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Comparative Study of Analgesic effect of Different Solvent Fraction of Alcoholic Extract of Psidium Guajava leaves on mice

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ABSTRACT
Background and objectives: The unrivalled substitute to synthetic medicines, available, for relieving of pain, are the natural
products found in plants. They are known to exhibit a variety of activities. The objective of the present study is screening of P.
guajava for its analgesic activity.
Material and methods: The methanol extracts of the leaves of P. guajava were tested three different pain models viz. Hot
plate and Acetic acid induced pain models in mice.
Results and discussion: The treatment of P. guajava at varying doses (100 mg/kg and 200 mg/kg) significantly (P>0.001)
reduced the pain induced by Hot plate (77.56%), Tail immersion (77.18%) and Acetic acid induced abdominal contraction
(56.75%) respectively and the potency was found to be equivalent as compared to the standarddrug,Pentazocin,Diclofenacsodium. The presence of flavonoids, tanins and saponins present in the extracts of P. guajava maybe accountable for the analgesic property manifested.
Conclusion: The results further suggest that P. guajava possess analgesic effect which might act through central and peripheral mechanisms.
Keywords: Psidium guajava, Pentazocin, Diclofenacsodium, flavonoids, Saponins, Acetic acid.

I. INTRODUCTION

Pain is an unpleasant sensation that can be acute or persistent and is the result of complicated neurochemical processes in the peripheral and central nervous system(1). Chronic pain and inflammatory disorders are two of the world's most serious health issues(2). When living tissues are injured, they respond with pain and inflammation(3). The wounded tissue initiates a complex enzymatic response that results in the production of mediators such as prostaglandins, fluid extravasation, cell migration, and tissue repair(4). Pain and inflammation management is one of clinical medicine's most difficult tasks(5). The majority of medications, such as NSAIDs and narcotic analgesics, are now commonly used to treat pain and inflammation(6). Medication dependence established by narcotic type analgesics as a result of side effects such as stomach ulcers produced by NSAIDs and drug tolerance(7). Most analgesic medicines, such as NSAIDs and opiates, have not been successful in all types of people due to the development of unwanted effects(5). As a result, novel analgesic drugs with similar side effects are being developed around the world as alternatives to NSAIDs and opioids(3). Most herbal medications, on the other hand, relieve pain and inflammation and have been proven to be both safe and effective in clinical trials(8). Herbal extracts have been found to generate a challenging effect in the treatment of pain and inflammation, making them one of the most appealing sources of new medications(9). Guajava (Psidium guajava Linn) is a plant that is frequently utilised in Indian folk medicine(10). Gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothaches, coughs, sore throats, swollen gums, and a variety of other ailments are all treated with extracts from the roots, bark, and leaves of this plant(9). The analgesic impact of P. guajava leaf extracts in various pain models is investigated in this study(11).

II. MATERIALS AND METHODS

Drugs and Chemicals

Diclofenac sodium and Pentazocin , methanol, acetic acid were used in this study(1). All substances were prepared immediately before use and the reagents were used as analytical grade(3).

Plant Materials

The leaves of P. guajava used in this study were collected from Hooghly (West Bengal, India.)(4). The plant was authenticated by
Botanical garden, West Bengal, India(12).

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Extract preparation

P. guajava leaves were shade dried and coarsely powdered(7). The powdered materials were extracted with methanol(4). The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator(10). The dried extract was weighed and then kept at -4ºC until ready for use(3). The yield of the extract was 26.4 % (w/w). In each experiment, the extract was diluted with water to desired concentration(1).

Phytochemical screening

A Preliminary phytochemical screening of P. guajava was conducted to determine the presence or absence ofalkaloids, tannins, phenols, saponins, volatile oil, ascorbicacid, carbohydrates and glycosides by suitable methods(5).

Animals

Adult male albino mice weighing about 20-25g were used in this study(8). They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum(6). The study was approved by the Institutional Ethical Committee, which follows theguidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPSCEA)(2).

Pain

Pain is described as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage," according to the International Association for the Study of Pain (IASP)(9). Pain is derived from the Latin word poena, which means punishment, fine, or penalty(10). Analgesia is described as a condition of reduced pain awareness, whereas analgesics are drugs that reduce pain perception by raising the threshold to painful stimuli(5).

Pain causes various different symptoms like –

- Restlessness
- Anxiety
- Fear
- Depression
- Spasm of abdominal wall
- Increased secretion of abdominal walls and catecholamine.

It can also cause a reduction in cerebral function, a restriction in mobility of the affected area, blood stasis, and muscular weakness(9).

Various effects of pains are-

- Physical effects ofpain
- Psychological effects of pain

Types of pain:

Various types of pain are-

- **Acute pain**
- **Chronic pain**
- **Neuropathic pain**
- **Visceral pain**
- **Cutaneous pain**
- **Somatic pain**

Acute pain:-

Acute pain has a recent onset and a brief duration(3). Acute pain is perceived very quickly, usually within 0.1 seconds of a stimulus being raised. Fast or sharp pain is another name for it(13). Acute pain serves as a warning system for the body(14). It means something is incorrect or you're in danger of getting hurt(5). Acute pain is not sensed in the body's deeper tissues(3). Acute pain is characterised by its fast onset and severity, yet the strength of the pain can range from minor to severe(8). Surgery, medical sickness such as myocardial infarction and sickle cell crisis, musculoskeletal pain such as rheumatoid arthritis, cancer, trauma, bums, and labour pain are all associated with acute pain(1).

Chronic pain:-

After a stimulus is raised, the experience of chronic pain develops within a second or more(10). After that, it progressively grows in intensity over a few seconds or minutes(9). It is mostly transmitted through slow-conducting type C fibres(5). Pain that lasts longer than three months is referred to as chronic pain(6). This pain lasts for more than three months and is caused by chronic conditions such as arthritis, cancer, migraine, headache, diabetic neuropathy, and nerve irritation(11).

Neuropathic pain:-

Neuropathic pain is linked to a lower quality of life and is frequently mismanaged(9). Around 7–8% of adults suffer from pain that has neuropathic characteristics(6). Neuropathic pain affects a quarter of patients with diabetes and 35% of those with HIV(1).

Visceral pain:-

Visceral pain is a diffuse type of pain that usually refers to nonvisceral tissues and isn't always linked to organ injury(14). Two nerves innervate each organ, with certain functions overlapping but, more significantly, different functions(5). Multiple modalities of stimulation, including visceral nociceptors, are commonly sensitive to sensory endings in viscera(14). Inflammation and disease sensitise visceral nociceptors, but so do low-threshold, non-

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nociceptor mechanosensors(9). Inflammation and disease sensitise visceral nociceptors, but so do low-threshold, non-nociceptor mechanosensors(6). Central sensitization, which may persist in functional visceral diseases, is revealed by expanded areas of referred visceral sensations and discomfort in the referral location, implying dysregulation of central, endogenous pain modulatory systems(12).

Cutaneous pain:-

Cutaneous discomfort is caused by injury to the skin or superficial tissues(1). Cutaneous nociceptors are nociceptors that live just beneath the skin's surface(13). Because of the high concentration of nerve endings, it creates a well-defined, localised pain with a short duration(9). Cuts on paper, little cuts, and minor burns are all examples of cutaneous pain(5).

Somatic pain:-

This term comes from the Greek and means "body"(13). Pain that is evoked by nociceptive input from any of the tissues that make up the body's structure is known as somatic pain(6). The bones, muscles, joints, ligaments, and tendons of the spine, trunk, and limbs would be included, but technically, the skull, the pachymeningeal coverings of the brain and spinal cord, and the teeth would also be included(5).Pain that is evoked by nociceptive input from any of the tissues that make up the body's structure is known as somatic pain(10). Somatic pain, on the other hand, is a phrase used to describe pain that does not originate in the body's viscera, or internal organs(7). Pain induced by particular structures is referred to as visceral nociception and pain(5). A headache is defined as pain in the brain or skull, while dental discomfort is defined as pain in the teeth(11). As a result, somatic pain is essentially limited to musculoskeletal systems such the limbs, spine, chest wall, and abdominal wall(9).

Pain Receptors

Nociceptive pain is defined as pain that is responsive to noxious stimuli (nocer - to injure or hurt in Latin)(5). Noxious stimuli are those that cause tissue injury and cause nociceptors to fire(3). Nociceptors are sensory receptors that sense signals from damaged tissue or the danger of injury, as well as substances generated by the damaged tissue, indirectly(9). The free (bare) nerve endings found in the skin, muscle, joints, bone, and viscera are known as nociceptors(8). Transient receptor potential (TRP) channels have recently been discovered in nerve endings, which feel and identify injury(13).TRP channels have six transmembrane domains with a pore between domains 5 and 6, comparable to voltage-gated potassium channels or nucleotide-gated channels(14). They convert a wide range of painful inputs into receptor potentials, which then trigger action potentials in pain nerve fibres(1). This action potential travels to the spinal cord, where it forms a synaptic connection in lamina I and/or II(3). Nociceptor cell bodies are mostly found in the dorsal root and trigeminal ganglia(11). Inside the CNS, there are no nociceptors(9).The sensitivity of nociceptors varies(2). They are classified into numerous groups based on how they react to mechanical, thermal, and/or chemical stimulation released by the injury, tumour, and/or inflammation(8).

Skin Nociceptors:-

Based on their function, skin nociceptors can be categorised into four groups(3). High threshold mechanonociceptors, also known as particular nociceptors, are the first category(1). Only strong mechanical stimulation, such as pinching, cutting, or stretching, causes these nociceptors to respond(4).

Joint Nociceptors:-

High-threshold mechanoreceptors, polymodal nociceptors, and "silent" nociceptors are all found in the joint capsules and ligaments(5). Many of the fibres that innervate these joint capsule ends contain neuropeptides including substance P (SP) and calcitonin gene-related peptide (CGRP)(6). The release of these peptides is thought to play a role in the onset of inflammatory arthritis(7).

Visceral Nociceptors:-

Mechanical pressure, temperature, chemical, and silent nociceptors are all found in visceral organs(13). The visceral nociceptors are dispersed, with several millimetres between them and several centimetres between each nociceptor in some organs(15). Many visceral nociceptors are deafeningly quiet. Different pathways carry unpleasant information from the visceral organs and skin to the CNS(1).

Silent Nociceptors:-

Additional nociceptors called "silent" or "sleep" nociceptors exist in the skin and deep tissues(7). These receptors are ordinarily insensitive to painful mechanical stimulation, but they become “awakened” (responsive) to it during inflammation and tissue injury(6).

Factors that activate Nociceptors:

- Globulin and protein kinases.

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- Arachidonic acid
- Histamine
- Nerve growth factor (NGF)
- Substance P (SP) and calcitonin gene-related peptide (CGRP)
- Potassium - K^+
- Serotonin (5-HT), acetylcholine (ACh), low pH (acidic) solution, and ATP
- Muscle spasm and lactic acid

Globulin and protein kinases:-

Damaged tissue is thought to release globulin and protein kinases, which are thought to be some of the most active pain-producing chemicals(3).

Arachidonic acid:-

One of the molecules released during tissue injury is arachidonic acid(5). It is then converted to prostaglandins (and cytokines)(2). The prostaglandins' effect is mediated by a G protein-protein kinase A pathway(14). Prostaglandins inhibit potassium efflux from nociceptors in response to injury, resulting in more depolarization(3). The nociceptors become more sensitive as a result of this(4).

Histamine:-

Mast cells are triggered by tissue injury to release histamine into the surrounding region(9). The nociceptors are stimulated by histamine. Pain is elicited by subcutaneous injections of histamine(5).

Nerve growth factor (NGF):-

The release of NGF is triggered by inflammation or tissue injury(10). NGF then binds to TrkA receptors on nociceptors' surfaces, causing them to become activated. Pain is elicited by subcutaneous injections of NGF(1).

Substance P (SP) and calcitonin gene-related peptide (CGRP) :-

Injury causes the release of substance P (SP) and calcitonin gene-related peptide (CGRP)(14). Inflammation of tissue damage causes the release of SP and CGRP, which stimulates nociceptors(2). Pain is elicited by a minute subcutaneous injection of substance P and CGRP(12). Both peptides cause vasodilation, which causes edoema to spread surrounding the initial injury(9).

Potassium - K^+ :-

Extracellular K+ levels rise as a result of most tissue injury(15). Pain intensity and local K+ concentration have a strong relationship(1).

Serotonin (5-HT), acetylcholine (ACh), low pH (acidic) solution, and ATP:-

When tissue is damaged, these chemicals are released(14). Nociceptors are stimulated by subcutaneous injections of these compounds in minute quantities(3).

Muscle spasm and lactic acid :-

Not only may smooth muscle spasms cause headaches, but ligament stretching can also cause discomfort(2). Lactic acid concentration rises and discomfort is generated when muscles are overactive or when blood supply to a muscle is limited(9).

Basic mechanism of pain

When noxious stimuli are present, the basic pain mechanism goes through three steps: transduction, transmission, and modulation(4). For example, along the nociceptive pathway, transduction occurs in the following order: (1) stimulus events are converted to chemical tissue events; (2) chemical tissue and synaptic cleft events are then converted to electrical events in neurons; and (3) electrical events in neurons are transduced as chemical events at synapses(9). Transmission would be the next mechanism once transduction was completed(1).

It occurs when electrical events are sent along neural pathways, and neurotransmitters in the synaptic cleft transport information from one cell's post-synaptic terminal to another cell's pre-synaptic terminal(9). Meanwhile, modulation occurs at all levels of nociceptive pathways via up- or down-regulation of the primary afferent neuron, DH, and higher brain center(13). All of this leads to the same conclusion: the pain pathway has been launched and finished, allowing us to experience the unpleasant sensation elicited by the stimulus(10).

Treatment of Pain:

Acute pain is treated by removing the source of the pain and administering analgesic drugs(15). Chronic pain can be difficult to manage since the underlying cause may be difficult to eliminate. In people with persistent pain, secondary depression is prevalent. As a result, treating chronic pain requires a multidisciplinary strategy that includes counselling, physical therapy, and medications such as antidepressants, anti-arrhythmics, and opioids(11).

Mainly Analgesics are divided into two main groups. These are –

- **Narcotic analgesics**
- **Non-narcotic analgesics**

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Narcotic analgesics

These agents are capable of reliving extreme degree of pain, but are also moderately or strongly addicted(7). Opioids are a class of drugs that connect to opioid receptors and operate centrally(9). Opioids are the most potent and widely used group, and their analgesic activity is linked to a higher rate of adverse medication events, the majority of which are dose dependent(3). Moderate pain is also treated with narcotic pain relievers such as codeine and hydrocodeine(2). Other narcotics used to treat severe pain include oxycodone, hydromorphone, morphine, fentanyl, methadone, pethidine, sufentanil, and meperidine(5).

Adverse effects

Adverse effects of narcotic analgesics are following-

- Nausea, vomiting
- Constipation
- Drowsiness
- Respiratory depression
- Bradycardia
- Decreased gastrointestinalmotility
- Increased intracranial pressure
- Bronchial spasm, and urinary retention

The use of narcotic analgesics on a regular basis might lead to tolerance and dependency(1).

Non-narcotic analgesics

Non-narcotic analgesics are deemed non-addictive and alleviate mild to moderate pain(10). This group comprises aspirin and other analgesics and antipyretics, and they have little affinity for the opoid receptors and it operates peripherally(14). NSAIDs (nonsteroidal anti-inflammatory medications) have a peripheral effect(6). Mild pain is usually treated with over-the-counter non-steroidal anti-inflammatory medicines (NSAIDs), such as aspirin, ibuprofen, and naproxen, as well as acetaminophen(9). Oral corticosteroids like prednisone and methyl-prednisolone are commonly used to treat inflammation-related discomfort(5).

Adverse effects

Adverse effects of non-narcotic analgesics are following-

Salicylate shows adverse effects like-

- Heart bum
- Nausea
- Gastric distress
- Hemorrhage
- Hypersensitivity
- Renal dysfunction
- Respiratory alkalosis followed by acidosis, skin rash.

Other NSAIDS shows-

- Dizziness, rashes
- Photosensitivity
- Anorexia
- Nausea, vomiting
- Hepato-toxicity

Role of Traditional medicine

On a global scale, traditional medicines play a critical role in resolving health issues(1). In both modern and traditional medicine, medicinal plants continue to deliver essential therapeutic substances(6). Traditional remedies are rising in popularity as a result of the negative side effects of modern medicine, and they are now being examined to determine the scientific basis for their therapeutic actions(5). The study of medicinal plants has progressed, and information about these plants has been shared(15). This study will contribute significantly to the scientific exploration of medicinal plants for human benefit, and it is anticipated to reduce reliance on synthetic medications(10).

The Indian people have been blessed with more medicinal plants than the natives of any other country on the planet, which is a huge credit to them(9). Natural materials have been used as a source of pharmaceuticals since ancient times, and around half of today's useful drugs come from natural sources(1). India is sitting on a rich mine of well-documented and historically practised herbal medicinal knowledge(2). This country is the world's largest producer of medicinal plants and is appropriately known as the "Botanical Garden"(11).

According to research, around 6000 plants are utilised in traditional, folklore, and herbal medicine in India, accounting for about 75% of the world's medicinal needs(9).

Because it has a diverse class of chemical ingredients, a significant variety of Indian medicinal plants are associated with distinct therapeutic effects(10). Current analgesics, such as opiates and non-steroidal anti-inflammatory medicines, are thought to be ineffective in some circumstances due to their adverse effects and limited potency(5).

Natural medications are gaining popularity in today's society since they are more affordable, readily available, and virtually free of most adverse effects(14).

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As a result, it's critical to do study to find different ways to alleviate pain(8). Medicinal herbs have been utilised for therapeutic purposes for ages(12). Many of these analgesic herbs had been utilised in the past with no negative consequences(5).

H. spinosa T. Anders, a plant with haematinic, antidiabetic, anti-nociceptive, hepatoprotective, antioxidative, and androgenic properties, is revered by the rural population(10).

Guava, also known as Psidium guajava Linn, is a popular traditional medicine in India(3). This plant's roots, bark, and leaves are frequently used to cure gastroenteritis, vomiting, diarrhoea, wounds, ulcers, toothaches, and a variety of other ailments(15).

The analgesic efficacy of P.guajava leaf extracts on various pain models is investigated in this work(2).

PHARMACOLOGICAL ACTIVITIES:

- **Anti-Inflammatory & Analgesic Activity:-**

Simmering fruit tree leaves is used to treat a variety of inflammatory conditions, including rheumatism, all over the world(2). Dose-dependent and significant analgesic effects were also observed with the leaf extract (50-800mg/kg, i.p.)(15). The anti-inflammatory and analgesic properties of a 70% ethanolic extract of Psidium Guajava leaves were also studied. The extracts were given orally at a rate of 300 mg/kg. Aspirin (300 mg/kg, p.o.) was used as a control drug(5).The anti-inflammatory action of Psidium Guajava leaves was found to be significant, with a 58 percent inhibition(7). The essential oil (0.8 mg/kg) considerably reduced oedema formation caused by carrageenan, and the oil also greatly reduced granuloma formation caused by cotton pellets at 0.4 mg/kg and 0.8 mg/kg(10).

- **Antimicrobial:-**

Three antibacterial compounds, all of which are derivatives of quercetin, have been discovered in the leaves(6). Fungicidal activity of methanolic extract from ripe fruit against Arthrinium sacchari M001 and Chaetomium funicola M002 strains(5). In the case of Candida albicans, the bark tincture demonstrated fungicidal efficacy at various concentrations but solely fungistatic activity(12).

- **Antitussive Effects:-**

Within 10 minutes of injecting the extract, a water infusion made from Psidium guajava leaves reduced the frequency of coughing generated by capsaicin aerosol compared to a control group(14).

- **Anti-Hyperglycemic:-**

Evaluation of anti-hyperglycemic action of the ethanol extract produced from the stem bark of guava on blood glucose levels of normal, alloxan-induced hyperglycemic rats & normal glucose loaded rats(7). Guava leaves extract demonstrated substantial antidiabetic effects via the suppression of tyrosine phosphatage & also can reduce in the quantity of lipid droplets in liver in type 2 diabetic(1).

- **Anti-cancer:-**

Extracts from Psidium guajava have the potential to be developed as novel chemotherapeutic medicines to prevent or reduce the growth of tumours(15).

- **Cardioprotective effect:-**

In an isolated rat, aqueous leaf extract of Psidium guajava showed cardioprotective properties against myocardial ischaemia-repurfusion damage(12). As a result, guava leaf extract may be useful in the prevention of cardiovascular disorders(1).

- **Hepatoprotective effect:-**

The guava leaf extract at levels of 500mg/kg produced considerable hepatoprotection(9).

Introduction to Psidium Guajava

Table 1- Botanical profile:

Botanical name	**Psidium guajava**
Common name	**Guava**
Kingdom	**Plantae- Plants**
Division	**Magnoliophyta Flower plants**
Subclass	**Rosidae**
Order	**Myrtales**
Family	**Myrtaceae**
Genus	**Psidium**
Species	**Psidium guajava**
Part Used	**Bark, root, fruit**

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❖ **Medicine properties:-**

Antidiabetic, Hepatoprotective, Anticancer, Antiinflammatory, Analgesic

❖ **Medicinal Parts Used:- Bark, Root, Stem, Leaves, Fruit**

❖ Guava leaves contain a variety of volatile chemicals, including limonene, isopropyl alcohol, and oleonic acid(6).

❖ The bark includes tannin 27.4 percent , polyphenols, resin & the crystals of calcium oxalate, bark is also an efficient astringent(12).

❖ Tannin is found in the roots(5).

❖ Vitamin A, C, iron, phosphorus, and calcium are all found in the guava fruit(13).

Literature Survey:-

➢ Ojewole et al. (2005) found that an aqueous extract of guava leaves given intra-peritonially at doses ranging from 50 to 800 mg/kg produced analgesic effects in rats and mice(15).

➢ According to Roy et al. (2006), the aqueous extract of P. gujava has hepatoprotective properties(11).

➢ In normal &alloxan-treated diabetes mice, Cheng et al. 1983 found that i.p. administration with 1 g/kg guava juice generated considerable hypoglycemic action(1).

➢ Grover et al. (1993) found that water and chloroform extract of guava leaves were efficient in activating Salmonella typhimurium mutagenicity(9).

➢ According to Jaiarj et al. (1999), guava leaf extract has anti-cough properties by lowering the frequency of cough caused by capsaicin aerosol(14).

➢ The hypoglycemic impact of plant extract was investigated in normal and diabetic rats utilising a Streptozotocin (STZ)-induced diabetes mellitus model, according to Ojewole, 2005(3).

The leaves of Psidium Guajava have been demonstrated to have analgesic properties(15). However, when compared to the conventional medicine pentazocine, the analgesic effect of alcoholic extracts of guava leaf has yet to be reported.

So the goal of my research is to compare the analgesic activity of various solvent fractions of alcoholic extracts of guava leaves with pentazocine in mice.

Guava Leaf **Guava Flower**

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Guava Fruit

- **Aim & Objectives of the study:-**
- **Aim of this study:-**

To compare analgesic activity of different solvent fraction of alcoholic extract of guava leaf with pentazocine on mice.

- **Objectives of the study:-**
- Collection of fresh Psidium Guajava leaves(6).
- Leaves were washed & completely dried in sunlight(9).
- Then the dried leaves were crushed in mixture grinder to form powered particles(1).
- Extraction of dried & powdered leaf through cold maceration process(5).
- To study the analgesic activity of the plant, Psidium Guajava(15).
- To test whether the methanolic extract of Psidium Guajava leaves have an effect of lowering pain(2).
- To study which fraction shows the better result in lowering pain(8).
- To find out comparatively which drugs give better result between test drug(methanolic extract of guajava leaves) & standard drug(pentazocin)(6).
- To test the differences between which the animals receiving the methanolic extract of Psidium guajava leaves & which the animals receiving the standard drug pentazocine in pain(5).
- To perform phytochemical analysis(chemical group test)(15)
- In the VIVO investigation, a) toxicity was determined by the LD50 value, and b) pharmacological activity was determined(14).

- **Materials & Methods:-**

1. Plant material collection:-

The plant's leaves will be collected in Hooghly, West Bengal's rural areas. The plant specimen is yet to be authenticated at the Botanical Survey of India, Botanical Garden, Howrah-711103, West Bengal.

2. Preparation of extract:-

- Psidium Guajava leaves were chosen while they were fresh and in the middle of their growth cycle(5).
- Extraneous materials were removed from the leaves by washing/rinsing them with tap water and drying them entirely in the sun/in the shade at room temperature(15).
- The powdered particles were then sieved through a 40 mesh sieve after being crushed by a mechanical grinder. Using the Cold Maceration extraction technique, the crushed materials were extracted with 70% ethanol(1).

3. Solvent Fraction:-

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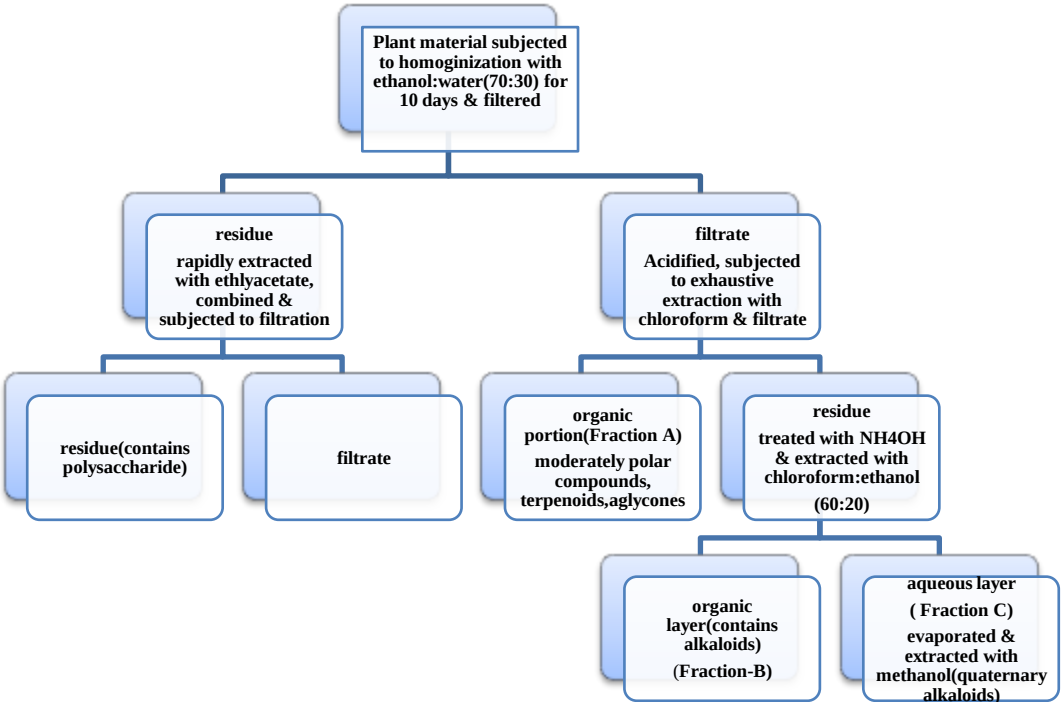

- **Materials used during the project work**

Chemicals used:-

Table-3

SL. NO.	NAME	DETAILS
1.	ETHANOL	Purchased From Sigma Laboratories, Germany
2.	CHLOROFORM	Purchased From Sigma Laboratories, Germany
3.	AMMONIA SOLUTION	Purchased From Sigma Laboratories, Germany
4.	HYDROCHLORIC ACID	Purchased From Sigma Laboratories, Germany
5.	ETHYL ACETATE	Purchased From Sigma Laboratories, Germany
6.	DISTILLED WATER	Purchased From Sigma Laboratories, Germany
7.	DIETHYL ETHER	Purchased From Sigma Laboratories, Germany

SUBJECTS USED FOR IN-VIVO STUDY:-

TABLE-4

SUBJECTS REQUIRED	RATS
NUMBER OF SUBJECTS REQUIRED	48
STRAIN	ALBINO MICE
SEX	MALE OR FEMALE
AVERAGE BODY WEIGHT	20-25 gm (Mice)
SUPPLIER	ANIMAL HOUSE, NSHM KNOWLEDGE CAMPUS, KOLKATA

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TABLE-5

Analytical instruments used:-

VACCUM FILTER	**ANALYSIS DONE IN- NSHM KNOWLEDGE CAMPUS, KOLKATA**

TABLE-6

Chemicals used for in vivo study:-

S.L. NO.	NAME	DETAILS
1.	PENTAZOCINE	Purchased from Sigma Laboratories, Germany
2.	ACETIC ACID	Purchased from Sigma Laboratories, Germany

TABLE-7

Equipments used:

S.L. NO.	NAMES	DETAILS
1.	ORAL FEEDING TUBE	Purchased from Sigma Laboratories, Germany
2.	SYRINGE	Purchased from Sigma Laboratories, Germany
3.	WEIGHING MACHINE	Purchased from Sigma Laboratories, Germany
4.	MICROPIPETTE	Purchased from Sigma Laboratories, Germany
5.	EDDY'S HOT PLATE	Purchased from Sigma Laboratories, Germany

TABLE-8

Apperatus used:-

S.L. NO.	NAMES	DETAILS
1.	BEAKER	BOROSIL, 200ml, 500ml
2.	PETRIDISH	BOROSIL
3.	GLASS ROD	BOROSIL
4.	ROUND BOTTOMED FLASK	BOROSIL, 500 ml
5.	SEPARATING FUNNEL	BOROSIL

- **COLD MACERATION PROCESS:-**

1. The whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and left to remain at room temperature for at least 3 days with frequent agitation until the soluble count has dissolved in this cold maceration process(9).
2. After standing, the combination is strained, the marc (wet solid material) is pressed, and the combined liquids are clarified by filtration or decantation(6). In maceration (for fluid extract), entire or coarsely powdered plant-drugs are kept in contact with the solvent in a stoppered container for a set amount of time, with regular agitation, until the soluble depend is dissolved(10).
3. This method is particularly well suited to the usage of thermolabile medicines. The technique was designed to soften and shatter the plant's cell wall, allowing the soluble phytochemicals to be released(8).
4. Maceration is a cold extraction process and an isocratic extraction technique. It can be used to extract thermolabile chemicals because it is soluble(2).
5. This method entails soaking the plant pattern in a specific solvent to remove constituent compounds from flowers. It is finished in a constant state at room temperature(4).

- **Phytochemical Analysis:-** The extracted solution of Psidium guajava used to perform Phytochemical analysis.

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1. Identification test of Alkaloids:-

Various alkaloid test are-

- **By Dragendroff's Reagent**
- **By Mayer's Reagent**
- **By Hager's Reagent**

By Dragendroff's Reagent:-

Process:

Sample+ Dragendroff's Reagent(Potassium Bismuth iodide Solution). Alkaloids gives reddish brown precipitate with Dragendroff's Reagent.

By Mayer's Reagent:-

Process:

Sample+ Mayer's Reagent(Potassium Mercuric Iodide Solution).Alkaloids give cream precipitate with Mayer's Reagent.

By Hager's Reagent:

Process:

Sample + Hager's Reagent (Saturated solution of Picric Acid). Alkaloids gives yellow precipitate with Hager's Reagent.

Observation table of alkaloid test:-

Test Name	Process	Observation	Inference
Dragendroff's Reagent	**Sample+ Dragendroff's Reagent (Potassium Bismuth Iodide Solution). Alkaloids gives reddish brown precipitate with Dragendroff's Reagent**	**Precipitate formed in fraction A, B, C**	**Alkaloid Present.**
Mayer's Reagent	**Sample+ Mayer's Reagent (Potassium Mercuric Iodide Solution). Alkaloids give cream colour precipitate with Mayer's Reagent**	**Precipitate formed in fraction A, B, C**	**Alkaloid Present.**
Hager's Reagent	**Sample+ Hager's Reagent(Saturated solution of Picric Acid). Alkaloids give yellow precipitate with Hager's Reagent.**	**Precipitate formed in fraction A, B, C**	**Alkaloid Present.**

2. Identification test of Flavonoids:-

Shinoda Test:-

Process:-

Sample + magnesium fillings + Concentrated Hydrochloric Acid. To the test solution add few magnesium fillings & concentrated hydrochloric acid is added dropwise , pink or crimson red or occasionally green to blue colour occurs after few minutes.

Zinc Hydrochroride Test:-

Process:

Sample + Zinc Test + Conc. Hydrochloride Acid. To the test solution add a mixture of zinc dust & conc. Hydrochloride acid. It gives red colour after few minutes.

Observation table of Flavonoids:

Test Name	Process	Observation	Inference
Shinoda Test	**Sample+ magnesium fillings+ Concentrated Hydrochloric Acid. To the test solution add few magnesium fillings and concentrated hydrochloric acid is**	**Crimson Red Colour observed in fraction A,B,C**	**Flavonoid Present**

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	added dropwise, pink or crimson red or occasionally green to blue coour occurs after few minutes.		
Zinc Hydrochloride Test	**Sample+ Zinc Dust+ Conc. Hydrochroric Acid. To the test solution add a mixture of zinc dust & conc. Hydrochloric acid. It gives red colour after few minutes.**	**Red Colour observed in fraction A,B,C**	**Flavonoid Present**

3. Identification test of steroids:-

Libermann-Burchard Test:

Process :-

Sample + Acetic Anhydride + Boil + Cool + Conc. H2SO4. Treat the extract with few drops of acetic anhydride, boil & cool. Then add concentrated sulphuric from the side of the test tube, brown ring is formed at the junction of two layer and upper layer turns green which shows the presence of steroids and formation of red colour indicates triterpenoids.

Observation table of steroid test:-

Test Name	Process	Observation	Inference
Libermann- Buchard Test	**Sample + Acetic Anhydride + Boil +Cool + Conc. H2SO4.**	**a) Brown ring formed. b) Upper layer turns green.**	**Steroids are present.**

4. Identification test of Amino Acids:-

Millon's Test:

Process :

Sample + Millon's Reagent . To the test solution add about 2 ml of Millon's Reagent, white precipitate indicates the presence of amino acids.

Ninhydrine Test:-

Process:

Sample + Nin-Hydrine Solution + Boil . To the test solution add Nin-Hydrine solution, boil , violet colour indicates the presence of amino acids.

Observation table of amino acid test:

Test Name	Process	Observation	Inference
Millon's Test	**Sample+ Millon's Reagent. To the test solution add about 2 ml of Millon's Reagent, white precipitate indicates the presence of amino acids.**	**White precipitate is observed in fraction A,B,C.**	**Amino acids are present.**
Nin-Hydrine Test	**Sample + Nin-hydrine Solution + Boil. To the test solution add Nin-Hydrine solution, boil, violet colour indicates the presence of amino acids.**	**Violate colour is observed in fraction A,B,C.**	**Amino acids are present.**

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5. Identification test of Carbohydrates:

Molisch's Test:-

Process:

Sample+ alpha –naphthol + Conc. H2SO4. To the test solution add few drops of alcoholic alpha-naphtol, then add few drops of conc. Sulpheuric acid through sides of the test tube, purple to violet colour ring appears at the junction.

Observation table of Carbohydrate Test:-

Test Name	Process	Observation	Inference
Molisch's Test	**Sample+ alpha-naphthol + Con. H2SO4. To the test solution add few drops of alcoholic alpha-naphthol , then add few drops of conc. H2SO4 acid through sides of the test tube, purple to violet colour ring appears at the junction.**	**Violet Colour Ring appears at the junction in fraction A,B,C**	**Carbohydrates are present.**

6. Identification test of Tannins:-

Ferric Chroride Test:-

Process:

Sample + Ferric Chloride. Treat the extract with ferric chroride solution, blue colour appears if hydrolysable tannins are present & green colour appears if condensed tannins are present.

Test Name	Process	Observation	Inference
Ferric Chloride Test	**Sample+ Ferric Chloride. Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.**	**Blue colour is observed.**	**Tannin is present.**

7. Identification of Acidic Compounds:

Test Name	Process	Observation	Inference
Unnamed	**Sample + NaHCO3. Alcoholic extract of the drug after treatment with sodium bicarbonate produces effervescence.**	**Effervescence is observed in fraction A,B,C**	**Acidic Compounds present.**
Unnamed	**Sample+ Warm Water + Filtration. Treat alcoholic extract of the drug with warm water & filter. Test the filtrate with warm**	**Litmus turn blue in fraction A,B,C**	**Acidic compounds present**

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	water & filter. Test the filtrate with litmus paper, it turns blue.		

- **Pharmacologicalstudies** :-

Analgesicactivity:-

Chemical and thermal methods will be used to assess the analgesic activity of various solvent extracts of P. guajava(9). Acetic acid induced writhing response will be employed in the chemical method, and hot plate reaction time will be used in the thermal way(5). Five groups of animals will be created(6). Different extracts will be given orally at doses of 200 and 400 mg/kg of body weight, with pentazocine as the reference medication at a dose of 100 mg/kg of body weight and a 1% (v/v) control group(3).

Acetic acid induced writhing test:-

All groups will receive acetic acid (0.6 percent v/v) intraperitonially in a volume of 10 ml/kg of body weight 60 minutes after receiving the test chemical(13). The number of writhes after a 10-minute injection of acetic acid will be counted to determine analgesic activity(15). The abdominal constriction and full elongation of the hind limb imply a writhing(6).

Hot plate test:-

The test will be carried out utilising Eddy's Hot Plate at a temperature of 55+- 10 degrees centigrade(12).

All animals' basal reaction times to thermal heat will be recorded(3). The animals who lick their forepaws or jump within 6-8 seconds were chosen for the study(8). The animals in all groups were individually exposed to the hot plate maintained at the temperature mentioned above 60 minutes after receiving the test and standard compounds, and the time taken for forepaw licking or jumping was recorded as reaction time(7).

Procedure:-

- **Experimental Design:-**

Male albino mice weighing 20-25 g were divided into five groups. Mice in group I will be served as normal controls.

Group I: Normal Saline

Group II: Standard drug (Pentazocine) (In case of Acetic acid induced contraction Diclofenac Sodium 50 mg/kg is given)

Group III: Test drug -I (Methanolic leaf extract Psidium guajava, Fraction-A)

Group IV: Test drug- II (Methanolic leaf extract Psidium guajava, Fraction-B)

Group V: Test drug- III (Methanolic leaf extract Psidium guajava, Fraction-C)

In case of Hot Plate Method:- (Procedure)

Male albino mice weighing 20-25 grammes were placed into four groups, each with five mice. Group 1 acted as a control, Group 2 received Pentazocin (10 mg/kg) as a positive control, and Groups 3–5 received alcoholic extracts of Psidium guajava at concentrations of 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively. The creatures were placed on Eddy's hot plate, which was set to 55+0.5C.

To avoid damaging the paw, a 15-second cut was observed. When animals licked their fore or hind paws or leaped prior to and 0, 30, 60, 90, and 120 minutes following oral administration of the samples, reaction time was observed. Using the student T-test, the average basal reaction time and the percent increase in basal reaction time were calculated.

In case of Acetic acid induced abdominal contraction test:-

Male albino mice Five sets of five animals each with a body weight of 20-25gm were formed. The first group of animals received acetic acid (0.1 ml of 0.6 percent v/v, intra-peritoneal) as a control, the second group received Diclofenac sodium (50 mg/kg) as a positive control, and the third, fourth, and fifth groups received alcoholic extracts of Psidium guajava (100, 200, and 300 mg/kg body weight, respectively).

15 minutes before the acetic acid injection, all of the extracts were given orally using an intragastric tube. The writhing effect indicator is determined by stretching the abdomen while simultaneously stretching at least one hind limb for roughly 10 minutes and calculating the percent of protection for analgesic activity.

- **Experimental Design:-**

Male albino mice weighing 20-25 g were divided into five groups. Mice in group I will be served as normal controls.

Group I: Normal Saline

Group II: Standard drug (Pentazocine) (In case of Acetic acid induced contraction Diclofenac Sodium 50 mg/kg is given)

Group III: Test drug -I (Methanolic leaf extract Psidium guajava, Fraction-A)

Group IV: Test drug- II (Methanolic leaf extract Psidium guajava, Fraction-B)

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Group V: Test drug- III (Methanolic leaf extract Psidium guajava, Fraction-C)

III. RESULTS

Statistical analysis

The data is presented as a mean + standard deviation. The presence of flavonoids, triterpenes, saponins, carotinoids, alkaloids, glycosides, and carbohydrates was also investigated using a one-way ANNOVA Tukey's Multiple Comparison Test. Up to 2 g/kg body weight, there were no indicators of toxicity in P. guajava acute toxicity trials. Because there was no evidence of animal mortality at large doses.

Hot plate test

Table 1 shows the results of the P. guajava hot plate test. When compared to the control, the plant extract exhibits a dose-dependent increase in reaction time. At 120mts, the percent inhibition of two different doses (100 and 200 mg/kg) was 62.50 and 77.56 percent, respectively. The results of five observations were compared to the Control group using the One Way ANNOVA Tukey's Multiple Comparison Test.**p< 0.05. This discrepancy is believed to be statistically significant.

**p< 0.05

Acetic and induced abdominal contraction test

The analgesic efficacy of P. guajava in an acetic acid-induced abdominal contraction test is shown in Table 2. In this way, P. guajava reduced the acetic acid-induced abdominal constriction in a dose-dependent manner. For both dosages (100 and 200 mg/kg), the percent inhibition was 37.34 and 56.75, respectively. The conventional drug diclofenac sodium was found to be more powerful than the plant extract at all dosing levels. The results are shown as mean SEM from five observations when compared to the Control group.

**p< 0.05

Tukey's Multiple Comparison Test (ANNOVA). This disparity is believed to be statistically significant.

Table 1-Effect on sidium guajava on heat(hotplate) induced pain in mice

Treatment Dose mg/kg	Mean increased reaction time (sec) Before and after Drug administration.					% Reaction time
	0mts	30mts	60mts	90mts	120mts	120mts
Normalsaline 10ml/kg	3.43 ±0.23	3.54 ±0.19	3.16 ±0.18	3.40 ±0.21	3.34 ±0.15	--
Pentazocine10mg/kg	3.74 ±0.21	6.56 ±0.48	10.82 ±0.86	14.43 ±1.1	16.52±1.45**	84.88**
P. guajava100mg/kg	3.36 ±0.16	4.74 ±0.53	5.82 ±0.61	6.34 ±0.61	7.24±0.56	63.50
P. guajava200mg/kg	3.14 ±0.17	5.82 ±0.63	8.36 ±0.54	8.26 ±0.67	10.43±0.74**	78.56**

Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test.
**p < 0.05.

Groups	Dose mg/kg	Number of abdominal constrictions in 10mts.	% inhibition of abdominal constriction in 10mts.
I	Normal saline 10ml/kg	43.36 ±1.73	--
II	Diclofenac sodium50mg/kg	11.21 ± 0.78	74.55**
III	P. guajava 100mg/kg	27.54 ±1.64	38.34
IV	P. guajava 200mg/kg	18.33±1.40**	57.75**

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Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test.
****p < 0.05.**

IV. DISCUSSION

Inflammation and pain are associated to a variety of clinical symptoms, including cancer, rheumatoid arthritis, and cardiovascular disease(11). Many additional natural chemicals are employed to counteract the symptoms of pain in various time-honored therapeutic systems(1). Thermal (Hot plate) and chemical (Acetic acid) produced pain models on mice were used to assess the analgesic effects of Psidium guajava in this study(9). These models represent some of the most common human sources of pain(14). The findings revealed that animals treated with Psidium guajava shown considerable activity in various pain animal models(6). The hot plate approach has been demonstrated to be a good paradigm for assessing centrally acting analgesics(10). This is one of the most common acute nociceptive models for assessing central nociceptive activity(7). In a dose-dependent manner, the extract of Psidium guajava showed an enhanced reaction time when compared to control and standard(13). According to these reports, the extract of Psidium guajava must have the ability to operate centrally like medicines(4). The method of evaluating peripherally acting analgesics using acetic acid generated belly contractions has been used(8). Because acetic acid-induced abdominal contraction in mice is linked to visceral discomfort, this research is crucial for the development of analgesic medications(12). In the acetic acid induced abdominal contraction method, pain is elicited by the release of free arachidonic acid from tissue phospholipids via the cyclooxygenase enzyme and prostaglandin production, which results in a localised inflammatory reaction(5). On the other hand, elevated levels of PGE2 and PGF2 in peritoneal fluids, as well as the lipogenase enzyme, have been linked to the acetic acid-induced abdominal contraction approach(9). By increasing capillary permeability, an increase in prostaglandin levels in the peritoneal cavity increases inflammatory discomfort(11). In an acetic acid-induced abdominal contraction (writhing) test in mice, the Psidium guajava extract showed considerable analgesic efficacy(14). In mice given acetic acid, the Psidium guajava extract significantly reduced the abdominal constriction reaction(2). Endogenous mediators such as prostaglandin and a portion of local peritoneal receptors are involved in the abdominal contraction response(10). As a result, it's possible that the Psidium guajava extract causes analgesia through interfering with prostaglandin synthesis and the peritoneal receptor(6). The results showed that the Psidium guajava extract had analgesic effect that was mediated by the peripheral nervous system(9). In Psidium guajava, phytochemical screening revealed the presence of carbohydrates, flavanoids, tannins, saponins, and phytosterol(1). These chemicals are thought to be responsible for the observed analgesic action(5). Flavanoids have been shown to have analgesic properties by working on the prostaglandin pathway(8). There is also having a report on the involvement of tannins in analgesic activity(11) . The current study found that the leaf extract of Psidium guajava inhibited pain in both the central and peripheral nervous systems in experimentally produced pain models(13). These findings support previous findings that Pergularia daemia, Lantana trifolia, and Pergularia daemia, Lantana trifolia, and Pergularia daemia, Lantana trifolia, and Pergularia daemia, Lantana trifolia, and Pergularia daemia(6). As a result, the analgesic effects of Psidium guajava leaf extract could be mediated by both central and peripheral mechanisms(7). However, more research is required to fully comprehend the mechanism(5).

Conclusion- I would like to conclude by saying that Psidium guajava leaf extract show sufficient central&peripherally acting analgesic mechanisms compared to the standard drugs Diclofenac &Pentazocine.

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