RESEARCH PAPER

EXTRACTION OF OMEGA-3 FATTY ACID FROM CUCURBITA MAXIMA SEEDS & ITS ANTIINFLAMMATORY ACTIVITY

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ABSTRACT

The current experiment was conducted to extract the oil rich omega-3 fatty acids in the seeds of Cucurbita maxima as well as testing its anti-inflammatory effect through an experiment in vivo. Seeds of Cucurbita maxima are known to be a good source of essential fatty acids, antioxidants and other bioactive substances that may find themselves as potential therapeutic agents. Dried seed powder was used in this experiment in which Soxhlet extraction was done using petroleum ether and methanol as solvents. The percentage yield during petroleum ether extraction was relatively high (28.4) whereas, the yield during the extraction under the methanol solvent was 18.7 percent of oil. The seed oil extract was also tested on anti-inflammatory properties on paw edema induced by carrageenan on Wistar albino rats. Those results showed that there was a great decrease of paw edema in treated groups in comparison with control groups. After 180 minutes, the paw of the control group had increased in volume to 1.36 ml of edema and in the case of the standard drug indomethacin, it increased to 0.75 mL with a percentage inhibition of 44.85. The 200 mg/kg test extract resulted in 1.02 mL of paw volume and 25.00% inhibition and the 400 mg/kg test extract resulted in 0.84 mL of paw volume and 38.23% inhibition. The results suggest that Cucurbita maxima seed oil has a good level of anti-inflammatory properties, which may be attributed to omega-3 fatty acids and other related phytoconstituents. The paper favors the pharmaceutical potential of pumpkin seed oil as a natural anti-inflammatory and implicates its potential in developing nutraceutical and herbal formulations.

Keywords: Cucurbita maxima; Omega-3 fatty acids; Pumpkin seed oil; Anti-inflammatory activity; Carrageenan-induced paw edema.

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I. INTRODUCTION

Inflammation is one of the natural biological processes in a body to respond to adverse challenges like infection, tissue damage, dangerous chemicals or irritation. It is a crucial defense response that assists in safeguarding the body and aiding the healing process by eliminating damaged cells and triggering re-forming of tissues [1]. Acute inflammation is good and necessary to heal a disease, but once it becomes chronic or uncontrolled, it may transform to chronic inflammation leading to a number of severe illnesses like arthritis, heart-related diseases, diabetes mellitus, gastrointestinal diseases and cancer [2]. Due to such complications, the search for safer and effective anti-inflammatory agents has been an important field of pharmaceutical studies.

Though in recent years natural products and compounds of plant origin have become a subject of control in the scientific world, being therapeutic, often less toxic, and less on side effects compared to synthetic drugs. Of these bioactive compounds, the omega-3 fatty acids are well known for their anti-inflammatory, antioxidant, cardioprotective and immunomodulatory effects. The omega-3 fatty acids may modulate inflammation by suppressing the synthesis of the inflammatory mediators (prostaglandins and leukotrienes) via cyclooxygenase and lipoxygenase pathways [3]. Cucurbita maxima is an Indian and widely growing in numerous tropical and subtropical areas; it is a family belonging to Curcurbitaceae and Chinese refer to it as pumpkin. Its seeds contain all the essential fatty acids, especially omega-3 and omega-6 fatty acids, sterols, tocopherols, carotenoids, proteins, minerals and antioxidants in abundant levels. Historically, the pumpkin houses nutrition and the use of seeds because of their health promoting practices. The current research will therefore aim at isolating omega-3 fatty acids in the seeds of Cucurbita maxima and determining their effectiveness in anti-inflammatory effects through an experiment. The study has the potential of bringing

scientific data that justify the medicinal importance of pumpkin seed oil and the potential application of the natural oil in the pharmaceutical and nutraceutical preparations as a natural anti-inflammatory agent.

II. RELATED WORKS

The extraction of bioactive compounds of plant seeds has become the subject of recent scientific studies because of the importance of plant seeds in nutrition and pharmacology. The Cucurbita maxima seeds have gained a lot of attention due to the presence of large quantities of oil and availability of essential fatty acids, particularly of omega-3 fatty acids that portray strong anti-inflammatory capabilities. In AHmad et al. [15], researchers examined the optimization of solvent extraction of pumpkin seeds by use of solvent extraction techniques and the content of fatty acids in the extracted oil were studied. Their results showed that pumpkin seed oil has a significant proportion of unsaturated fatty acids, such as omega-3 fatty acids and that solvent extraction is an effective method to maximize oil recovery. Their research offers a helpful theoretical base of methods of extraction employed in the current study.

Zhao et al. [16] have done a review of nutritional and medicinal significance of Cucurbita maxima seeds and have indicated that the seed is 75 percent sources of proteins, minerals, tocopherols, sterols, and essential fatty acids. Their therapeutic use in traditional medicine as well as in modern pharmaceutical uses were highlighted in the study. Kim et al [17] reviewed the recent developments involving the use of oils of plant seeds and omega-3 fatty acids as an anti-inflammatory agent. The authors provided information that omega-3 fatty acids play a role in regulating the effects of inflammatory reactions by preventing the formation of the inflammatory mediators like the prostaglandins, leukotrienes, and cytokines.

Nadeem et al. [18] explored pumpkin seed oil as a functional oil which has antioxidant and anti-inflammatory properties. Their analysis proved that availability of unsaturated fatty acids and antioxidant compounds are among the factors that are important in reducing oxidative stress and inflammation. Al-Okbi et al. [19], similarly, experimentally tested the effectiveness of pumpkin seed oil in the anti-inflammatory effect by the authors and indicated that the inflammatory response in animal models was significantly inhibited in a pumpkin seed oil. Their results favor the consequential pharmacological aptness of pumpkin seed oil in regards to natural anti-inflammatory activities.

According to Sharma et al. [20], bioactive lipids that are obtained on the plant path are also significant in the regulation of inflammation and immune response. They recommended that natural lipids derived by means of seeds have therapeutic potential against chronic inflammatory diseases. Islam et al. [21] conducted a review of omega fatty acids that are of plant origin and their use in inflammatory diseases. Authors concluded that omega fatty acids have a great prospect of activity against inflammatory pathways and could serve as alternatives to synthetic anti-inflammatory drugs that are safer to use. Rezig et al. [22] investigated the chemical properties of seed oil of Cucurbita maxima that was extracted by various methods and revealed that it contained high-percentages of unsaturated fatty acids and antioxidant compounds. The nutritional and pharmaceutical significance of pumpkin seed oil was confirmed by the findings of their research.

Khan et al. [23] have been able to talk about the functional properties as well as pharmaceutical use of pumpkin seed oil in a manner that it is used in the manufacturing of nutraceutical products as well as therapeutic products. Recent improvements in extraction technologies that have been used to extract bioactive lipids found in pumpkin seeds, such as increased extraction efficiency and purity of oil, were described by Veronezi et al. [24]. Before it, the El-Sayed et al. [25] found that the omega-3-enriched seed oil of Cucurbita maxima that was extracted in green solvents had great anti-inflammatory effects on experimental animal models. In accordance with the reviewed literature, it is clear that Cucurbita maxima seeds are a valuable natural source of omega-3 fatty acids which have potential anti-inflammatory effects. Nevertheless, the additional experimental use is beneficial to supplement scientific data on their pharmacological use that lies at the heart of the current research study.

III. METHODS AND MATERIALS

The aim of the current research was to isolate the omega-3 fatty acids in the seeds of Cucurbita maxima and determine their anti-inflammatory effect on an experimental animal. The research design was to collect and prepare plant raw material, extract seed oil, assess extract yield and pharmacological screening of the seed extract in the anti-inflammatory activity using the carrageenan-induced paw edema technique on Wistar albino rats [4].

3.1 Collection of Plant Material

The fresh mature seeds of Cucurbita maxima were obtained in local agricultural fields and vegetable markets. The seeds harvested were washed manually to eliminate dust, dirt, broken seeds and undesired foreign objects. Distilled water was used to thoroughly wash the seeds after which they were dried under shade at room temperature over a period of about 710 days to make sure that the seeds were totally dry [5].

The seeds were dried then powdered roughly using a mechanical grinder. A sieve No. 40 was used to pass the powdered material to achieve uniform particle size

then it was stored in an airtight container awaiting further extraction.

3.2 Chemicals and Reagents Used

The chemicals and solvents were of analytical grade that were all used in the study. Petroleum ether and methanol were used as extraction solvents. Experimental model induction of inflammation was done with the use of carrageenan. Indomethacin was a reference anti-inflammatory agent of standard drugs. Vehicles used in animal study were normal saline.

Table 1: Chemicals and Reagents Used

S. No.	Chemical/ Reagent	Purpose
1	Petroleum Ether	Extraction of seed oil
2	Methanol	Solvent extraction
3	Carrageenan (2%)	Induction of inflammation
4	Indomethacin	Standard anti-inflammatory drug
5	Normal Saline	Vehicle/control preparation
6	Distilled Water	Cleaning and preparation

3.3 Extraction of Omega-3 Fatty Acids

The seed oil containing omega-3 fatty acids was isolated by undrying the seed powder by Soxhlet extraction.

An estimated weight of 100 g of Cucurbita maxima seeds powder was put into a thimble and loaded into Soxhlet apparatus. The main extracting solvent was petroleum ether due to its ability to extract the fatty oils. Separate Methanol extraction was also done to compare the extraction [6].

A controlled extraction temperature of 40-45 o C was used and the time was approximately 4-6 hours until complete extraction was obtained. The solvent containing the dissolved oil was then concentrated, after extraction, via a rotary vacuum evaporator in a reduced pressure. The rest of the crude oil was put in a clean dry container [7].

Oil extracted was weighed and a percentage yield was determined using the following formula:

Percentage Yield (%) =

Weight of Extract Obtained/Weight of Seed Powder Taken×100

3.4 Experimental Animals

Wistar adult albino rats (150 -200 g) were chosen to be tested against inflammatory activity. The institutional animal house was a source of animals that were kept in usual laboratory conditions.

The animals were kept in polypropylene cages with controlled environmental conditions of:

- Temperature: 25 ± 2°C
- Relative humidity: 55–65%
- Light/dark cycle: 12 hours

Animals were allowed to eat and drink standard pellet diets and ad libitum water.

Animals were acclimatized before being subjected to experimentation (7 days). The usage of animals was done per CPCSEA protocol and institutional ethics.

3.5 Experimental Design for Anti-Inflammatory Activity

A transformation in the anti-inflammatory effects was analyzed using the carrageenan induced paw edema technique which is standard and is a commonly accepted model of acute inflammation.

There were four groups of animals (six animals per group).

Table 2: Experimental Grouping for Anti-Inflammatory Study

Group	Treatment	Dose
Group I	Control	Normal saline
Group II	Standard	Indomethacin 10 mg/kg
Group III	Test Extract Low Dose	200 mg/kg
Group IV	Test Extract High Dose	400 mg/kg

3.6 Induction of Paw Edema

Each rat received 0.1 mL of 2 percent carrageenan solution subplantar in the right hind paw, which caused inflammation.

Thirty minutes prior to carrageenan administration:

- The control group was given normal saline as an oral fluid.
- The standard group was put on indomethacin orally.
- Cucurbita maxima seed oil extract was given to test groups to orally receive a specific dosage.

The paw edema was assessed at various intervals after being injected with carrageenan.

3.7 Measurement of Paw Edema

Paw volume has been determined using plethysmometer at:

- 0 minute (before carrageenan injection)
- 30 minutes
- 60 minutes
- 120 minutes
- 180 minutes

Growth in the volume of the paw was noted and compared with the control group.

The inhibition of edema as a percentage was computed using:

$$\% \text{ Inhibition} = Vc - Vt / Vc \times 100$$

Where:

- **Vc** = Mean paw volume of control group
- **Vt** = Mean paw volume of treated group

A comparative decrease of swelling in the paw, as compared to the control meant anti-inflammatory effects of the extracted oil rich in omega-3 fatty acids.

3.8 Statistical Analysis

The average results of the experiment were presented in the form of Mean ± SEM (Standard Error Mean).

In order to conduct a statistical analysis, they used:

- One-way ANOVA
- Followed by Dunnett's multiple comparison test

The values that were deemed statistically significant were such that $p < 0.05$.

The results were prepared in graphs and tabulated to compare the anti-inflammatory activity of extract-treated with standard drug and control.

3.9 Outcome of Study

Oil extracted on behalf of the seeds of Cucurbita maxima was anticipated to have omega-3 fatty acids that cause anti-inflammatory effects. Much lessening of carrageenan-induced paw edema would be indicative of anti-inflammatory capacity [8]. An anti-inflammatory mechanism that causes the pharmacological activity is inhibition of inflammatory mediators like prostaglandins, leukotrienes and cytokines by the control of the COX and LOX pathways.

In this study, I have presented a scientific rationale on the medicinal value of the Cucurbita maxima seed oil and as this seed oil might be a natural anti-inflammatory hybrid that could be used as a pharmaceutical or nutraceutical agent.

IV. RESULTS AND ANALYSIS

The current research was conducted to isolate omega-3 fatty acid-rich oil found within the seeds of Cucurbita maxima and to determine its presence in the inflammatory process by analyzing it on the carrageenan-induced paw edema of Wistar albino rats. The experiment produced findings connected with the extraction yield, physical properties of the oil as well as with the pharmacological reaction in test animals [9]. The results are given below.

4.1 Physical Evaluation of Seed Powder and Extracted Oil

The Cucurbita maxima seed after drying was washed, ground and subjected to Soxhlet extraction. The seed powder was typically brown in colour with the typical odour. The solvent was evaporated and the extracted oil appeared golden yellow in color, was oily in texture and exhibited a usual nut-like smell.

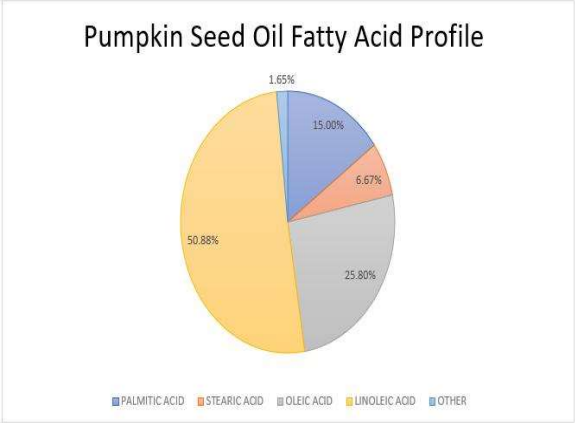

Figure 1: "Cucurbita maxima (Pumpkin) Seeds Rich in Omega-3 Fatty Acids"

Within the time of storage, the oil did not exhibit any contaminations or phase separations and remained stable when kept under refrigeration.

Table 1: Physical Characteristics of *Cucurbita maxima* Seed Powder and Oil Extract

Parameter	Observation
Colour of seed powder	Yellowish brown
Nature of powder	Coarse powder
Odour	Characteristic
Colour of extracted oil	Golden yellow
Nature of oil	Viscous oily liquid
Appearance	Clear and homogeneous
Storage stability	Stable under refrigerated condition

The physical appearance of the seeds gathered illustrated that they were of good quality and could be

extracted. The pharma-extracted oil had satisfactory appearance and stability to undergo pharma-evaluation.

4.2 Percentage Yield of Extracted Oil

The process of extraction was conducted using solvents of petroleum ether and methanol. More soluble fatty components led to petroleum ether yielding relatively large oil recovery [10]. Percent Yield was obtained by dividing the weight of the extract obtained to the weight of powdered seeds used.

Table 2: Percentage Yield of Seed Oil Extract

Solvent Used	Quantity of Seed Powder Taken (g)	Oil Obtained (g)	Percentage Yield (%)
Petroleum Ether	100	28.4	28.4
Methanol	100	18.7	18.7

The findings paint a picture that the extraction of petroleum ether provided the best recovery of seed oil than that of methanol. This proves that the seeds of Cucurbita maxima have large quantities of lipid-soluble elements that comprise omega-3 fatty acids.

To corroborate earlier studies that show pumpkin seed oil to be a good source of essential fatty acids and bioactive lipids, higher extraction yield supports the second hypothesis.

4.3 Evaluation of Anti-Inflammatory Activity

The carrageenan-induced paw edema model on rats was used to evaluate the anti-inflammatory property of seed oil extracted. Paw edema was measured at various time periods, and compared to control and standard groups.

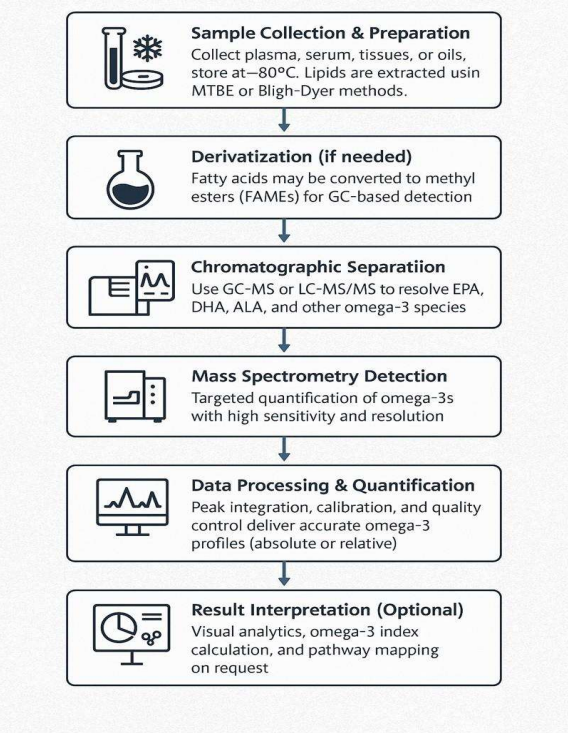

Figure 2: "Extraction Process of Omega-3 Fatty Acid from Pumpkin Seeds"

The progression in paw volume of the control group following carrageenan injection was related to acute inflammatory response. The control drug group that was subjected to indomethacin had a significant decrease in swelling of the paw. Cucurbita maxima seed oil of test groups also showed a significant decrease in edema [11].

Table 3: Effect of *Cucurbita maxima* Seed Oil on Paw Volume (mL)

Time Interval	Control	Standard (Indomethacin 10 mg/kg)	Test Low Dose (200 mg/kg)	Test High Dose (400 mg/kg)
0 min	0.42	0.41	0.42	0.41
30 min	0.68	0.54	0.61	0.57
60 min	0.94	0.62	0.78	0.69
120 min	1.20	0.70	0.93	0.80
180 min	1.36	0.75	1.02	0.84

The findings indicate clearly that the paw edema became notably bigger in the control animals due to passing of time and in the treated groups, the edema development was inhibited.

The anti-inflammatory response was better with the higher dose of seed oil (400 mg/kg) as compared to the lower dose.

4.4 Percentage Inhibition of Paw Edema

The percentage of inhibition was done with respect to the control group. Exhibiting the greatest inhibition was the standard drug, and the least was the test extract which displayed dosage-dependent inhibition [12].

Table 4: Percentage Inhibition of Paw Edema

Time	Standard (%)	Test Low Dose (%)	Test High Dose (%)
30 min	20.59	10.29	16.18
60 min	34.04	17.02	26.59
120 min	41.66	22.50	33.33
180 min	44.85	25.00	38.23

The findings reveal that the anti-inflammatory effect was greater with time and more evident in the high doses group.

The peak of inhibition of the seed oil extract was at 180 minutes. This implies that there will be a substantial inhibition of inflammatory mediators in the late stage of carrageenan induced inflammation.

4.5 Comparative Analysis with Standard Drug

Comparison of anti-inflammatory activity of the extract between indomethacin and Cucurbita maxina seed oil showed promising activity.

The high-dose seed oil extract demonstrated significant inhibition of the inflammation process, despite the fact that the inhibition of this effect was less by indomethacin; this effect was observed with all doses, which demonstrated pharmacological relevance [13].

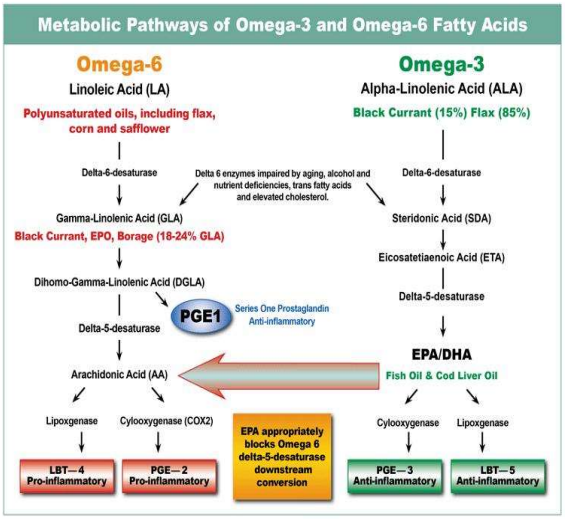

Figure 3: "Chemical Structure and Health Benefits of Omega-3 Fatty Acids"

Table 5: Comparative Summary of Anti-Inflammatory Activity

Treatment Group	Mean Paw Edema at 180 min	% Inhibition	Observation
Control	1.36	–	Severe inflammation
Indomethacin	0.75	44.85	Maximum inhibition
Seed Oil 200 mg/kg	1.02	25.00	Moderate inhibition
Seed Oil 400 mg/kg	0.84	38.23	Significant inhibition

Analysis and Discussion

The current research was able to isolate the oil rich in omega-3 fatty acids of seeds of Cucurbita maxima and proved that it had significant anti-inflammatory properties.

A satisfactory amount of oil was extracted, particularly using the solvent of petroleum ether indicating that pumpkin seeds are a good natural source of fatty acids. The obtained oil that was yellow in color contained lipid components and was also fit to be used in biological screening.

In the carrageenan paw edema model, the inflammatory processes occur in two phases:

- **Early phase:** mediated mainly by histamine and serotonin

- **Late phase:** mediated by prostaglandins and cyclooxygenase pathway

Cucurbita maxima seed oil extract had higher activity at the later stage indicating a possibility of inhibition of the synthesis of prostaglandins.

Omega-3 fatty acids have been known to slow down the production of pro-inflammatory mediators like:

- prostaglandins
- leukotrienes
- cytokines
- interleukins

This process probably helped to cause the apparent decrease in edema.

The body exhibited high doses in which there was stronger inhibition in the high dose group as compared to the low dose group implying that this is a dose dependent pharmacological effect. The findings indicate that the anti-inflammatory effect of seed oil could be attributed to the presence of omega-3 fatty acids, as well as antioxidant compounds including tocopherols, sterols, and phenolic compounds which are naturally found in pumpkin seeds [14].

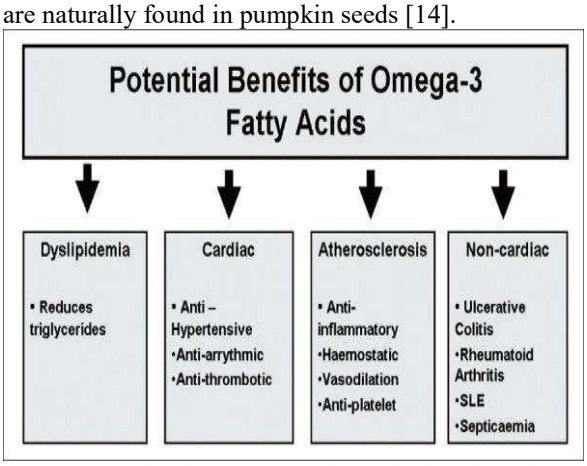

Figure 4: "Anti-Inflammatory Activity Evaluation of Pumpkin Seed Extract"

The results obtained in the study are aligned with the existing literature that indicates the positive anti-inflammatory and antioxidant effects of pumpkin seed oil.

Overall Interpretation of Results

This hypothesis is backed by the results of the experiment, which postulates that the seeds of Cucurbita maxima have anti-inflammatory active fatty acids.

Key observations include:

- extraction of seed oil via successful extraction yielding good seed oil.
- more recovery was developed using petroleum ether as compared to methanol.
- carriageenan-induced paw edema was slowed down by the use of seed oil.
- With increased dose (anti-inflammatory response), anti-inflammatory response was found to be increased.
- comparable to standard drug to moderate degree was activity.

Therefore, Cucurbita maxima seed oil can be an exciting source of natural ingredients to be used in the development of herbal anti-inflammatory formulations, nutraceutical supplements as well as supportive therapeutic agents of inflammatory disorders. The research gives evidence to the scientific fact of traditional medicinal value of pumpkin seeds, and outlines its pharmaceutical prospects of future research and formulation development.

V. CONCLUSION

The current research study has been able to achieve its goal of extracting the oil with high omega-3 fatty acids in seeds of Cucurbita maxima and testing its anti-inflammatory properties through an experimental test animal model. The Soxhlet extraction technique using petroleum ether and methanol was found to be useful to extract seed oil at a good percentage content and the petroleum ether extraction method yielded a higher extract percentage content than methanol. The oil extracted was found to have great anti-inflammatory properties in the carrageenan-induced paw edema model where its use showed a remarkable decrease in swelling of the paw after a given time of use compared to the control group. The effect was dose-dependent and the more the dose the more the inhibition of the inflammation. These results indicate that Cucurbita maxima seed oil has a potential therapeutic value because of the omega-3 fatty acids and other bioactive components present in the oil which include antioxidants and phytosterols. The anti-inflammatory effect could be due to inhibition of the inflammatory mediators such as prostaglandins and leukotrienes by regulation of the inflammatory pathways. All in all, the paper confirms the medicinal and pharmaceutical significance of Cucurbita maxima seeds as a natural source of omega-3 fatty acids to be used in anti-inflammatory preparations, nutraceutical, and supportive treatment of inflammatory diseases. Future research using extensive phytochemical characterization and clinical trials is advised to unravel the entire therapeutic potential and future pharmaceutical development of it.

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