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EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF ARGYREIA NERVOSA LEAF EXTRACTS

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Abstract : Herbal therapy has been increasingly popular recently, signalling a "Return to Nature" from modern medication. The use of medicinal plants as a rich source of therapeutic substances for the treatment of sickness and other afflictions has been recognised for millennia and is highly regarded around the world. Nature has given us a very varied collection of plants that are grown wild around our nation in a wide variety of settings. Since ancient times, it has been popular in India to treat specific illnesses with various components of various medicinal plants. Common medicinal plant Argyreia nervosa is utilised in a number of ethno-medical formulations. It has historically been utilised as an antibacterial, antifungal, analgesic, anti-inflammatory, etc. substance. The anti-inflammatory efficacy of the leaf from Argyreia nervosa was examined in the current study using both an aqueous extract and a methanol extract. Aqueous and alcoholic leaf extracts of Argyreia Nervosa, 400 mg/kg body weight, shown considerable analgesic and anti-inflammatory action in the current investigation. However, at a dose of 200 mg/kg body weight, the same extracts showed little analgesic and anti-inflammatory efficacy. In conclusion, the presence of flavonoids and steroids in both aqueous and alcoholic extracts may be the cause of the analgesic and anti-inflammatory effects.

IndexTerms **- Argyreia Nervosa, Carrageenan, Plant extracts, Tail flick, Anti-Inflammatory, Analgesic**

INTRODUCTION

Since ancient times, medicinal plants have been employed as treatments for human ailments. Traditional medicine is acknowledged and respected for its role in the preservation of health and the treatment of diseases as the knowledge, skills, and practice of holistic health care. It is based on theories, beliefs, and experiences that have persisted over generations among the indigenous peoples. People, especially those from rural and developing areas, are frequently influenced to utilize traditional medicines by the expensive expense of allopathic therapy and its potential side effects. The growing demand for plant extracts in the cosmetics, food, and pharmaceutical industries suggests that methodical research on medicinal plants could significantly advance the identification of new bioactive chemicals and their application as drugs to treat a range of ailments. (1) Current developments have opened up new fields of study for the biological functions of medicinal plants due to the bacterial resistance to available antibiotics and the acceptance of traditional medicine as an alternative form of healthcare. (2) Almost 80% of the world's population in underdeveloped nations uses herbal medicines for primary healthcare. Because to their effectiveness, safety, cultural acceptability, and less side effects, they have withstood the test of time. They are thought to have higher compatibility with the human body because the chemical components they contain are a part of the physiological processes of living flora. It's interesting to note that ancient literature also has references to various herbal treatments for age-related illnesses like memory loss, osteoporosis, diabetic wounds, immunological, and liver ailments. Ayurveda, Unani, and Siddha are just a few of the many medical systems that use plant-derived oils and extracts because India is such a rich supply of medicinal herbs. Only a few of these plant-based treatments have, however, received scientific investigation. Natural substances originating from plants, such as flavonoids, terpenes, and alkaloids, have drawn a lot of interest recently because of their wide range of pharmacological capabilities, which include anti-inflammatory, antipyretic, analgesic, and wound healing effects. The goal of the current study is to investigate how Argyreia Nervosa Leaf Extract works as an anti-inflammatory and analgesic. For a better understanding of the potential mechanism of pharmacological action, efforts have also been made to support the traditional claim of plant by utilising relevant animal models. (3)

INFLAMMATION: To survive, all organisms must get rid of outside invaders like pathogens and damaged tissues. Inflammation is the name of a sophisticated host response that mediates these actions. The goal of inflammation, a protective response involving host cells, blood vessels, proteins, and other mediators, is to remove the original insult that caused the cell injury as well as the necrotic cells and tissues that resulted from it. It also serves to start the repair process. (4) Inflammation first neutralises dangerous substances by dilution, destruction, or other means before carrying out its protective function (e.g. microbes, toxin). It subsequently initiates the processes necessary for eventual wound healing and restoration. Infections wouldn't be stopped by inflammation, and wounds wouldn't ever recover. One element of the defence mechanism known to immunologists as innate immunity is inflammation. Despite the fact that inflammation promotes repair and aids in the removal of infections and other harmful stimuli, both the inflammatory response and the following repair process can themselves be quite harmful. Inflammatory reactions have a component that can harm healthy tissues in addition to destroying and eliminating germs and dead

tissue. Therefore, damage may accompany perfectly healthy, beneficial inflammatory reactions, and it may even overpower them if they are excessively strong (such as when an infection is extremely severe), persistent (such as when the eliciting agent resists eradication), inappropriate (such as when they are directed against self-antigens in autoimmune diseases), or against typically benign environmental antigens (e.g. in allergic disorders). Disorders that are the result of improper, frequently chronic inflammation are some of the most troublesome diseases that affect humans.

The aim of the inflammatory response is to move the host defence molecules and cells, such as leucocytes and plasma proteins, which normally circulate in the blood, to the site of infection or tissue damage. The extracellular matrix (ECM), which is made up of cells and proteins, as well as the resident cells of vascular walls, are also engaged in inflammation and repair. (5) The side effects of the various pain medications ranged from unpleasant to dangerous. (6) Non-steroidal anti-inflammatory medications (NSAIDs), opioids, and corticosteroids, which are frequently used in contemporary medicine to decrease pain and inflammation, only offer symptomatic relief. Moreover, using these medicines is linked to significant side effects. (7) So, there is justification for the quest for new, safe, and efficient analgesic and anti-inflammatory medications. Signs of inflammation are Rubor(redness), Tumor(swelling), Colar(heat), Dolar(pain) and Functiolaesa(loss of function). (8)

Agents which cause to inflammation:

- Bacteria, viruses, and their toxins are examples of infectious agents.
- Agents of immunology include cellular responses and antibody responses to antigens.
- Physical agents include radiation, heat, cold, and mechanical stress.
- Organic and inorganic toxins undergo chemical transformations.
- Inert substances: foreign objects.

Types of inflammation: (9)

Acute inflammation: It has a brief duration (less than 2 weeks), indicates the body's initial response, clears up rapidly, and is typically followed by healing. Here, plasma proteins and leucocytes are quickly transported to the site of injury. At this point, the leukocyte eliminates the invaders and starts the process of breaking down and eliminating necrotic tissues.

Chronic inflammation: It has a longer duration and either happens after the acute inflammation's primary cause has lasted for a long time or because of stimulation that causes chronic inflammation right away. The existence of chronic inflammatory cells including lymphocytes, plasma cells, and macrophages, the creation of granulation tissue, and in some circumstances, granulomatous inflammation, are the defining characteristics of chronic inflammation. The difference between acute and chronic inflammation as shown in table no. 1 and chemical mediators for inflammation as shown in table no 2.

Table no 1: Features of acute and chronic inflammation

Feature	Acute	Chronic
Onset	Fast: minutes or hours	Slow: days
Cellular infiltrate	Mainly neutrophils	Monocytes/macrophages and Lymphocytes
Tissue injury, fibrosis	Usually mild and self-limited	Often severe and progressive
Local and systemic Signs	Prominent	Less prominent; may be subtle

Table no 2: Chemical mediators for inflammation: (10)

Mediator	Source(s)	Actions
	Cell derived	
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Serotonin	Platelets	Vasoconstriction
Prostaglandins	Mast cells, leucocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leucocytes	Increased vascular permeability, emotaxis, leukocyte adhesion and activation
Platelet activationfactor	Leucocytes, mastcells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Reactive oxygenspecies	Leukocytes	Killing of microbes, tissue damage
Nitric oxides	Endothelium,macrophage	Vascular smooth muscle relation, killing of microbes
Chemokinase	Leucocytes, activated macrophages	Chemo taxis, leukocyte activated

Methodology:

Collection and Authentication of plant material: The leaves of Argyreia nervosa were obtained from a reputable botanist in the district of Washim, Maharashtra, and the plant material was authenticated by Dr. Pramod Kumar Proffesor and the Head of the Department of Pharmacognosy at the V.L.College of Pharmacy, Raichur. (NETPC-2021/09 is the number for the herbarium specimen.) The collected plant material was washed with water, and samples were then air-directed with a dehumidifier at room temperature. The samples were put into a sealed plastic container after being mechanically ground to powder.

Preparation of Aqueous extract:

Method of Maceration: In this procedure, the entire or coarsely powdered crude medication is put in a stoppered container (1:6 ratio) with the solvent and let to stand at room temperature for at least 6 days while being frequently stirred to dissolve the soluble material. Then, the combined liquids are clarified by filtration or decantation after standing. The mixture is then strained, the marc (the moist solid material) is pressed, and the mixture is clarified. (11-12)

Preparation of Alcoholic extract:

Soxhlet extraction: When there are insoluble contaminants present and the solid sample has a limited solubility in a solvent, soxhlet extraction has historically been used. The main chamber of the Soxhlet extractor is filled with a porous thimble containing a solid sample. The extraction cycle is frequently repeated by refluxing the solvent through the thimble using a condenser and a syphon side arm. Soxhlet extraction is a reliable, time-tested method that allows for unattended extraction. Unfortunately, it necessitates a protracted extraction period and substantial solvent use. The solvent is reconstituted in a CE separation solution after evaporation and then injected into the capillary because the extract volume is significantly bigger than a usual injection volume for CE. (13)

Preliminary phytochemicals screening:

Using the following substances and reagents, the extract's phytochemical characteristics were examined (Trease and Evans, 1983). With the aid of Mayer and Dragendoff's reagents, alkaloids, saponins (frothing test), tannins ($FeCl_3$), glycosides (NaCl and Fehling's solution A and B), cardiac glycosides (Salkowski test), flavonoids (NaCl and HCl), anthraquinones (Borntrager's reaction), phenols ($FeCl_3$ and $K_3Fe(CN)$ (filter paper). (14)

Acute oral toxicity studies (OECD 423)

The goal of the acute toxicity test is to determine the therapeutic index, or the ratio of the lethal dose to the therapeutically effective dose (LD_{50}/ED_{50}) for the same strain and species. The safety of a substance increases with dosage level. (15)

According to the OECD-423 recommendations, the acute toxicity research of extracts of EEAN and AEAN was conducted. Following a 16-hour fast, rats received oral dosages of extracts ranging from 5, 50, 300, and 2000 mg/kg body weight. After receiving the medicine, the animals were closely monitored for the first 30 minutes. They then had ongoing monitoring for 14 days. No dead animals were discovered during this observation. Up to 2000mg/kg of body weight, there were no clinical symptoms and no cases of toxicity. According to their touch, pain, illumination, pinna, corneal, and grasping reflexes, the animals have demonstrated a typical response to awareness. The movement of the eyes, salivation, colour of the skin, lacrimation, movement of the fur, respiration, and sleep were also found to be normal. Throughout the observation period, no coma, tremor, convulsive, or writhing reflexes were observed. The extracts are therefore thought to be non-toxic. No discernible behavioural alterations were apparent in the extracts. (16)

ANALGESIC ACTIVITY:

Analgesia is an ill defined, uncomfortable sensation known as analgesia is typically brought on by noxious stimuli, either internal or external. Analgesic is a term used to describe a medication that selectively relieves pain by impacting the CNS or peripheral pain mechanisms without significantly affecting consciousness. (17)

Eddy's hot plate method: The hot plate method developed by Eddy is used to test the central analgesic activity. The approach makes use of heat as a pain reliever. Animals are placed one at a time on heated plates that are kept at a constant temperature of 55°C to 10°C. The animal's reaction time, or the amount of time it takes to lick its rear paw or leap out of the way before it touches the hot plate, is interpreted as the animal's response to painful stimuli. The reaction time is sped up by analgesics. Selected albino mice of either sex were separated into six groups of varying animal sizes. These animals had 18 hours of testing before the experiment. Animals from group-1 were used as controls and received 5% gum acacia. Group-2 was treated with a regular medication, Pentazocine (10mg/kg), and was regarded as the control group. The remaining four groups received aqueous and ethanolic leaf extracts of Argyria Nervosa in doses of 200 mg/kg and 400 mg/kg, respectively, body weight. At 0, 30, 60, 90, and 120 minutes following the administration of the standard medication and the aqueous and ethanolic extract of Argyria Nervosa leaf extract, the response time for each minute was recorded. (18)

ANTI-INFLAMMATORY ACTIVITY:

An ordinary protective reaction to tissue damage brought on by physical stress or harmful chemicals is inflammation. It is the body's response to tissue repair and is brought on by the release of chemical mediators from the wounded tissue and migratory cells. Depending on the type of inflammatory activity, several chemical mediators are used, including histamine, serotonin, lipids like prostaglandins, and tiny peptides like resins. Three primary purposes are served by the acute inflammatory response. The acute inflammatory exudate was present in the afflicted area for a brief period of time. Proteins, fluid, and cells from nearby blood arteries are carried by the exudate into the wounded location to facilitate local defences. If an inductive causal agent (such as a bacteria) is present in the damaged location, it will be killed and removed by the exudate's components. The debris from the injury location can be removed as the injured tissue is broken down and partially liquified. (19)

Carragenan induced rat paw oedema model: We chose albino rats of either sex, weighing 150–200g, and kept them on a regular diet with free access to water. There were six animals in each of the groupings of the animals. Standard group, aqueous, and ethanolic Argyria Nervosa extracts were used to treat the various groups. Thirty minutes before the injection of 0.1 ml of 1% carrageenan suspension in normal saline, the rats received the standard control, diclofenac, aqueous extract, and ethanolic extract. The carrageenan suspension was injected into the subplanar region of the left hind paw using a no. 26 gauge needle, while the right hind paw served as a reference. Afterwards, using the mercury displacement method, the

oedema volumes of the injected paws were measured plethismographically. The volume of oedema was assessed at several predetermined time intervals for comparison purposes. The mean oedema volume was derived from the difference between the treated animals' paw volumes, and the formula was used to calculate the percentage of oedema volume reduction. (20)

Percentage = V0 - Vt / Vo× 100

Where V0 =Volume of the paw of control at time 't'

V1 =Volume of the paw of drug treated at the time 't'

From the obtained data, the mean oedema volume and the percentage reduction inoedema was calculated.

Result and Discussion:

Preliminary phytochemical screening: Argeria Nervosa leaf extracts in aqueous and ethanol show the presence of fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins, and phenolic compounds, whereas extracts in methanol show the presence of carbohydrates, protein, amino acids, fixed oil, fats, phytosterols, glycosides, flavonoids, and alkaloids, as well as tannins and phenolic compounds. The result of Preliminary Qualitative Phytochemical examination of Leaf Argeria Nervosa as shown in table no 3.

Table no 3: Preliminary Qualitative Phytochemical examination of Leaf Argyreia Nervosa.

S.No:	Tests	Ethanol extract	Water extracts
1	**Alkaloids**		
	Dragendroff's test Mayer's test Hager's test Wagner's test	+ ve + ve + ve + ve	+ ve + ve + ve + ve
2	**Carbhohydrate**		
	Fehling's test Molish test	+ ve + ve	+ ve + ve
3	**Gums/Mucilage**		
	Water Alcohol	- ve -ve	ve ve
4	**Tannins**		
	Aq. FeCl3 TestAlc. FeCl3 Test	+ ve + ve	+ ve + ve
5	**Flavonoids**		
	Lead acetate test Shinoda test Mg/Hcl	+ ve + ve + ve	+ ve + ve + ve
6	**Saponins**:		
	Foam Test Lead acetate test	+ ve + ve	- ve + ve
7	**Sterols**:		
	Salowaski test Libbermam Burchad	+ ve + ve	+ ve + ve

ANALGESIC ACTIVITY: A search for analgesic action was conducted to Argyreia Nervosa leaf extracts. In comparison to normal pentazocin, the 400mg/kg aqueous and ethanolic extracts demonstrated significantly higher analgesic efficacy. When compared to standard, the same extract exhibits a moderate analgesic effect of 200mg/kg. The outcome of the analgesic activity of Argyreia Nervosa leaf extracts is depicted in table no. 4.

Table no 4. ANALGESIC ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF ARGYRIA NERVOSA LEAF EXTRACT.

GROUP	TREATMENT	DOSE mg/kg	BASAL REACTION TIME (Sec)				
			0	30	60	90	120
1	Control	10 mg/kg	3.5±0.22	3.5±0.22	4.0±0.36	3.6±0.21	4.16±0.30
2	Standard	10 mg/kg	5.1±0.30	6.66±0.33	9.5±0.42	9.6±0.21	9.33±0.42
3	AEAN	200 mg/kg	3.16±0.30	3.33±0.21	4.16±0.30	4.66±0.21	4.66±0.21
4	EEAN	200 mg/kg	3.83±0.16	3.5±0.22	4.5±0.02	5.33±0.33	5±0.36
5	AEAN	400 mg/kg	5.16±0.30	5.83±0.30	8.33±0.33	**8.9**5±0.67	9.31±0.21
6	EEAN	400 mg/kg	4.66±0.33	5.83±0.30	8.16±0.30	8.66±0.33	9.66±0.33

Anti-inflammatory activity: By employing carrageenan-induced paw edema, the current investigation was conducted to assess the anti-inflammatory efficacy of aqueous and ethanol extracts of Argyreia nervosa leaf extracts. According to the acute toxicity investigation, different groups of rats received an oral dose of the extracts at 400 mg/kg.

Table 5 displays the outcomes of the anti-inflammatory activity. Aqueous and methanolic preparations of Argyreia nervosa leaf extract shown substantial activity when compared to the common medication diclofenac, according to the findings. It is possible that the active components in the ethyl acetate and ethanolic extracts of whole aerial parts from Argyreia nervosa showing significant activity may be involved in the inhibition of some of these inflammatory mediators since the carrageenan-induced paw edoema model was used for evaluation of anti-inflammatory activity of the compounds involving several chemical mediators such as prostaglandins, serotonin, histamine, and bradykinin.
side some empirical results showed that high risk is not associated with high return (Michailidis et al. 2006, Hanif, 2009). Mollah and Jamil (2003) suggested thatrisk-return relationship is notlinear perhaps due to high volatility.

GROUP	TREATMENT	DOSE Mg/kg	PAW OEDEMA VOLUME							
			ZERO HOURS	AFTER 1ST HOUR		AFTER 2ND HOUR		AFTER 3RD HOUR		AFTER 4TH HOUR	
			NORMAL PAW VOLUME	MEAN± SEM	% ROV	MEAN±SEM	% ROV	MEAN±SEM	% ROV	MEAN±SEM	% ROV
1	Control	10ml/kg	0.75	0.45±0.125	----	0.55±0.112	----	0.58±0.110	-------	0.55±0.112	------
2	Standard Diclofenac	50	0.81	0.22±0.017	51.11	0.12±0.011	78.18	0.08±0.017	86.20	0.14±0.02	74.54
3	AEAN	200	0.98	1.21hrs	19.33	1.09*	31.44	0.91*	52.10	1.21hrs	28.82
4	EEAN	200	0.96	1.19hrs	20.66	1.20*	24.52	0.93*	51.05	1.10*	35.29
5	AEAN	400	0.90	1.15*	23.33	1.13*	28.93	0.98	48.42	0.95	44.11
6	EEAN	400	0.92	0.97*	35.33	0.90**	43.39	0.83**	56.31	0.84*	50.58

Conclusion:

Experimental animals were used to study the phytochemical and pharmacological effects of aqueous and alcoholic extracts of Argyreia Nervosa leaf. Aqueous and alcoholic extracts both contain carbohydrates, alkaloids, flavonoids, saponins, tannins, and steroids, according to phytochemical research.

Aqueous and alcoholic leaf extracts of Argyreia Nervosa, 400 mg/kg body weight, shown considerable analgesic and anti-inflammatory action in the current investigation. However, at a dose of 200 mg/kg body weight, the same extracts showed little analgesic and anti-inflammatory efficacy. In conclusion, the presence of flavonoids and steroids in both aqueous and alcoholic extracts may be the cause of the analgesic and anti-inflammatory effects.

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Referances:

1. Kirtikar, K. R. and Basu, B. D. (1994) “Indian medicinal plants. Deharadun: Shiva Offset Press,” pp. 1707–1709.
2. Warrier, P. K., Nambiar, V. P. and Ramankutty, C. (1994) “Indian medicinal plants: A compendium of 500 species. Delhi: Orient Longman,” pp. 191–194.
3. Sharma, P.C., Yelne, M.B., Dennis, T.J., Joshi, A. and Billore, K.V., 2000. Database on medicinal plants used in Ayurveda.
4. Kumar, K.H. and Elavarasi, P. (2016). Definition of pain and classification of pain disorders. *Journal of Advanced Clinical & Research Insights*, [online] 3(3), pp.87–90. doi:https://doi.org/10.15713/ins.jcri.112.
5. Medzhitov, R. (2010) “Inflammation 2010: New adventures of an old flame,” *Cell*, 140(6), pp. 771–776. doi: 10.1016/j.cell.2010.03.006.
6. Stucky, C.L., Gold, M.S. and Zhang, X., 2001. Mechanisms of pain. *Proceedings of the National Academy of Sciences*, *98*(21), pp.11845-11846.
7. Tamrat, Y., Nedi, T., Assefa, S., Teklehaymanot, T. and Shibeshi, W. (2017). Anti-inflammatory and analgesic activities of solvent fractions of the leaves of Moringa stenopetala Bak. (Moringaceae) in mice models. *BMC Complementary and Alternative Medicine*, 17(1). doi:https://doi.org/10.1186/s12906-017-1982-y.
8. Takeuchi, O. and Akira, S. (2010) “Pattern recognition receptors and inflammation,” *Cell*, 140(6), pp. 805–820. doi: 10.1016/j.cell.2010.01.022.
9. Ferrero-Miliani, L. *et al.* (2007) “Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation,” *Clinical and experimental immunology*, 147(2), pp. 227–235. doi: 10.1111/j.1365-2249.2006.03261.x.
10. Chertov, O., Yang, D., Howard, O.M. and Oppenheim, J.J. (2000). Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. *Immunological reviews*, [online] 177, pp.68–78. doi:https://doi.org/10.1034/j.1600-065x.2000.17702.x.
11. Handa, S.S., 2008. An overview of extraction techniques for medicinal and aromatic plants. *Extraction technologies for medicinal and aromatic plants*, *1*(1), pp.21-40.
12. Azmir, J. *et al.* (2013) “Techniques for extraction of bioactive compounds from plant materials: A review,” *Journal of food engineering*, 117(4), pp. 426–436. doi: 10.1016/j.jfoodeng.2013.01.014.
13. Badmus, J.A., Odunola, O.A., Obuotor, E.M. and Oyedapo, O.O., 2010. Phytochemicals and in vitro antioxidant potentials of defatted methanolic extract ofHolarrhena floribunda leaves. *African Journal of Biotechnology*, *9*(3), pp.340-346.
14. Patel, S., Patel, D.K. and Nayak, B., 2019. Pharmacognostical & physicochemical evaluation of Parthenium hysterophorus plant. *Journal of Pharmacognosy and Phytochemistry*, *8*(3), pp.604-607.
15. Halim, S.Z., Abdullah, N.R., Afzan, A., Rashid, B.A., Jantan, I. and Ismail, Z., 2011. Acute toxicity study of Carica papaya leaf extract in Sprague Dawley rats. *Journal of Medicinal Plants Research*, *5*(10), pp.1867-72.
16. Akhila, J.S., Shyamjith, D. and Alwar, M.C., 2007. Acute toxicity studies and determination of median lethal dose. *Current science*, pp.917-920.
17. Tita, B., Abdel-Haq, H., Vitalone, A., Mazzanti, G. and Saso, L., 2001. Analgesic properties of Epilobium angustifolium, evaluated by the hot plate test and the writhing test. *Il Farmaco*, *56*(5-7), pp.341-343.
18. O'Callaghan, J.P. and Holtzman, S.G., 1975. Quantification of the analgesic activity of narcotic antagonists by a modified hot-plate procedure. *Journal of Pharmacology and Experimental Therapeutics*, *192*(3), pp.497-505.
19. Winter, C.A., Risley, E.A. and Nuss, G.W., 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the society for experimental biology and medicine*, *111*(3), pp.544-547.
20. Asongalem, E.A., Foyet, H.S., Ngogang, J., Folefoc, G.N., Dimo, T.H.É.O.P.H.I.L.E. and Kamtchouing, P.I.E.R.R.E., 2004. Analgesic and antiinflammatory activities of Erigeron floribundus. *Journal of ethnopharmacology*, *91*(2-3), pp.301-308.