown below in ~~table~~ Table 2. No groups showed statistically significant results in this test.

Table 5: Analgesic effect of different doses of *Manilkara zapota* *Manilkara zapota* and Aspirin aspirin by acetic acid writhing test (*presents the ~~level of significance~~ significance level of result).

Group specification	Dose	Number of writhing	% Inhibition
Ace		93.71±6.42	
As₁₀₀+Acetic Acid	100	70.42±5.84	
MZ₅₀₀+Acetic Acid	500	92.46±7.14	1.33%
MZ₇₅₀+Acetic Acid	750	92.21±6.19	1.6%
MZ₁₀₀₀+Acetic Acid	1000	89.19±5.39	4.82%

~~Tail flick~~ Tail-flick test (TFS): Table 3 shows the ~~outcomes of the exam~~ exam outcomes. Treatment with MZ improved the pain threshold in a dose-dependent way in ~~both~~ non-diabetic and diabetic rats; however, the impact was less than that of the ~~gold-gold~~ standard medication, aspirin. Only 4 hours after the 750 mg/kg dosage showed statistically significant results (p<

0.05). Statistical significance ($p < 0.05$) was seen at ~~both the 3 and 4 hour time points for a dosage of 1000 mg/kg~~the 3- and 4-hour time points for a 1,000 mg/kg dosage.

Table 6: Analgesic activity of ~~Manilkara zapota~~*Manilkara zapota* and ~~Aspirin~~*aspirin* by the tail-flick test method.

Group No	Group Specification	Basal Reaction	Reaction time in second			
			After 30 minutes	After 1 Hour	After 2 Hour	After 4 Hour
1	TFS	3.93±0.86	4.11±0.93	4.24±1.01	4.45±0.93	4.76±0.80
2	As ₁₀₀ +TFS	3.44±0.77	3.50±0.84	3.73±0.89	3.89±0.69	4.10±0.96
3	MZ ₅₀₀ +TFS	3.77±0.81	3.96±0.73	4.14±0.84	4.02±0.91	4.19±0.86
4	MZ ₇₅₀ +TFS	3.99±0.94	4.21±0.91	4.49±0.81	4.87±0.95	5.53±1.01
5	MZ ₁₀₀₀ +TFS	3.92±0.92	4.47±0.87	4.99±0.94	5.86±0.84	6.24±0.91

Discussion:

The healing potential of plants has been known for a long time. Traditional herbal therapy has been used by indigenous peoples all over the globe for hundreds of years to ~~eure-treat a wide variety of various~~ illnesses. In this research, we looked at the effectiveness of *Manilkara zapota* leaves as an analgesic and ~~as an anti-~~inflammatory. At a time interval of 4 hours, the effects of a dosage of 1,000mg/kg on anti-inflammatory activity were statistically significant ($p < 0.05$). However, no statistically significant differences were seen with any other dosage. High levels of flavonoids [34, 35] are responsible for their potent anti-inflammatory actions. Two investigations using ~~Manilkara zapota~~*Manilkara zapota* reported similar outcomes [19, 36]. The number of writhing rats in the acetic acid writhing technique dropped across all tested dosages, although this trend was not statistically significant relative to the positive control groups. In the ~~tail-tail-~~flick test, 1,000 mg/kg was ~~shown to be~~ statistically significant ($p < 0.05$) at both the 3 and 4 ~~time~~ periods. Statistical significance ($p < 0.05$) was seen only 4 hours after the 750 mg/kg dose. Previous investigations [37, 38] indicate that the high alkaloid and flavonoid content is responsible for the pain-relieving effects. Further research revealed the same ~~thing~~ about ~~Manilkara zapota~~*Manilkara zapota* [39, 40].

More research is needed to pinpoint the specific molecule responsible for the analgesic and anti-inflammatory effects of *Manilkara zapota*.

Conclusion:

~~This study demonstrated, using a rat model and varying doses of ethanolic extract and reference medications.~~ Using a rat model and varying doses of ethanolic extract and reference medications, this study demonstrated that the *Manilkara zapota* plant leaf extract has ~~had~~ anti-inflammatory and analgesic properties. More study is required to identify the component of the extract responsible for the intended effect. The next step in the research process is to isolate the active compounds.

References:

1. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, et al. (2020) The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain* 161(9): 1976-1982.
2. Rupak MA, Chowdhury MM, Shurovi FS, Ferdous J, Tahsin MR, Sarif S, Hasan MM, Chowdhury JA, Kabir S, Chowdhury AA, Aktar F. An Evaluation of Analgesic and Anti-inflammatory Activity of Ethanolic Extract of *Cynodon Dactylon* on Stressed Rodent Model. *Biomedical Journal of Scientific & Technical Research*. 2022;42(3):33550-7.
3. Twycross RG. Analgesics. *Postgrad Med J*. 1984 Dec;60(710):876-80. doi: 10.1136/pgmj.60.710.876. PMID: 6514647; PMCID: PMC2418085.
4. Munir MA, Enany N, Zhang JM. Nonopioid analgesics. *Anesthesiol Clin*. 2007 Dec;25(4):761-74, vi. doi: 10.1016/j.anclin.2007.07.007. PMID: 18054143.
5. Ricardo Buenaventura, M., Rajive Adlaka, M., & Nalini Sehgal, M. (2008). Opioid complications and side effects. *Pain physician* *Physician*, 11, S105-S120.
6. Bjarnason, I., & Hayllar, J. (1993). Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology*, 104(6), 1832-1847.

7. Lazzaroni, M., & Bianchi Porro, G. (2004). Gastrointestinal side-effects of traditional non-steroidal anti-inflammatory drugs and new formulations. *Alimentary pharmacology & therapeutics*, 20, 48-58.
8. Aye, M. M., Aung, H. T., Sein, M. M., & Armijos, C. (2019). A review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected Myanmar medicinal plants. *Molecules*, 24(2), 293.
9. Petrovska BB. Historical review of medicinal plants' usage. *Pharmacogn Rev.* 2012 Jan;6(11):1-5. doi: 10.4103/0973-7847.95849. PMID: 22654398; PMCID: PMC3358962.
10. Yang L, Stöckigt J (2010) Trends for diverse production strategies of plant medicinal alkaloids. *Natural product reports* 27(10): 1469-1479.
11. Saxena M, Saxena J, Nema R, Singh D, Gupta A (2013) Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry* 1(6): 168-182.
12. Shendye, N. V., & Gurav, S. S. (2014). *Cynodon dactylon*: A systemic review of pharmacognosy, phytochemistry and pharmacology. *Int J Pharm Pharm Sci*, 6(8), 7-12
13. Ghannadi A, Hajhashemi V, Jafarabadi H. An investigation of the analgesic and anti-inflammatory effects of *Nigella sativa* seed polyphenols. *Journal of medicinal food*. 2005 Dec 1;8(4):488-93.
14. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ. Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. *Journal of ethnopharmacology* *Ethnopharmacology*. 2003 Dec 1;89(2-3):277-83.
15. Adeyemi OO, Okpo SO, Ogunti OO. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia*. 2002 Aug 1;73(5):375-80.
16. Chan K, Islam MW, Kamil MA, Radhakrishnan R, Zakaria MN, Habibullah M, Attas A. The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. *sativa* (Haw.) Celak. *Journal of ethnopharmacology* *Ethnopharmacology*. 2000 Dec 1;73(3):445-51.

Formatted: Font: (Default) Times New Roman, 12 pt, Portuguese (Brazil)

Formatted: Portuguese (Brazil)

Formatted: Font: (Default) Times New Roman, 12 pt, Portuguese (Brazil)

17. Chanda SV, Nagani KV. Antioxidant capacity of ~~Manilkara zapota~~Manilkara zapota L. leaves extracts evaluated by four in vitro methods. Nature and science. 2010;8(10):260-6.
18. Kaneria M, Chanda S. Evaluation of antioxidant and antimicrobial properties of ~~Manilkara zapota~~Manilkara zapota L.(chiku) leaves by sequential soxhlet extraction method. Asian Pacific Journal of Tropical Biomedicine. 2012 Jan 1;2(3):S1526-33.
19. Ganguly A, Al Mahmud Z, Uddin MM, Rahman SA. In-vivo anti-inflammatory and anti-pyretic activities of ~~Manilkara zapota~~Manilkara zapota leaves in albino Wistar rats. Asian Pacific Journal of Tropical Disease. 2013 Aug 1;3(4):301-7.
20. Ganguly A, Al Mahmud Z, Kumar Saha S, Abdur Rahman SM. Evaluation of antinociceptive and antidiarrhoeal properties of ~~Manilkara zapota~~Manilkara zapota leaves in Swiss albino mice. Pharmaceutical biology. 2016 Aug 2;54(8):1413-9.
21. Ganguly A, Rahman SA. Evaluation of the cytotoxic, antimicrobial, antioxidant, anthelmintic and CNS depressant activities of ~~Manilkara zapota~~Manilkara zapota leaf (Sapotaceae). World Journal of Pharmaceutical Research. 2014 Oct 31;4(1):272-83.
22. Khalek MA, Khatun Z, Habib MR, Karim MR. Antitumor activity of ~~Manilkara zapota~~Manilkara zapota (L.) fruits against Ehrlich ascites carcinoma in mice. Biologija. 2015 Dec 22;61(3-4).
23. Singh M, Soni P, Upmanyu N, Shivhare Y. In-vitro anti-arthritis activity of ~~Manilkara zapota~~Manilkara zapota Linn. Asian Journal of Pharmacy and Technology. 2011;1(4):123-4.
24. Yong KY, Shukkoor MS. ~~Manilkara Zapota~~Manilkara zapota: A phytochemical and pharmacological review. Materials Today: Proceedings. 2020 Jan 1;29:30-3.
25. Islam MR, Parvin MS, Banu MR, Jahan N, Das N, Islam ME. Antibacterial and phytochemical screening of ethanol extracts of ~~Manilkara zapota~~Manilkara zapota leaves and bark. Int J Pharma Sci. 2013;3:394-97.
26. Jain AK, Jain CP, Gaur K, Jain A, Nema RK. 2010. Evaluation of antinociceptive and anti-inflammatory activity of leaves of *Cassia grandis* (L.). Int J Pharm Clin Res. 2:106–108.

27. Mulla WA, Kuchekar SB, Thorat VS, Chopade AR, Kuchekar BS. 2010. Antioxidant, antinociceptive and anti-inflammatory activities of ethanolic extract of leaves of *Alocasia indica* (Schott.). *J Young Pharm.* 2:137–143.
28. Vasudevan M, Gunman KK and Parle M: Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. *J Ethnopharmacol* 2007; 109: 264-270
29. Tahsin MR, Tithi TI, Mim SR, Haque E, Sultana A, Bahar NB, Ahmed R, Chowdhury JA, Chowdhury AA, Kabir S, Aktar F, Uddin MS, Amran MS. In Vivo and In Silico Assessment of Diabetes Ameliorating Potentiality and Safety Profile of *Gynura procumbens* Leaves. *Evid Based Complement Alternat Med.* 2022 Jan 19;2022:9095504. doi: 10.1155/2022/9095504. PMID: 35096119; PMCID: PMC8791719.
30. C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, *Proc. Soc. Exp. Biol. Med.* 111 (3) (Dec. 1962) 544–547.
31. O. O. Adeyemi, S.O. Okpo, O.O. Ogunti, Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae), *Fitoterapia* 73 (5) (Aug. 2002) 375–380.
32. S. Ahmed, A. Naved, R. A. Khan, and S. Siddiqui, 'Analgesic Activities of Methanol Extract of *Terminalia chebula* Fruit', *Pharmacol. Amp Pharm.*, vol. 6, no. 12, Art. no. 12, Dec. 2015, doi: 10.4236/pp.2015.612056
33. F. E. D'amour and D. L. Smith, 'A Method for Determining Loss of Pain Sensation', *J. Pharmacol. Exp. Ther.*, vol. 72, no. 1, pp. 74–79, May 1941
34. Falodun A, Okunrobo LO, Uzoamaka N (2006) Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn (*Euphorbiaceae*). *African Journal of Biotechnology* 5(6): 529-531.
35. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, et al. (2009) Mechanism of action of flavonoids as anti-inflammatory a

36. Konuku K, Karri KC, Gopalakrishnan VK, Hagos Z, Kebede H, Naidu TK, Noyola PP, Palleti JD, Rao Duddukuri GR. Anti-inflammatory activity of ~~Manilkara zapota~~*Manilkara zapota* leaf extract. Int J Curr Pharm Res. 2017 Jul 14;9(4):130-4.
37. Abdullahi MH, Anuka JA, Yaro AH, Musa A (2014) Analgesic and anti-inflammatory effects of aqueous leaf extract of Combretum micranthumg.Don (Combretaceae). Bayero Journal of Pure and Applied Sciences 7(2):78-82.
38. Uche FI, Aprioku JS (2008) The Phytochemical Constituents, Analgesic and Anti-inflammatory effects of methanol extract of Jatropha curcas leaves in Mice and Wister albino rats. Journal of Applied Sciences and Environmental Management 12(4).
39. Yong KY, Shukkoor MS, Chin JH. Analgesic activity of chloroform and methanolic leaf extracts of ~~Manilkara zapota~~*Manilkara zapota*. Materials Today: Proceedings. 2020 Jan 1;29:20-5.
40. Sultana F, Chowdhury MA, Hossain MT, Imran-Ul-Haque M. In vivo assay of analgesic activity of methanolic and petroleum ether extracts of ~~Manilkara zapota~~*Manilkara zapota* leaves. British Journal of Pharmaceutical Research. 2014 Jan 15;4(2):186.