PHYTOCHEMICAL ANALYSIS AND THE ANTI-INFLAMMATORY ACTIVITIES OF METHANOL EXTRACT OF CRATEVA ADANSONII

BY

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CERTIFICATION PAGE

This is to certify that this project work was fully carried out by Uwah Lynda O. of the Department of Biochemistry, Faculty of Natural Science Caritas University –Nike Enugu State.

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DEDICATION

This project work is dedicated to my creator in heaven and to my lovely parents and siblings. Who thought me to be hardworking and to my supervisor M.O Ezenwali and my humbly lecture Dr V. Ikpe.

ACKNOWLEDGEMENT

I want to thank and acknowledge God's almighty for his blessings in my life. I am grateful for his endless love, protection, guidance, grace upon me and my family.

My sincere appreciation goes to my parents Mr. and Mrs Stephen Uwah for their love, care, prayers, advice and financial support. I also appreciate my siblings for their love. Ambrose Okeke,, friends and well-wisher.

I also acknowledge the untiring effort to my supervisor Mr. Moses O. Ezenwali (H.O.D), my lecturers Dr V. Ikpe Mr P. Ugwudike, Mr Yusuf Omeh, Dr Charles Ishiwu, Mr Steve Eze Peter, who brought out their time to assist me and make suggestions to make this work a success.

May God guide and reward you abundantly amen.

ABSTRACT

Inflammation is a complex biological response of vascular tissue to harmful stimuli such as pathogen, damage cells or irritants. The urgency generated by in increased rate of stroke, atheroselerosis attributed to prolong use of cyclooxygenase-1 and 2 inhibitors have acceralated anti-inflammatory drug research over the last decade while synthetic pharmaceutical agents continued to dominate research, attention increasingly has been directed to natural products. These are often more affordable and available and sometimes are perceived as more effective than conventional anti-inflammatory drugs.

Anti-inflammation was carried out using 11 rats. That was divided into three groups of four rats each. Group 1 and 2 served as the positive and negative control respectively. Groups 3 and 4 received 200mg/kg b.w and 600mg/kg b.w. of the extracts respectively. However, it was discovered that the stem bark of *crateva Adansonii* showed greater significance anti-inflammatory activity when compared with the standard.

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1.0 INTRODUCTION

Anti inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti –inflammatory drugs make up about half of analgesics, reducing pain by inhibiting inflammation as opposed to opioids, which affect the central nervous system published by Artemis Morris, molly Rossiter.

Inflammation (Latin, inflammo, “I ignite, set alight”) is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of inflammation are pain, heat, redness, swelling and loss of function by Dr Weil. Inflammation is a protective attempt by the organism to remove the injurious stimuli and initiate the healing process.

Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, and organism pathogen.

Inflammation is a setereotyped response and is considered as a mechanism of innate immunity by Dr. Weil.

1.1 TYPES OF INFLAMMATION

Inflammation can be classified as either acute or chronic. Acute inflammation, is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues.

Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissues from the inflammatory process from Wikipedia, the free encyclopedia.

1.2. **AIMS AND OBJECTIVES**.

Evaluation of the anti –inflammatory activity of *crateva adansonii* (plant). The purpose of this study was to evaluate and compare the anti-inflammatory activity of the aqueous sten bark extract of crateva adansonii in experimental acute and chronic inflammatory animal models. And to evaluate the phytochemical constituents and pharmacological evaluation of the effect of crateva adansonii on albino rats.

1.3 RATIONAL STUDY.

Crateva adansonii was described in 1824 by Augstin Pyramus de candolle.

Crateva adansonii is a specie of a flowering tree in the capparancea family, which is equally called the “sacred garlic pear and temple plant,” and many other names in a variety of dialects, including Balai lamoke, barnaj Vanina and bidasi. The tree is sometimes called the spider tree because the showy flowers bear long, spidery stamens. It is native to Japan,

Australia, much of South East Asia and several south practice Islands India it is grown else where for fruit, especially in parts of the African continents.

The fruit of the tree is edible. The flowers are attractive to a multitude of insects and birds. The butterfly (Hebomoia glaucippe) is a frequent visitor to this plant by Dr. Weil.

1.4 TAXONOMY

Crateva Adansonii is a species in the genus *crateva*, which contains 8 species and belongs to the family of Capparacea (caper family)

The taxonomy of *crateva adansonii* for classification includes the following;

Kingdom:	Plantae
Division:	Angosperms
Class:	Eudicots
sub-class:	Rosids
Order:	Brassicales
Family:	Capparaceae
Genus:	Crateva
Species:	C. religiosa

1.5 PHYSICAL DESCRIPTION OF *CRATEVA ADANSONII*

Unarmed, small deciduous tree, 6-15m tall, glabrous leaves 3 – foliolate, petiolate, leaflet shorty petiolulate, elliptic, elliptic – lanceolate, more or les olique, acuminate/ narrowed towards the

base, 5-12m long, (1-) 2.5 -6cm broad, herbaceous to subcoriaceous, often turning brownish when dry, petiole (2.5) 4 - 8cm long flowers. 3-6cm across, sub irregular greenish white turning yellowish after opening appearing before or just after the flush of new leaves, pedicle 2-4cm long, 10-17mm wide, 2 adoxial ones generally what larger stamens usually 20-25, gynophore generally 3-5cm long, slender thickened in fruit. Fruit globose, 3-5cm in the development of fruit. Yellowish seeds reinform at about 3-4mm in diam smooth brown. The flowers are green and are arranged in racemes. The fruits are berries.

1.6 DISTRIBUTION

Crateva adansonii is native to tropical Africa, India, Srilanka, Myanmar, Japan, Australia and much of South East Asia.

1.7 CULTIVATION

Crateva Adansonii can withstand temperatures only above 1 - 2^0C. The plants bloom from March to May.

1.8 USES OF *CRATEVA ADANSONII*

Crateva adansonii is used in traditional medicine in the West Africa. The crude Hexane (CAN -1) and ethyl acetate activity has African trypanosome, Trypansomia brucei brikes and forms blood stream. The crude extracts showed moderate anti-trypansomal activity. It's use alone or in combination with other natural/ semi –synthetic drugs for the treatment of human Africa.

Crateva adansonii fruit serves as an edible fruit which contains berries Published by Flora of Taiwan, National Taiwan University.

1.9 SOURCES

From wikipedia,

The free encyclopedia

bing

pdf

and articles of Health.

CHAPTER TWO

LITERATURE REVIEW

2.1 Inflammation according to medilexicons medical dictionary inflammation is a fundamental pathologic process consisting of a dynamics complex of histological apparent cytologic changes, cellular infiltration & mediator release that occurs in the affected blood vessels..

2.1.1 EVENTS IN ACUTE INFLAMMATION

Acute inflammation begins within seconds to minutes following the injury of tissues. The damage may be purely physical or it may involve the activation of an immune response.

2.1.2 THREE MAIN PROCESSES WHICH OCCUR IN ACUTE INFLAMMATION

1. Increased blood flow, due to dilation of blood (arterials).
2. Increased permeability of the capillaries allowing fluid and blood proteins to move into the interstitial space.

3. Migration of neutrophils (and perhaps a few macrophages) out of the capillaries and venules and into interstitial spaces.

2.1.3 INCREASED BLOOD FLOW AND FORM.

Edema swelling caused by the acids produced from surgery or an injury seems to go faster when people take grapes seed extract. Edema or acidosis of the tissues is common after breast cancer surgery and one double blind, placebo controlled study found that breast cancer patients who took 600mg of grape seed extract daily after surgery for 6 months had le edema & pain than those who took placebo. Another study found that people who took grape seed extract after experiencing a sports injury had less swelling than those who took placebo by Robert O. Young D.Sc Ph.D.

The first two of the above effects are readily visible within a few minutes following a scratch and does not break the skin. At first, the scratch is visible as a pale red line which will later become red as blood flow increases locally. Finally, the area swells as additional fluid accumulates in the interstitial space of the region known as edema. The

increased permeability of the capillaries occur because the endothelial cells separate from one another at their edges.

2.1.4 CHEMOTAXIS

Once outside the blood vessel, a neutrophil is guided towards an infection by various diffusing chemotactic factors examples include the chemokines and the complement peptide C_5a, which is released when the complement system is activated either the specific Immunity or Innate Immunity.

2.1.5 EOSINOPILS

In some circumstances eosinophils rather than neutrophils predominate in acute inflammation. This tends to occur with parasitic worms, against which neutrophils have little success, or with a response involving the antibody 1gE.

Eosinophils release several proteins, such as major basic protein which are often effective against parasite. Eosinophils are linked to certain types of allergies.

2.1.6 CAUSES OF INFLAMMATION

Burns

Chemical irritant

Toxins

Infection by pathogens

Immune reactions due to hypersensitivity

Ionizing radiation.

Stress

Trauma

Alcohol.

2.1.7 CARDINAL SIGNS

Acute inflammation is a short term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It is characterized by five cardinal signs.

The acronym that may be used for this PRISH for pain redness, immobility, swelling and heat.

Redness and heat are due to increased blood flow at body temperature to the inflamed site, swelling is caused by accumulation of fluid, pailn is due to release of chemicals that stimulate nerve endings.

2.1.8 PROCESS OF ACUTE INFLAMMATION

The process of acute inflammation is initiated by cells already present in the tissues, mainly resident macrophages, dendritic cells, histocytes, and mastocytes.

These cells present on their surface certain receptors (PRRs) which recognize molecules that are shared by pathogens but distinguishable from host molecules, referred to as pathogen –associated molecular

patterns (PAMPS). At the onset of an infection, burn, or other injuries, the cell undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. vasodilaiton and its resulting increased blood flow causes the redness and increased heat. Increased permeability of the blood vessels results in an exudation of plasma proteins and fluid into the tissue (edema) which manifests itself as swelling (tumor). The mediator molecules alter the blood vessels to permit the migration of leukocytes, mainly neutrophils, outside of the blood vessels into the tissues. The neutrophils migrate along a chemotactic gradient crated by the local cells to reach the site of injury. Acute inflammatory response requires consistent stimulation to be sustained. Acute inflammation ceases once the stimulus has been removed.

2.1.9 EXUDATIVE COMPONENT

It involves the movement of plasma fluid, containing important proteins such as fibrin and immunogloblins (antibodies) into inflamed tissues. The increased collection in fluid into the tissue causes it to swell. This extravasted of fluid is funneled by lymphatics to the regional lymphnodes, flushing bacteria along to start the recognition and attack phase of the adaptive immune system by Houghton Mifflin company.

2.1.10 VASCULAR CHANGES

Acute inflammation is characterized by vascular changes, including vasodilation, increased permeability and increased blood flow, which are induced by the actions of various inflammatory mediators. Vasodilation occurs first at the arteriole level progressing to the capillary level, and brings about a net increase in the amount of blood present, causing the redness and heat of inflammation by Houghton Mifflin company.

2.1.11 MORPHOLOGIC PATTERNS

Specific patterns of acute and chronic inflammation are seen during particular situation that arise in the body such as when inflammation occurs on an epithelial surface or pyrogenic bacteria are involved by Houghton Mifflin company.

2.1.12 GRANULOMATOUS INFLAMMATION

Characterized by the formation of grabulomas, they are the result of a limited but diverse number of diseases, which include among others tuberculosis leprosy sacroidosis, and syphilis by Houghton Mifflin company.

2.1.13 FIBRINOUS INFLAMMATION

Inflammation resulting in a larger increase in vascular permeability allows fibrin to pass through the blood vessels by Houghton Mifflin company.

2.1.14 PURULENT INFLAMMATION

Inflammation resulting in large amount of pus which consists of neutrophils, dead cells, and is characteristic of this kind of inflammation.

2.1.15 SEROUS INFLAMMATION

Characterized by the copious effusion of non-viscous serous fluid, commonly produced by mesothelial cells of serous membranes, but may be derived from blood plasma. Skin blisters exemplify this pattern of inflammation.

2.1.16 ULCERATIVE INFLAMMATION

Inflammation occurring near an epithelium can result in the necrotic loss of tissue from the surface, exposing lower layers.

2.1.17 INFLAMMATORY DISORDERS

Inflammatory disorders are a large group of disorders which underlie a vast variety of human disease. The immune system is involved with

inflammatory disorders, demonstrated in both allergic reactions and some myopathies, with many immune system disorders resulting in abnormal inflammation.

2.1.18 EXAMPLES OF DISORDERS ASSOCIATED WITH INFLAMMATION INCLUDES:

Acne vulgaris

Asthma

Auto immune disease

Celiac disease

Chronic prostates

Glomerulonephritis

Hypersensitives

inflammatory bowel disease

Pelvic inflammatory disease

Rheumatoid arthritis

Sarcoidosis

Transplant rejection

Interstitial cystitis etc.

2.1.19 ALLERGIES

An allergic reaction, formally known as types 1 hypersensitivity, is the result of an inappropriate immune response triggering inflammation. A common example is hey fever which is caused by a hyper sensitivity response by skin mast cells to allergens.

2.1.20 MYOPATHIES

Myopathies are caused by the immune system inappropriately attacking components of muscle, leading to signs of muscle inflammation. They may occur in conjunction with other immune disorders such as systemic sclerosis, polymyositis, and inclusion body myositis.

2.1.21 LEUKOCYTES DEFECT

Due to the central role of leukocytes in the development and propagation of inflammation, defects in leukocyte function often result in a decreased capacity for inflammatory defense with subsequent vulnerability to infection.

Dysfunctional leukocytes may be unable to correctly bind to blood vessel due to surface receptor mutations digest bacteria, or produce microbicides.

2.1.22 PHARMACOLOGICAL

Certain drugs or exogenic chemical compounds are known to affect inflammation. Vitamin A deficiency causes an increase in inflammatory responses and anti inflammatory drugs work specifically by inhibiting normal inflammatory components.

2.1.23 RESOLUTION INFLAMMATION

The inflammatory response must be actively terminated when no longer needed to prevent unnecessary “by stander” damage to tissues. Failure to do so results in chronic inflammation, and cellular destruction

2.1.23.1 Mechanisms which serve to terminate inflammation include

i. Short half life of inflammatory mediators
ii. Production and release of transforming growth factors beta from macrophages.
iii. Production and release of interleukin
iv. Production of anti inflammatory lipoxins
v. Down regulation of pro-inflammatory molecules, such as leukotrienes.
vi. Up regulation of pro-inflammatory molecules such as inter leukin

2.1.24 EXERCISE AND INFLAMMATION

Acute inflammation of the muscle cells, can result after induced eccentric and concentric muscle training. Participation in eccentric training and conditioning, including resistance training and activities that emphasie eccentric lengthening of the muscle including downhill running on a moderate to high incline can result in considerable soreness within 24 to 48 hours, though blood lactate levels, previously thought to cause muscle soreness, were much higher with level running. This delayed onset muscle. Soreness results from structural damage to the contractile filaments and Z-disks, which ahs been noted especially in marathon runners whose muscle fibers revealed remarkable damage to the muscle. Fibers affect both training and marathon competition. The onset and timing of this gradient damage to the muscle parallels the degree of muscle soreness experienced by the runner's disruption of the thick and thin filaments in parallel groups of sacromers as a result of force of eccentric actions or stretching of the trightened muscle fibers. This disruption of the muscle fibers triggers

white blood cells to increase the induced muscle soreness, leading to the inflammatory response observation from the induced muscle soreness.

One SGP –T derivative is a three amino acid sequence shown to be a potent anti –inflammatory molecule with systemic effects. This three amino acid peptide is a phenylalanine glutamine glycine (FEG) and its D-Isomeric form (FEG) have become the foundation for the IMSAID category. The cellular effects of the IMSAIDS are characterized in a number of publications. Peptides are known to modulate leukocyte (white blood cells) activity by influencing cell surface receptors to inhibit excessive activation and tissue infiltration.

IMSAIDS, the tripetide FEG (Phe –GIV –Gly) and its D-Isomer FEG are known to alter leukocyte adhesion involving actions on MB2integrin, and inhibit the binding of CD16b (FcyRiii) anti body to human neutrophils. FEG decreases circulating neutrophil and eosinophil accumulation, decreases intracellular oxidative activity, and reduce the expression of CD49d after antigen exposure.

2.1.25 LONG TERM EFFECTS

Anti inflammatory treatment trials for existing Alzheimer's disease have little to no effect on halting or reversing the disease. Two studies from 2012 and 2013 found regular use of aspirin for over ten years is associated with an increase in the risk of macular degeneration.

2.2.0 TYPES OF INFLAMMATION

Acute inflammation

Chronic inflammation.

2.2.1 Acute Inflammation: is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of bio-chemicals events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells with the injured tissues. Acute inflammation occurs over seconds,

minutes hours and days. Dictionary/thesaurus legal, encyclopedia, Wikipedia

2.2.2 CHRONIC INFLAMMATION

It is a prolonged inflammation, which leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of tissue from the inflammatory process.

2.2.3 COMPARISON BETWEEN ACUTE AND CHRONIC INFLAMMATION

	ACUTE	**CHRONIC**
Causative agents	Bacterial pathogens, injured tissues	Persistent acute inflammation due to non degradable pathogens, viral infection, persistent foreign bodies and acute immune reaction.
Major cells involved	Neutrophils (primary), basophils and eosinophils	Lymphocytes, mononuclear cells and fibroblasts.
Primary mediators	Vasoactive amines, eicosanoids	IFN –Y and other cytokines growth factors, reactive oxygen specie.
Onset	Immediate	Delayed
Duration	Few days	Up to many months or years
Outcomes	Resolution, chronic inflammation	Tissue destruction.

2.3 HEALTH SUPPLEMENTS.

Some herbs and health supplements have anti inflammatory qualities, including devils claw (Harpagophytum procumbens) hyssop, ginger (zingiber officinale), turmeric (curcuma longa), Arinca Montana (containing helenalin) and willow bark (containing salicyclia acid) other anti –inflammatory dietary sources include pomegranate (punica granatum), green tea (camellia sinensis), cats claw (uncaria tometowsa and uncaria guianensis), Indian olibaum (boswelia serrata) and pineapple bromelain (Ananas comosus). A cannabinoid also has anti inflammatory effect.

2.4 EXERCISE AS A TREATMENT FOR INFLAMMATION.

Regular physical activity is reported to decrease makers of inflammation although the correlation is imperfect and seems to reveal differing results contingent upon training intensity. While baseline measurements of circulating inflammatory makers do not seem to affect greatly between healthy trained and untrained adults, long-term chronic

training may help reduces chronic low-grade inflammation levels of inflammatory makers (IL-6) remained elevated longer into the recovery period following an acute bout of exercise in patients with inflammatory disease.

Moderate intensity training has milder and less-established anti-inflammatory benefit.

2.5 OUTCOME OF THE INFLAMMATION

The outcome in a particular are circumstance will be determined by the tissue in which the injury has occurred and the injurious agent that is causing it.

2.5.1The possible outcomes to inflammation by Arthur Schopenhauer (1788-1860) are as follows;

1. **Resolution**.

   The complete restoration of the inflamed tissue back to a normal status. Inflammatory measures such as vasodilatation, chemical

production, and leukocyte infiltration cease, and damaged parenchyma cells regenerate.

II **Fibrosis.**

Large amounts of tissue destruction, or damages, in tissues unable to regenerate, cannot be regenerated completely by the body. Fibrous scarring occurs in these areas of damage, forming ascar composed primarily of collage. The scar will contain any specialized structures, such as parenchyma cells,

III. **Abscess Formation**

A cavity is formed containing pus, an opaque liquid containing dead white blood cells and bacteria with general debris from destroyed calls.

2.6 Benefit of inflammation are;

- Activation of immune system
- Destruction of pathogens
- Delivery oxygen and nutrients
- Toxin dilution.
- It helps the body to heal from injuries or fight infection but excessive inflammation can lead to health problems.
- Inflammation helps in the development of diseases such as inflammatory bowel disease rheumatoid agents arthritis, coronary heart disease, diabetes, asthma, lumps, cancer, dementia, cystic fibrosis and multiple sclerosis

2.7 ANTI- INFLAMMATORY AGENTS

A number of medications are used to prevent and control the symptoms of asthma and to reduce the frequency and severity of episodes. Asthma medications are categorized into three main types; anti-inflammatory agents, which suppress inflammation bronchodilators which relax smooth muscle constriction and open the always, and leukotriene receptor antagonists (LTRAS, sometimes called leukotriene.

2.8 STEROIDS

Steroids is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other.

Glucocorticoids reduce inflammation or swelling by binding to glucocoriticoid receptors. These drugs are refereed to as corticosteroids.

2.9 NON – STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

Non-steroidal anti-inflammatory drugs (NSAIDS), are type of pain reliever that inhibit the cycloxygenase (cox) enzyme. cox enzymes synthesizes prostaglandins, that cause inflammation.The prostaglandins from ever being synthesized, reducing or eliminating the pain. From Wikipedia, free encyclopedia.mht

Examples of NSAIDS are: aspirin, ibuprofen, and naproxen.

Non steroidal anti-inflammatory drugs that can be purchased over the counter include.

BRAND NAME	GENERIC NAME
Advil Motrin	Ibuprofen
Aleve	Naproxen sodium
Ascriptin, Bayer, Ecoirin	Aspirin

There are analgesics that are commonly associated with ant-inflammatory drugs but have no anti-inflammatory effects. An examples is paracetamol, called acetaminophen in the U.S and sold under the brand name of Tylenol, which reduce pain and inflammation by inhibiting Cox enzymes, paracetamol block the reuptake of endocann abinoids, which only reduces pains explaining why it has minimal effect on inflammation.

Long-term use of NSAIDS can cause gastric erosions, which can become stomach ulcers and in extreme cases can cause severe hemorrhage, resulting in death.

The risk of death as a result of use of NSAIDS is I in 12,000 for adults aged 16-45. the risk increases almost twenty fold for those over 75. other dangers of NSAIDs are exacerbating asthma and causing kidney damage. Apart from aspirin. Prescription and over the counter NSAIDS also increase the risk of myocardial infraction and smoke.

2.10 IMMUNE SELECTIVE ANTI-INFLAMMATORY DERIVATIVES (IMSAID)

Immune selective Anti-inflammatory derivatives are a Therapeutics, LLC, which were discovered to have diverse biological properties, including anti-inflammatory properties.

IMSAIDS work by altering the activation and migration of inflammatory cells, which are immune cells responsible for amplifying the inflammatory response. IMSAIDS represent a new category of anti-inflammatory and are unrelated to steroid hormones or non-steroidal anti-inflammatory.

The IMSAID were discovered by scientist evaluating biological properties of he submandibular gland and saliva. It is now accepted that the Immune, nervous, and endocrine systems communicate and interact to control and modulate inflammation and tissue repair. One of the neuroendocrine pathways, when act ated, results in the release of Immune – regulating peptides from the subsmandibular gland uron

neuronal stimulation from sympathetic nerves. This pathway is referred to as the cervical sympathetic tunic-submandinular gland (CST-SM6) axis, a regulatory system that plays a role in the system control of inflammation.

2.11 ANTI-INFLAMMATORY DIET

They anti-inflammatory diets includes the following:-

1. Eat list of fruits vegetables, and wild seafood. Add generous portions of deeply – pigmented vegetables to every meal and snack for their fiber and natural anti-inflammatory compounds. Many herbs and foods such as turmeric, oregano, garlic, greentea, blueberries, nutmeg, ginger contain bioflavonoid and polyphenols that limit free – radical production in the body.
2. Add essential fatty acids (EFA'S) to your diet: because omega -3 fatty acids are in shorted supply in our modern diet. Its good to take omega- 3 supplements daily to rebalance you diet. This is

one of the simplest, safest, yet most effective steps you can take to quell chronic inflammation in your body.

Some practitioners also recommend adding an omega 6-supplements called gamma linolenic acid (GLA), if you have rheumatoid arthritis

Eliminate certain foods and additive from diet:

By trans-fats hydrogenated oils. Next would be the sugars, refined carbohydrates, and gluten – containing foods that woman often crave when their systems are off-balance. Examples nuts, seeds and certain spices such as ginger.

Anti-inflammatory diets, prostaglandins are hormone like substances that affect the body in variety of ways, also regulating inflammatory mediation. An anti-inflammatory diets includes less foods that create inflammation causing prostaglandins (PGE_2) in the body, and more foods that create anti-inflammatory prostaglandins. (PGE_1 and PGE_3)

2.12 NATURAL ANTI-INFLAMMATORY SUPPLEMENTS

1. Add a high-quality daily multivitamin /mineral complex. A connection between higher blood levels of certain nutrients blood levels of certain nutrients and lower risk. Of health addition caused by inflammation like arthritis, cardioscular disease and insulin resistance. Along with the benefits of folic acid other vitamin B, Vitamin D also have anti- inflammatory effects. And vitamin C,A and E are powerful antioxidants, countering the effects of free radical damage

2. Bioflavonoid. Also called flavones or flavonoids, is a class of chemicals that our bodies metabolize in away that offers strong anti-inflammatory effects.

   Examples includes Tea, Cocoa, Vegetable and citrus fruits etc.

Among the best for soothing the inflammatory cascade are quercetin, rutin and procyanidins (opc's) such as those found in pine bark extract (pycnogenol) and grape seed extract.

3. Anti-inflammatory herbs: Aside from other large groups of bioflavonoid others includes:

Boswellia (Boswellia serrata) also known as India frankincense, Boswellia serrata has been recognized in Ayurvedic medicine for its anti-inflammatory benefits. It can switch off key cell. Signalers and pro-inflammatory mediators known as cytokines in the inflammatory cascade.

Geiger: It is analgesic, anti-inflammatory, antinausea, and sugar-moderating effects in the body. Geiger extract can inhibit or deactivate genes in our body that encodes the molecules involved in chronic inflammation.

iv. Glucosamine – chondrotin; Glucosamine sulfate and chondrotin sulfate are important building blocks of healthy cartilage. Glucosamine – chondrotin supplements may help repair damages tissues, they have strong impact on the underlying cause of chronic inflammation.

2.13 BENEFITS OF NON – STEROIDAL ANTI-INFLAMMATORY DRUGS

Drugs have potential harms as well as benefits doctors would like to be able to balance any risks against benefits to derive a therapeutic ratio for each patient, formal trials can tell a lot about the efficacy of a drug in specific context, but unless they are huge and pragmatic they tell less about a drug's toxicity.

Furthermore extrapolation of the efficacy or toxicity of a drug in one disease or group of patients to those associated with different diseases or groups can be difficult and misleading.

2.14 MEDIATORS OF INFORMATION

Once leucocytes have curved at a site of infection or inflammation they release mediators which control the later accumulation and activation of other cells.

However, in inflammatory reactions initiated by the immune system, the ultimate controls extended by the antigen itself, in the same way as it controls the immune response itself.

Cellular accumulation at the site of chronic infection, or in autoimmune reactions is quite different from that at sites where the antigenic stimulus is rapidly cleared.

Inflammatory Mediators.

Are soluble, dififusible molecules that act locally at the site of tissue damage and infection, and at more distant sites. They can be divided into exogenous and endofenous mediators.

Bacterial products and toxins can act as exogenous mediators of inflammation,

Mediators of inflammatory responses are released at the site of injury by a number of cell type that either contain them as reformed molecules within storage granules, e.g histamine, or which can rapidly switch on the machinery required to synthesize the mediators when they are required, for example to produce metabolite of arachidonic acid.

2.14.1 Mononuclear phagocytes: (Monocytes & macrophages) are central to inflammation, as they produce many components which participate in or regulate the different plasma enzyme systems and hence the mediators of the inflammatory response. They are also actively phagocytic and are involved in microbial killing, as are neutrophils.

Mononuclear phagocytes are long lived and some can proliferate in situ.

2.15 Cytokines

Cytoines (Greek cyto-, cell; and – kinos, movement are small signaling molecules used for cell signaling. Cytokines can be classified as proteins, peptides, or glycoproteins.

The term “Cytokine” has been use to refer to the immunodulating agents, such as interleukins and interferons.

Adverse reactions to cytokines are characterized by local information and/or ulcerations at the injection sites.

2.15.1 Nomenclature of cytokines

Cytokines have been classed as lymphokines, interleukins, and chemokines, based on their presumed function, cell of secretion or target of action.

- The term interleukin was initially used by researchers for those cytokines whose presumed targets are principally leukocytes. The vast majority of this group are produced by T-helper cells.
- The term chemokine refers to a specific class of cytokines that mediates chemo-attraction (chemotaxis) between cells.

2.16 Histamine

Histamine a chemical found in some of the body's cells-causes many of the symptoms of allergies, such as a runny nose or sneezing. When a person is allergic to a particular substance, such as a food or dust, the immune system mistakenly believes that this usually harmless substance is actually harmful to the body. In the attempt to protect the body, the immune system states a chain reaction that promotes some of the body's cells to release histamine and other chemicals into the blood stream.

The histamine then acts on a persons eyes, nose, throat, lungs, skin, or gastro intestinal tract, causing allergy symptoms.

Antihistamine medications help to fight symptoms caused by the release of histamine during an allergic reaction.

2.17 Arachidonic acid

Is a polyunsaturated omega -6 fatty acid 20:4 (10-6). It is the counter part to the saturated arachidic acid found in peanut oil.

Chemistry

In chemical structure, arachidonic acid is a carboxylic acid with a 20-carbon chain double bond is located at the sixth carbon from the omega end.

Arachidonic acid is a polyunsaturated fattyacid present in the phospholipids Arachidonic acid in the human body usually comes from dietary animalsources, meat, eggs, dairy or is synthesized from linoleic acid.

Arachidonci acid s not one of the essential fatty acids. It does not become essential if there is a deficiency in linoleic acid or if there is an inability to convert linoleic acid to arachidonic acid, which is required by most mammals.

Arachidonic acid is a type of omega 6 fatty acid that is involved in inflammation. Like other omega -6 fatty acids, arachidonic acid is essential to health.

Omega-6 fatty acids helps to maintain the brain function and regulate growth. Eating diet that has a combination of omega 6 and omega -3 fatty acids belower lower the risk of developing heart disease.

Arachidonic acid in particular helps regulate neuron activity.

2.17.1 Arachidonic acid and Eicosanoids Eicosanoids, derived from arachidonic acid are formed when cells are damaged or are under threat of damage. This stimulus activates enzymes that transform the arachidonic acid into eicosanoids such as prostaglandin, thomboxane and leukotrienes.

Eicosanoids cause inflammation the more arachidonic acid that is present, the greater capacity the body has to become inflamed. Eicosanoids tend to act locally rather than circulate throughout the body because they degrade quickly

2.18 Nitric oxide

Nitric oxide or nitrogen oxide also known as nitrogen monoxide is a molecule with chemical formula. (NO)it is a free radical

In mammals including humans, No is an important cellular signaling molecule involved in many physiological and pathological processes.

It is a powerful vasodilator with a short half-life of a few seconds in the blood.

Low levels of nitric oxide production are important in protecting organs such as the liver from ischemic damage.

Nitric oxide Is a molecule that plays a crucial role in vascular disease. Nitric oxide is also known as endothelium – derived relaxing factor. It dilates blood vessels, reduces blood pressure, and have can be of benefit in those with angina, heart failure , pulmonary hypertension and erective dysfunction.

In addition, its powerful antioxidant, anti-inflammatory and antithrombotic actions can reduce atherosclerosis.

Low levels of (NO) are characterized by cells in the body's aging quicker, oxidative stress, inflammation, endothelial dysfunction, vascular disease, insulin resistance and type 2 diabetes mellitus.

(NO) is formed from the conversion of L-arginine by an enzyme caused nitric oxide synthase.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipments /Apparatus used for this study areas Follows:

- ❖ Beaker
- ❖ Conical flask
- ❖ Measuring cylinder
- ❖ Weighing balance
- ❖ Soxhlet extractor
- ❖ Water bath
- ❖ Test tubes
- ❖ Syringe (1ml and 5ml)
- ❖ Grinder
- ❖ Burette
- ❖ Retort stand
- ❖ Uvlight.

3.1.2 Chemicals, solvents and Reagents

Chemical	**Manufactures**
Absolute ethanol	BDH
Tween-80	Kermel
Ibuprofen	MC cure industry ltd
Ferric chloride	Qualikems
Concentrated ammonium	
Concentrated hydrochloric acid	
Methanol	Lobal chemie
Olive oil	
Dichloromethane	
N-hexane	May and beaker limited
Potassium bismuth (drangendroffs reagent)	
Distilled water	
Benzene	
Ammonia	
Potassium mercuric iodide (mayers reagent)	
Glacial acetic acid	
H2SO4 Hydrogen tetraoxosulphate (vi) acid.	
Ethyl acetate	
Acetone	Analar
Chloroform	Bio lab limited

3.1.3 ANIMALS

The experimental animals used in carryout this research study were wistar albino rats of both sexes, about 8 to 12 weeks old within an average of 140-250g.

The animals were rared in the animal house at Brain-Phosphorylation Research and Training Center No. 9 Ogui Raod, 5 Floor Right Wing Former ACB Building Enugu State and maintained on standard pellets, water and lieitun. A short period was allowed for acclimazation.

3.2 METHODOLOGY

3.2.1 COLLECTION AND PREPARATHION OF PLANT MATERIAL

Fresh stem barks of *crateva Advansonii* were collected in Asata Street Enugu State, Nigeria in the month of April 2013.

Extracts were obtained from the leaves, the leaves were plucked from the stem with sharp knife and chipped into pieces, which was air dried under room temperature and ground into powder using a

blender (grinder), the resulting powder was used for extraction. 2 bowl of grounded *crateva adansonii* was measured and power inside a bowl/bucket and 250ml of methanol measured and poured inside *crateva adansonii* and was stirred and left for 48hours.

After 48hours the mixture of *crateva advansoii* and methanol was filtered using filter cloth, after which the extract and the filtrate was gotten.

Part of the filtrate was boiled inside soxhlet extractor to get the extract.

3.4 COLUMN CHROMATOGRAPHY

Column chromatograph in the chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms upto kilograms. Is the relatively low cost and disposability of the stationary phase used in the process.

The latter prevents cross-contamination and stationary phase degradation due to recycling.

The classical preparative chromatography column, is a glass tube with a diameter from 5mm to 50mm and a height of 5cm to 1m with a tap and some kind of filter (glass wool plug-to prevent the loss of the stationary phase) at the bottom.

Two methods are generally used to prepare a column: the dry method, and the wet method.

3.4.1 Dry method: The columns is first filled with dry stationary phase, powder, followed by the addition of mobile phase, which is flushed through the column until it is completely wet, and from this point of never allowed to run dry.

3.4.2 WET METHOD

A slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles.

A solution of the organic materials is pipetted on top of the stationary phase. This layer is usually topped with a small layer of

cotton or galas wool to protect the shape of the organic layer from the velocity of newly added eluent.

Eluent is slowly passed through the column to advance the organic material often a spherical eluent reservoir or an eluent-filled and stopped separation funnel is put on top of the column.

The individual components are retained by the stationary phase different and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column with the event. At the end of the column they elute one at a time.

During the entire chromatography process the eluents is collected in a series of fractions, fractions can be collected automatically by means of fraction collectors. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds example by analytical chromatography, uv absorption or fluorescence. Colored compounds can be seen through the glass wall as moving bands

3.4.3 Column chromatography of *crateva adansonii* using each of the following: methanol, N-hexane, Dichloromethane, ethyl acetate.

3.4.4 METHANOL

Methanol, also known as methyl alcohol, wood alcohol, wood naphtha or wood spirits, is a chemical with the formula CH_30H.

Methanol acquired the name "wood alcohol" because it was once produced chiefly as a by product o the destructive distillation of wood.

Modern methanol is produced in a catalytic industrial process directly from carbon monoxide, carbon dioxide and hydrogen.

Methanol is the simplest alcohol and is a light, volatile, colourless, flammable liquid with similar to, but slightly sweeter than, that of ethanol (drinking alcohol).

Methanol is produced naturally in the anaerobic metabolism of many varieties of bacteria, and is ubiquitous in small amounted in the environment.

Over the course of several days, atmospheric methanol is oxidized with the help of sunlight to carbon dioxide and water.

Methanol burns in oxygen including open air, forming carbon dioxide and water.

$CH_30H + 30_2$-2 $\rightarrow$ 2C02 +4H20.

Methanol has a high toxicity in humans if a little as 10ml of pure methanol is ingested for example, it can breath down into formic acid, which can cause permanent blindness by destruction of the optic nerve.

3.4.5 n-HEXANE

n-hexane is a chemical made from crude oil pure, n-Hexane is a colorless liquid with a slightly disagreeable odor. It is highly flammable, and its vapors can be explosive.

Pure n- Hexane is use in laboratories. most of the n –hexane used in industry is mixed with similar chemicals called solvents.

The major use of solvents containing n-Hexane is to extract vegetables oils from corps such as soybeans. These solvents are

also used as cleaning agents in the printing, textile, Furniture and shoe making industries.

STRUCTURES OF HEXANE

3.4.6 ETHYL ACETATE

STRUCTURE OF ETHYL ACETATE

Ethyl acetae is the organic compound with the formula CH3-C100-CH2-CH3. This coloreless liquid has a characteristics sweet smell and is used in glues, nail polish removers, decaffeinating tea and coffee and cigarettes.

Ethyl acetate is the ester of ethanol and acetic acid.

Ethyl acetate is fairly volatile at room temperature and has a boiling point of 77^{o}c.

3.4.7 DICHLOROMETHANE

STRUCTURE OF DICHLOROMETHANE

Dichloromethane is an organic compound with the formula CH2CL2. This colorless volatile liquid with a moderately sweet aroma is widely used as a solvent. Although it is not miscible with water, it is miscible with many organic solvents.

3.4.8 COLOUMN CHROMATOGRAPHY OF *CRATEVA*

ADANSOII USING METHANOL

1. Use a 5 inch disposable glass pipette a your column
2. Choose a solvent, which I made you of 2500ml of methanol

3. Place a cotton plug at point where the pipette narrows, and pack with silica gel just as you would use a normal glass column leaving an inch or two of silica free space at the top.
4. I applied the compound that is the extract of *crateva adansonii* and elute as usually, using either a pipette bulb or compressed air source to flash the solvent through.

    You will have to refill often, and experiment with fraction size, depending on how difficult the separation is. It is possible to separate components of very similar relative fraction.

    Finally, the filtrates was system and the extract was equally obtained after boiling part of the filtrate to dryness with water bath.

    Both the filtrate and extract were used in phythochemcal analysis/screeing

3.5 PHYTOCHEMCIAL ANALYSIS OF THE EXTRACT

Photochemical screening for *crateva adasonii* extract test for the presence of alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponin, phlobatannins, tannins was carried out.

3.5.1 TEST FOR ALKALOIDS

0.4g of *crateva adansonii* extract was stirred with 8ml of 1% Hcl and the mixture was warmed and filtered. 2ml of filterate was treated separately with a few drops of potassium mercuric iodide (mayers reagent).

Turbidity or precipitation with either of these reagents was taken as evidence for existence of alkaloids.

3.5.2 TEST FOR SAPONINS

The ability of saponins to produce emulsion with oil was used for the screening test. 10mg of the plant extract was boiled in 20ml of distilled water in a water with, for 5min and filtered.

10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for froth formation.

3 drops of olive, oil were mixed with froth, shaken vigorously and observed for emulsion development.

3.5.3 TEST FOR ANTHRAQUINONES

20mg of plant extract was boiled with 6ml of 1% Hcl and filtered . The filtrate was shaken with 5ml of benzene filtered and 2ml of 10% ammonia solution was added to the filtrate.

The mixture was shaken and the presence of a pink, violet or red colour in the ammonical phase indicated the presence of free hydroxyl anthraquinones.

3.5.4 CARDIAC GLYCOSIDES DETERMINATION

5ml of glacial acetic acid having one drop of $fecl_3$ (ferric chloride solution)

To the mixture obtained 1ml of concentrated, H2SO4 was added to form a layer.

The presence of brownring of the interface indicated deoxy sugar-characteristics of cardiac glycosides.

3.5.5 TEST FOR COUMARINS

In a small test tube, 30mg of plant extract was covered with filter paper; it was examined under Uv light yellow florescence indicated the presence of coumarins.

3.5.6 TEST FOR PHILOBATANNINS

40mg of extract was boiled in 15 aqueous Hydrochloric acid, the deposition of a red precipitate indicated the presence.

3.5.7 TEST FOR FLAVONOIDS

20mg of extract was suspended in 20ml of distilled water to get the filtrate. 5ml of dilute ammonia solution was added to 5ml of filtrate followed by few drops of con.c H2S04. Presence of flavonoids was confirmed by fellow coloration.

3.5.8 TEST TO TANNIS

50mg of extract was boiled in 20ml of distilled H20 and filtered.

A few drops of 0.1% fecl3 was added in filtrate and observed for colour change brownish green or a blue-black coloration was taken as evidence for the presence of tannins.

5.5.9 TEST FOR RESINS

A. Colour Test

A quantity, 0.12g of the extract was extract with chloroform and the extract come to dryness.

The residues was re-dissolved in 3ml of acetone and 3ml con.c HCL added.

This mixture was heated in a water bath for 30minutes.

A pink colour that changes to mayers red indicates the presence of resins.

3.6 THIN LAYER CHROMATOGRAOPHY (TLC)

TLC is a simple, quick, and in expensive procedure that gives chemist a quick answer as to ho many components are in a mixture.

TLC is used to support the identity of a compound is compared with the Rf of a known compound.

A TLC plate is a sheet of glass, metal or plastic which is coated with a thin layer of a solid adsorbent.

A small amount of the mixture to be analyzed is spotted near the bottom of this plate.

3.7 ANTI-INFLAMMATORY ACTIVITY TEST

The rats paw edema method of winter et al, (1962) was used for these test.

The increased in the right hind paw volume induced by the sub planter injection of fresh egg albumin (Akah and Njike, 1990) was used as a measure of acute inflammation. Adult wistar albino rates of either sex were divided into 4 groups of 4 animals each. The first two groups received different doses of extracts (100mg/kg and 500mglkg b.w) in 3% Tween- 80 administered intraperitoneally. The control group received equivalent volume of 3% Tween-80, while the standard group received 100mglkg of ibuprofen.

One hour after administration of test substances, acute inflammation was induced by injection of 0.1ml of undiluted fresh egg album into the sub plantar of the right hind paw of rats. The volume of the paw as measured by mercury displacement before and at 30s, 1.00 2.00, 3.00,

4.00 hours after egg albumin injection. Edema formation was assessed in terms of the different in the zero time paw volume of the injected paw and its volume at the different times after egg albumin injection for each doses of extract percentage inhibition of edema was calculated using the relation (Perez, 1996) inhibition of edema (%) 100 (1-(a-x)(b-y) where:

A = mean paw volume of treated rats after egg albumin injection

A = mean paw volume of treated rats before egg albumin injection

B = mean paw volume of control/rats after egg albumin injection

Y = mean paw volume of control rats before egg albumin injection.

3.8 THIN LAYER CHROMATOGRAPHY (TLC)

Solvents for developing TLC are:

40ml of chloroform = 16ml
30ml of methanol = 4ml
10ml of diethylether = 4ml

which serves as buffer solution

A small sample of the evaporated dichloromethane was dissolved with a little dichloromethane liquid.

Using a capillary tube, the dissolved sample was loaded (placed) on chromatography paper on the line which had been created with pencil towards the end. It is then placed in the TLC chromatography containing the mixed solvents (serving as buffer solution).

The solvent in the bottle is allowed in such a way that it does not touch the marked line on the TLC paper (TLC plate).

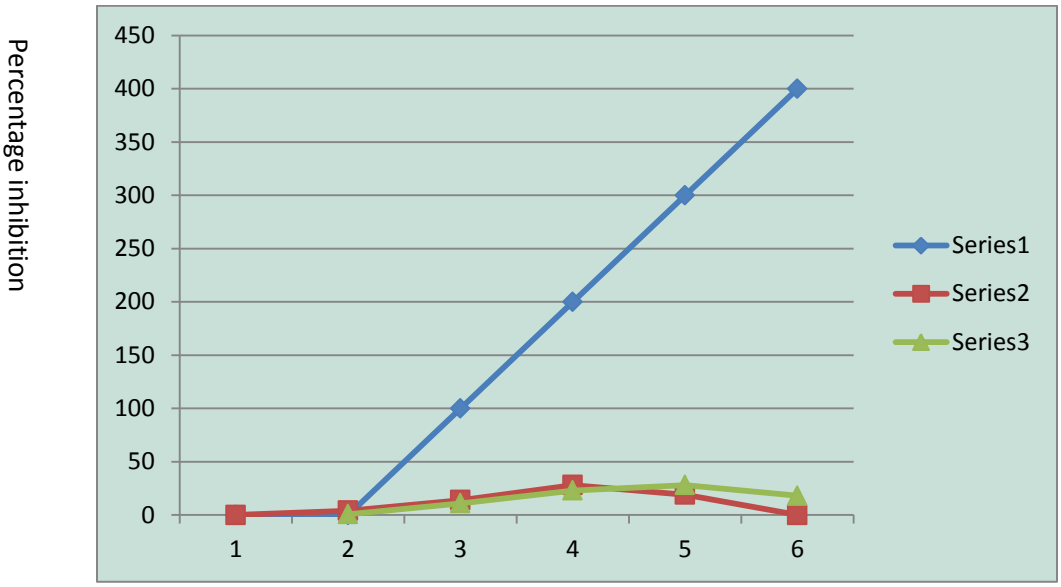
If the content containing more than one band, it means that more than one constituent or compound is present.

RESULT

Only one compound was found (one band) which mean that the dichloromethane extract contains a pure compound.

CHAPTER FOUR

4.1 The effect of methanol fraction of methanol extract of crateva adansonii on acute inflammation of rat paw.



4.2 PHYTOCHEMICAL ANALYSIS OF EXTRACT

Photochemical constituents of methanol fraction of *crateva adansonii.*

s/n	Constituent	Experimental method	Relative presence in methanol extract
1	Tannin	Ferric chloride test	-
2	Flavonoids	ammonic test	+++
3	Alkaloids	Mayers test	++
4	Resins	Colour test	+++
5	Saponins	Emulsion test	_
6	Phlobatannins	Hydrogen chloride	+
7	Glycosides	Ferric chloride test	+
8	Coumarins	Sodium hydrogen test	_
10	Anthraquinnones	Ammonium test	-

+:Present in little concentration

++:Present in very high concentration

-:Absent

4.3 PHYTOCHEMCIAL ANALYSIS OF DICHLOROMETHANE FRACTION

Phytochemcal constituents of dichloromethane fraction of methanol bark extracts of *crateva adansonii.*

s/n	Constituents	Experimental method	Relative presence in methanol extract
1	Alkaloids	Mayers test	-
2	Saponin	Emulsion test	-
3	Anthraquinones	Ammonia test	-
4	Glycosides	Sulphuric acid test	+
5	Coumarins	Sodium hydroxide test	-
6	Phlobotannins	Hydrogen chloride test	-
6	Tannins	Ferric chloride test	-
7	Resins	Colour test	-

CHAPTER FIVE

5.1 DISCUSSION

This study investigates the pytochemical and methanolic extract of *crateva adansonii*. (winter et al; 1962).

This study established pytochemically that methanol extract of *crateva adansonil* bark contains glycoside, Resins, oil, saponins, alkaloids, flavonoids, phlobathannins, coumarins, anthraquinones, tannin,.

The result of this study indicates that methanol extracts of *crateva adansonii* administered at 100mglkg.b.w and 500mglkg b.w suppressed egg albumin induced to the rat paw edema. This in acute edema of model. The methanol extract showed significant effect by suppressed inflammation.

The ibuprofen exhibited lower level of inhibition at three hour and has less inhibition in control at 4hour which is also at the end of the experiment.

Egg albumin ha been known to induce acute inflammation as it macroscopically characterized by swollen pains, heat and redness

which are mediated by prostaglandin, cytokine, chemokines, histamine, serotonin mast, basophile, macrophages etc.

5.2 CONCLUSION

Following the experimental analysis and result, the methanol extract stem bark of *crateva adansonii* has greater significant in the treatment of acute inflammation in white albino rats. It is recommended or such cases of inflammation sited in these research. Further researchers are encouraged to use other parts of the plants.

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APPENDIX

1.1 Table on the effect of extract on acute inflammation of rat paw.

Time	30s	100	200	300	400
Inhibition%	4	14	28	19	0
	0.8	11	23	28	18

Group 1 (control)

s/n	Mark	Bodyweight	Dosage	Volume (ml)
1	Head	91.37		0.5
2	Two hands	90.19		0.5
3	Two hands two legs	91.79		0.5
4	Base of tail	92.03		0.5
				2.0ml

s/n	Mark	0.00	30	1.00	2.00	3.00	4.00
1	Head	5.20s	4.60	4.60	4.60	4.80	4.80
2	Two hands	5.20	4.60	4.50	4.60	4.60	4.80
3	Two hands two legs	5.30	4.60	4.40	4.50	4.60	4.70
4	Base of tail	5.20	4.60	4.70	4.70	4.80	4.90

s/n	0.00	30s	1.00	2.00	3.00	4.00
1	0.80	1.40	1.40	1.40	1.20	1.20
2	0.80	1.40	1.50	1.40	1.40	1.20
3	0.70	1.40	1.60	1.50	1.40	1.30
4	0.80	1.40	1.40	1.30	1.20	1.10

GROUP 2 (STANDARD)

s/n	Mark	0.00	303	1.00	2.00	3.00	4.00
1	Dot at back	5.20	4.00	4.60	4.70	4.80	4.80

2	1 leg	5.20	4.60	4.60	4.80	4.80	4.90
3	Stomach	5.33	4.70	4.70	4.80	5.00	5.00

s/n	30s	1.00	2.00	3.00	4.00
1	0.60	0.60	0.50	0.40	0.40
2	0.60	0.60	0.40	0.40	0.30
3	0.65	0.65	0.55	0..35	0.35
X	0.62	0.62	0.48	0.38	0.35

	30	1.00	2.00	3.00	4.00
X	0.62	0.62	0.48	0.38	0.35
Y	0.625	0.70	0.625	0.525	0.425
	0.80	11	23	28	18

Formular for calculating the

$\%I = 100\ (1-\frac{x}{Y})$

METHANOL FRACTION GROUP 3

s/n	Mark	0.00	30s	1.00	2.00	3.00	4.00
1	1 hand	5.20	4.50	4.60	4.60	4.60	4.60
2	2 legs	5.20	4.70	4.60	4.70	4.80	4.80
3	2 slides	5.30	4.80	4.80	5.00	5.00	5.00
4	Lear	5.30	4.60	4.60	4.90	4.90	4.90

s/n	Mark	Body weight	Dosage	Volume (ml)
1	1 hand	106.70	53.35s	0.50
2	2 legs	106.27	53.14	0.50
3	2 sides	106.40	53.20	0.50
4	1 ear	105.72	52.86	0.497
			212.55	2.0ml

s/n	Mark	0.00	30s	1.00	2.00	3.00	4.00

1		0.80	1.50	1.40	1.40	1.40	1.40
2		0.80	1.30	1.40	1.30	1.20	1.20
3		0.70	1.20	1.20	1.00	1.00	1.00
4		0.70	1.40	1.40	1.10	1.10	1.10

s/n	Mark	0.00	30s	1.00	2.00	3.00	4.00
1			0.70	0.60	0.60	0.60	0.60
2			0.50	0.60	0.50	0.40	0.40
3			0.50	0.50	0.30	0.30	0.30
4			0.70	0.70	0.40	0.40	0.40
			0.60	0.60	0.45	0.42	5

	Mark	30s	1.00	2.00	3.00	4.00
X		0.60	0.60	0.45	0.475	0.425
Y		0.625	0.70	0.625	0.525	0.425
%		4	14	28	19	0