



Evaluation of Anti-inflammatory Activity of *Psidium guajaya* and *Moringa oleifera* by using In-vitro Method

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Abstract-

The condition known as hyperlipidemia is characterized by blood lipid levels (fats, cholesterol, and triglycerides) that are higher than usual and can have potentially fatal consequences. The development of atherosclerosis and its associated conditions, including peripheral vascular disease, cerebral vascular disease, and brain strokes, are typically linked to these consequences. The build up of these lipids, particularly cholesterol, in the walls of the arteries, which causes the vessels to constrict, reduce blood flow through the impacted areas, and cause atherosclerosis. When other common diseases including hypertension, diabetes mellitus, and renal problems are present, the rates of morbidity and mortality rise.

Leaves and fruits of guava treat pain of stomach and intestinal, increased sugar level, and wound healing. *Moringa oleifera*'s include antibacterial, antifungal, and anti-inflammatory effects are shown by gums and roots . It belongs to the Moringaceae family. β -carotene, protein, vitamin C, calcium, and potassium, present in *Moringa oleifera* which can act as pure antioxidants.

The aim of our project was to develop polyherbal cream containing extracts of Guava and drumstick leaves which can give anti-inflammatory effect.

Key words –

Inflammation, COX-2, Cream, Protein denaturation, heat induced hemolysis, Soxhlet, Extraction

Materials and Methods –

Taxonomical classification of plants *Psidium guajaya*, *Moringa oleifera* were explained in tabular form.

This Cetyl alcohol, Propylene glycol, Sodium benzoate, Emulsifying wax, Sodium stearate, Glycerin, Stearic acid, ingredient required for the preparation of cream are enlisted with the required quantity.

Procedure required for preparation of w/o type emulsion cream were explained.

Evaluation parameter for the cream are measured and the procedure is performed as given.

INTRODUCTION

Consumers are more think about their health in today's rapidly shifting socioeconomic landscape. Over time, the use of natural plant-based products with less adverse reactions has grown in day today life.

There is a huge potential for foods to provide health advantages beyond standard nutrients. Several nutraceuticals or food deliver advantage to health. These is not only but also "functional foods." Functional foods can be whole, fortified, enriched, or improved. [1]

Functional food include, Flavanoid minimizes the threat of coronary heart disease, Calcium lowers the threat of osteoporosis, beta carotene scavenge free radicals, potassium lowers the danger of high blood pressure, fatty acids decreases the risk of coronary heart disease. Creams made with herbal extract with artificial preservatives utilised in cosmetics industry have adverse effects. [2]

Redness on skin, warmth, and swollen at the location are some of the main indicators of inflammation, which is a general mechanism by which the body responds to any wound, infection, or irritation. It is believed to have distinct pathways for every kind of infection[13].

Macrophages secretes pro-inflammatory mediators and proteins such as TNF-alpha COX-2, IL-6, and nitric oxide production. Major role played by the macrophages in the immunity system response and a variety of inflammatory conditions[18].

Guava consist of leaves and fruits used for pain of stomach and intestine, Increased sugar level and to heal the wound. Fruit is used to treat hypertension which belong to family Myrtaceae[19].

Drumstick tree consists of root, bark has medicinal value and used for dyspepsia, eye disease and heard complaints. Gum and roots also have antibacterial antifungal and anti-inflammatory properties. It belong to family Moringaceae. β -carotene, protein, vitamin C, calcium and potassium that are present in *Moringa oleifera* that may be act as the antioxidant. Cream of Moringa leaf extract can be applied topically to prevent the treatment of stress disease, anti-inflammatory as well anti-aging. [7-9]

Though allopathic medicine are very costly, Extracts of plants are still utilized in alternative and complementary medicine, and they are seen to be healthy substitutes for synthetic and organic medications. [16]

Their potential to prevent the common adverse event of allopathic medications, herbal medicines are becoming more and more popular with patients. It makes sense that one-fourth of the world's population or 1.42 billion people, rely on traditional method of medicine to cure a type of illnesses. One of the main sources of cures for human illnesses has been medicinal plants. The use of environmentally friendly and bio-friendly plant-based products for the treatment and prevention of various diseases has received a lot of attention recently, and conventional medicine has been used by the majority of people worldwide[16].

India has a wide range of plants with therapeutic qualities. It is possible to discover efficient substitutes for chemical drugs by using medicinal plants. Globally, the utilization of herbal remedies as a source of treatment is steadily gaining popularity[16].

The aim of our project was to develop polyherbal cream containing extracts of Guava and drumstick leaves which can give anti-inflammatory effect.

There are two kind of creams exists not only Oil in H₂O O/W but also Water in oil W/O, O/W creams which are formed of droplet of oil disseminate in the continuous disseminate in continuously oil Phase and H₂O in oil W/O creams that is formed of tiny droplet of H₂O.

Materials and Methods –

Herbal drug profiles :-

Psidium guajaya and *Moringa oleifera* –

Taxonomical Classification –

Plant name	<i>Psidium guajaya</i>	<i>Moringa oleifera</i>
Kingdom	Plantae	Plantae
Sub – kingdom	Viridi plantae	Tracheobionta
Super – division	Embryo phyta	Spemtophyta

division	Trache phyto	Magnoliophyta
Class	Magnolio psida	Magnoliopsida
Sub class	Spermato phytia	Dilleniidae
Order	Myvtales	Capparales
Family	Myrtaceae	Moringaceae.
Genus	Psidium	Moringa
Species	Guajava	Olifera

Table.no. 1 Taxonomical classification of Plant

Psidium guajaya–

Fig no :-1. P. guajaya leaves

Chemical constituent [4,6] –

The chemical constituent of *P.guajaya* quercetin, avicularin, apigenin, guajaverin, kaempferol, hyperin, myricetingallic acid, catechin, epicatechin, chlogenic acid, caffeic acid.

Uses-

- It magnify the health of heart.
- It assist in relieving dysmenorrhea's symptoms.
- It helps digestive System to improve itself.
- It assists in Loosing weight.
- Anticancer Effect were shown by the plant extract.

Moringa oleifera –

Fig no :-2. M. Oleifera plant

Chemical constituent of *Moringa oleifera* [4] –

Flavonoids, Phenolic acids, glucosinolates, Alkaloids, saponins, tannins, steroids, and terpenes this all present in the extract

Uses-

It regulates sugar levels in blood.

It assists in uplifting the immunity.

Bone density were upgraded.

Drumsticks aid in purification of blood.

They are better in prevention of diseases related to respiratory track.

Anti-inflammatory activity

MATERIAL AND METHOD:- [2].**Material:-**

Ingredient	Procured from
Alcoholic extract of <i>Psidium guajaya</i> and <i>Moringa oleifera</i>	-
Stearic acid	Loba Chem
Cetyl alcohol	Loba Chem
Emulsifying wax	Loba Chem.
Propylene glycol	Loba Chem
Sodium benzoate	Sigma Aldrich Fine Chem, Mumbai.
Sodium stearate	Loba Chem
Propyl paraben	Loba Chem
Glycerin	Loba Chem

Table.no. 2 Ingredients table

Method-

Ingredient	Quantity (gm)
Alcoholic extract of <i>Psidium guajaya</i> and <i>Moringa oleifera</i>	250
Stearic acid	1
Cetyl alcohol	0.7
Emulsifying wax	2
Propylene glycol	5
Sodium benzoate	0.8
Sodium stearate	5
Propyl paraben	0.2
Glycerin	1.7

Table.no.3 Formulation table [1]

Sample collection of *Psidium guajaya* and *Moringa oleifera*[1,3] –

The leaves samples which are required for experiment is collected from residential area of Karad. The leaves were kept for drying four to five days and the powder is made using cutter mill. Collected plant material recognized and authenticated by Department of Botany, Yashwantrao Chavan College of Science, Karad.



Fig no :- 3. Extraction of Guava & Drumstick using Soxhlet apparatus



Fig no :-4. Preparation of plant extract

Formulation of cream :-[1]

Preparation of oil phase-

Ingredient which are soluble in oil like as stearic acid, emulsifying wax, propylene glycol, propyl paraben were taken in first beaker.

Preparation of aqueous phase[2].-

Alcoholic extract of *P.guajaya*, *moringa oleifera* were taken in another beaker with Glycerin, cetyl alcohol, sodium stearate.

Inclusion of aqueous phase into oil phase[1] [2].-The alcoholic extract's aqueous phase were combined with the oily phase while continuously shaking at 70° C. After reaching room temperature, perfume was incorporated before transferring the mixture to a container, followed by the evaluation of the cream.



Fig No :- 5. Anti- inflammatory cream

Evaluation parameter of formulated cream[1], .[2],[5]:-

Determination of organoleptic properties[11]-

Colour , odour and appearance of cream were checked.

Determination of PH[3]-

0.5gm weighed cream mixed with 50 ml of H₂O and dissolved. P^H of the prepared cream were checked by using PH meter.

Determination of spreadability [1,2,10,11] –

Spreadability was assessed by measuring the duration it had taken for two different slides to distinguish when placed between the cream.

A sample were put in between two slides of glass and pressed one on another together with a 30 gm weight to create a uniform film. It was kept for the 5 min.

Then, an additional 30 gm weight was added, and the upper slide was dragged with a thread which is holded to a holder. Duration it took for the slide above one another to travel 5cm across the other was noted.

Measure of how easily cream spreads (S) could be determined with the help of the above formula:

$$S=m \times L/T$$

Determination of smear's type [5] –

The categories of film or residue left on the skin part was examined following with the cream application.

Determination of emolliency[5]-

Slipperiness and emolliency of cream were checked of formulated cream.

Determination of category of emulsion –

Dilution Test - If an emulsion is of the H₂O -in-oil (w/o) kind and it's diluted with oil, it will remain stable since oil serves as disperse phase. However, if it is mixed along with H₂O, the emulsion will separate it's phases.

Irritancy Test[6,10] - Cream was administered onto the lower surface of the left hand, covering an area of 1 square centimeter. Edema was then monitored at periodically for up to 1day.

Phase separation test[3]-

3 gm of cream is weighed accurately. It is filled in cuvette and cuvette is placed in centrifugation machine. Centrifugation machine is rotated at 3000 rpm for 5 min. Separation of phase is observed after 5 min .

Suppression of protein denaturation [1,11,13] :-

- The 10 ml mixture comprised (0.4 ml) of an egg white, 5.6 ml solution of Phosphate Buffered (PBS) with a pH of 6.4, and 4 ml of test samples, resulting in a final concentration of 100 µg/ml.
- On behalf of extracts, distilled water were used in the mentioned mixture to act as a control.
- Diclofenac were kept as reference.
- Reaction mixtures was then kept in a H₂O bath at a temp of $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and allowed to incubate for 15–20 minutes.
- Afterward, the reaction was heated upto 70°C and kept at this temperature for 5 minutes.
- Following that, the reaction were kept for cool to room temperature, and this cooling process was sustained for the 15 minutes.
- Reaction mixture's absorbance were assessed at a wavelength (680 nm) utilizing a UV spectrophotometer.
- Each test was did for the three times, and the average absorbance were recorded.

The percentage of protein suppression was Estimated in a percentage relative to an control using the following formula:

$$\% \text{ inhibition} = \frac{A_t - A_c}{A_c} \times 100$$

Where,

A_t = Absorbance of Test sample.

A_c = Absorbance of Control sample.

Heat induced hemolysis[14] [15]:-

- The reaction, totaling two ml, presence of an 1 ml of a (RBCs) and 1 ml of the test sample
- Saline were kept as control in the test tube.
- Diclofenac were kept as standard in the test tube.
- The reaction were stored in a H₂O bath at temp of 56°C at 30 minutes.
- Reaction were halted with cooling the reaction mixture by using running water. Subsequently, the speed for centrifugation kept for 2500 revolution/min for 5 minutes.
- Supernatant,s absorbance were measured at the 560 nm wavelength.
- The test was performed thrice.
- The average % of hemolysis suppression was determined in the below manner -

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

A_c – Absorbance of control

A_s – Absorbance of sample.

Result and Discussion –**Estimation of Anti-inflammatory cream:-**

Recognition of organoleptic properties [11]-

Sr. No.	Parameter	Inference
1	Colour	Green
2	Odour	Aromatic
3	Appearance	Semisolid

Table No. 4 Organoleptic properties recognition

Determination of PH:-

The cream's pH was measured at 5.8. pH is a crucial factor in assessing the efficacy of the emulsion. Typically, the common pH of skin of person falls from 4.5 to the 6.0.

Determination of spreadability:-

$$S = m \times L / T$$

Where,

S – Spreadability

m- Weight tied to upper glass slide.

L - Length moved on a glass slide

T - Time taken.

Triplicate reading is taken as 20 sec, 22 sec and 20 sec

$$a) \quad S = 30 \times 5 / 20$$

$$= 150 / 20$$

$$= 7.5$$

$$b) \quad S = 30 \times 5 / 22$$

$$= 150 / 22$$

$$= 6.81$$

$$c) \quad S = 30 \times 5 / 20$$

$$= 150 / 20$$

$$= 7.5$$

Sr. No.	Test	Observation	Mean
1	Spreadability	7.5	7.27
2		6.81	
3		7.5	

Table No. 5 Observation for spreadability

The lesser the duration for the distinguish of the two slides, the higher the ability to spread. The cream's spreadability was determined to be 7.27.

Determination of type of smear [5] –

Different types of films or smudges that developed on the skin were examined following the application of the cream. The cream resulted in a skin surface that appeared non-greasy.

Determination of category of emulsion:-

w/o category of emulsion were found.

Irritancy test :-

There was no irritancy observed when applied on the skin.

Phase separation test [3]- No phase separation was observed.

Inhibition of protein denaturation :-

$$\% \text{ inhibition} = \frac{A_t - A_c}{A_c} \times 100$$

Where,

A_t = Absorbance of Test sample

A_c = Absorbance of Control sample

Test	Sample	% inhibition of protein denaturation	Mean
Protein Denaturation test	Test sample	71.2	71.8
		72.3	
		71.9	
	Diclofenac standard	76.2	76.06
		75.9	
		76.1	

Table No. 6 -Inhibition of protein denaturation

The lack of biological properties in molecules of protein is a consequence of protein denaturation. This denaturation has been linked to the onset of inflammatory conditions such as rheumatoid arthritis, diabetes, and cancer. Thus, substances capable of preventing protein denaturation may also aid in preventing these inflammatory disorders. An anti-inflammatory activity of 71.8% was observed.

Heat induced hemolysis :-

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Test	Sample	% inhiton of Heat induced hemolysis	Average
Heat induced hemolysis	Test sample	58.2	63.53
		61.4	
		60.5	
	Diclofenac standard	71	71.30
		71.3	
		71.6	

Table No.7 Heat induced hemolysis

The extract of plant demonstrated activity in stabilizing red blood cell (RBC) membranes, offering a notable percentage of protection against heat-induced lysis. The process of releasing inflammatory mediators involves the degranulation of sub-cellular organelles, and thus, stabilizing their membranes can reduce the inflammatory process.

Conclusion-

The extraction of Psidium guajava and Moringa oleifera plants was conducted, followed by the formulation of a cream using these extracts. Prepared cream was then tested for various factors including pH, spreadability, type of smear, type of emulsion, irritancy, phase separation, activity of anti-inflammatory through Suppression of protein, and hemolysis by heat-induced . Results indicate that the formulated cream exhibits favorable properties such as good spreadability, absence of irritancy, no phase separation, and enhanced anti-inflammatory activity. Consequently, the extracts is considered to be as natural sources of membrane stabilizing agents, Presenting promise as a possible alternative treatment for the management of inflammatory related to disorders and diseases.

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