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CHARACTERISATION AND FORMULATION OF ANTI INFLAMMATORY CREAM FROM EXTRACTED FROM *PSIDIUM GUAJAVA L*

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ABSTRACT

Background: *Psidium guajava* Leaves L. (Common name: Guava/Yellow Guava/ Lemon Guava) have been verified for some medicinal entities. The plants are worn in Ayurvedic, Unani and Siddha medicine for a variety of ailments. **Objective:** The purpose of the current research work was compare with Anti-inflammatory side effect ethanolic *Psidium guajava* extract. **Methodology:** To assess The phytochemicals and anti-inflammatory properties of the aqueous leaf extract For *P. guajava* L. Phytochemical investigations were conducted using normal qualitative tests and exposed the existence of terpenoids, alkaloids, carbohydrates, flavonoids, tannins, phenols, glycosides. The anti-inflammatory activity was resolute by *In-vitro* model: The percentage inhibition of denaturation of egg albumin ($R2 = 0.612$, $p = 0.01$) was dose reliant. The maximum inhibition was observed at 50 μg/ml (%MIC = 69.79) for egg albumin and at 100 μg/ml (%MIC =71.06) for Bovine Serum Albumin (BSA). **Result:** The anti-inflammatory effect obtained in BSA denaturation test was equivalent to reference drug Aceclofenac. **Conclusion:** It is concluded that the *P. guajava* L. ethanolic leaf extract possesses marked anti-inflammatory activity *In-vitro* and this is a novel finding.

Keywords: - *Psidium guajava* L., cream, *In-vitro*- anti-inflammatory Activity.

INTRODUCTION

India is a country with a rich assortment of medicinal flora and a rich history of Ayurvedic and traditional medicine. The earliest reference of Ayurvedic medicine in India dates back to prehistoric times and it was the main method of medication for decades.[1] Is an injury, infection or disturbance pathophysiological reaction characterized by heat, redness, pain, swelling and disrupted functions. Inflammation is a natural tissue injury-protective response caused by physical damage, noxious chemical or microbial agents. [2] Macrophages is main in various inflammatory diseases and in the immune response where they release proinflammatory mediators and proteins, including interleukin-6 (IL-6), tumor necrosis factor-a (TNF-a), cyclooxygenase-2 (COX-2), And inducible synthesis of nitric oxides (iNOS).1 The RAW264.7 mouse macrophage cell line, once activate by lipopolysaccharide (LPS) they produce proinflammatory cytokines and other inflammatory mediators, including nitric oxide (NO) and prostagland in E2 (PGE2) are synthesized by iNOS and COX-2, correspondingly.[3] The herbal creams containing artificial preservatives are used in skincare cosmetics contain side effects. Herbal cosmetics are used in crude form and powdered form mixed with a variety of ingredients and directly applied on the skin for instant relief although it cannot be preserved and stored for long period. Herbal cosmetics can avoid the skin from unusual, skin allergic reactions, skin conditions and skin diseases. Pflanzen cosmetics are favored more than synthetic cosmetic, they have smaller number of safe on skin, efficacy side, effects and quality and the cost of these products are also cost-effective and reasonable to customers.[4] *Psidium guajava* is a tree-like evergreen plant and is 6 to 25 ft tall. Figure 1 shows different parts of the plant, i.e., leaves, flowers, berries, seeds, and bark. The fruit contains vitamin A, C, iron, phosphorus and calcium. It has additional vitamin C than the orange. The fruit contains guaijavarin, quercetin, saponin, oleanolic acid, arabopyranoside, lyxopyranoside, and flavonoids.[5] As of the medicinal significance of *P. guajava*, a variety of works concerning to its ethnobiological olden times, as well as scientific investigations involving to confirmation of its common pharmacological effects, have been made. Present work will be more precise, exclusively addressing the medicinal use for treatment of diseases and symptomologies related to the action of opportunistic/pathogenic microorganisms, whether these may be protozoa, bacteria, fungi or viruses to recapitulate the scientific estimation attesting to the potential of this species beside such organisms. In a global background, where numerous populations feel the impact of scarcity, pharmaceutical

industry inattention and resistance of microbial pathogens to conventional antimicrobials, this study provides an pharmacological profile of the species *P. guajava* in the clinical scenario, pointing out interesting results on biological activities as a starting point for the deepening of scientific research in this field of pharmacology.

MATERIALS AND METHODS

Plant Collection:

Leaves of *P.guajaya* were collected from the residential areas of Karad. These leaves were dried under shade for 4-5 days and then reduced to powder form in a mortar.

Preparation of Plant Extract:

The dried leaves were powdered and passed through sieve no. 44 and the fine powder (60gm) was extracted by soxhlet extraction using ethanol (200ml) as a solvent for 24hours. The extract was filtered and solvent was evaporated to obtain powdered extract of leaves.[7]

Phytochemical Screening of *P. guajaya* extract:

Phytochemical screening tests of *P.guajaya* extract were performed according to the standard procedure from literature.

Physico-chemical characteristics of *P. guajaya* extract:

The *P. guajaya extract* sample was subjected to preliminary test (e.g.Sulphuric acid test, Sodium hydroxide test, Lead acetate test) were estimated according to the standard procedures.[8]

Formulation of Skin Cream

P. guajaya extract Mixed with 2 g of emulsifying wax, 1 g of stearic acid and 0.7 g of ceyl alcohol (4.76 g). The mixture was melted at 700C and a mixture was applied with constant stirring of 15 ml water, 1.7 g glycerin and 5 g Sodium stearate. The mixture was added with 0.8 g of sodium benzoate, 0.2 g of methylparaben and 0.5 ml of propylene glycol.

Table No. 1: Formulation of Anti-inflammatory cream

Sr. No.	Ingredients	Quantity
1.	*P. guajaya extract*	4.76 g
2.	Stearic acid	1 g
3.	Cetyl alcohol	0.7 g
4.	Emulsifying wax	2 g
5.	Propylene glycol	5 ml
6.	Sodium benzoate	0.8 g
7.	Sodium Stearate	5 g
8.	Propyl Paraben	0.2 g
9.	Glycerin	1.7 g

Physical Evaluation of Anti-inflammatory Cream -

1) Physical properties: Colour, odor and appearance of the Cream were observed. Check for thermal.

2) Stability: The regulated humidity chamber assessed the thermal stability of the formulation at 60-70 percent RH and 37 ± 10C.

3) Determination of pH: Measuring correctly 5 ± 0.01 g of the Cream in a 100 ml beaker. The Cream was applied 45ml of water and dispersed in it. The pH of the suspension was determined at 27^0 C using the pH meter.

4) Stability studies: Studies: The stability testing of drug products begins as part of the development of drugs and ends with the death of a compound or commercial product. Stability studies were conducted in compliance with International Conference of Harmonization (ICH) recommendations to determine drug and formulant stability. The stability studies were conducted according to ICH guidelines. The cream was packed in a bottle and held in a moisture chamber at 30 ± 2 ° C/65 ± 5 percent RH for two months. At the end of studies, samples were analyzed for the physical properties and viscosity[10].

Figure No. 1: Extraction and Formulation of *P. guajaya*

Biological Evaluation:

***In- vitro* anti-inflammatory activity**

1. Control DMSO
2. Standard Aceclofenac (50 µg/ml)
3. Test compound 50 µg/ml, 100 µg/ml.
4. Device used UV spectrophotometer

Evaluation of *in vitro* anti-inflammatory activity by protein denaturation:

The mixture (10 ml) consisted of 0.4 ml of egg albumin (from fresh hen's egg), 5.6 ml of Phosphate Buffered Solution (PBS, pH 6.4) and 4 ml of varying concentration of test samples so that final concentration become 50 µg / ml,100 µg / ml. Similar volume of DMSO served as control. Then the mixtures were incubated at (37 ° C ± 2) for 15 min. and then heated at 70 ° C for 5min. After cooling, their absorbance was measured at 660 nm (JASCO UV Spectrophotometer) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Aceclofenac at the final concentration of 50 µg / ml and 100 µg / ml were used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula.[1]

$$\% \text{ Inhibition of Protien Denaturation} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical screening:

An optimistic observation for Saponin, Flavonoids, and Steroids were recognized using phytochemical test in the *Psidium guajava* leaf.

Physical Evaluation of Anti-Inflammatory Cream

The pH of the prepared cream and it was found that extract is 5 which are appropriate for topical purpose because the pH of the skin is within 5-6. The outcomes of Physical Properties are summarizing in table 2. The outcome of Thermal Stability and pH of cream are summarized in table 3. The stability studies of the different parameters like nature, visual appearance and pH of the formulations showed that there was no considerable distinction after two months of the study period and the outcome are summarize in table 4. These formulations are harmless to use for skin.

Table No. 2: Physical Properties of cream:

Sr. No.	Test	Observation
1	Color	Green
2	Odour	Characteristic
3	Appearance	Semi- solid

Table No. 3: Thermal Stability and pH of cream

Sr. No.	Test	Observation
1	Thermal Stability	Stable
2	pH	5.71

Table No. 4: Stability studies of cream

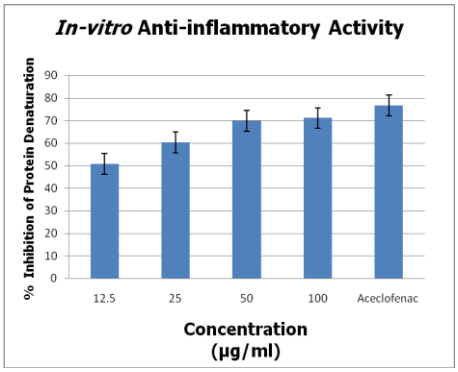
TESTS	30 ± 2°C/ 65 ± 5 % RH and			40 ± 2°C / 75 ± 5 % RH		
	Initial month	**After – 1 month**	**After – 2 month**	**Initial month**	**After – 1 month**	**After – 2 month**
Physical appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Texture	Fine	Fine	Fine	Fine	Fine	Fine
Colour	Green	Green	Green	Green	Green	Green
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
pH	5.7	5.8	5.8	5.4	5.4	5.7

Evaluation of *in vitro* anti-inflammatory activity by protein denaturation:

The formulated cream was tested for the presence anti-inflammatory activity by using protein denaturation method in-vitro. The present work covers study on anti-inflammatory and wound healing activity of the leaves *Psidium guajava* L. The anti-inflammatory activity was firm by *In-vitro* model: The percentage inhibition of denaturation of egg albumin (R2 = 0.665, p = 0.21) and it was dose reliant. The maximum inhibition was observed at 50 µg/ml (%MIC = 69.79 µg/ml) for egg albumin and at 100 µg/ml (%MIC = 71.06 µg/ml) for egg albumin shown in Table 5.

Table No. 5: Effect of *P. guajava* L. on *In-vitro* protein denaturation of egg albumin

Concentration (µg/ml)	% inhibition of protein denaturation	Viscosity (cps)
12.5	50.79	0.69
25	60.29	0.65
50	69.79	0.72
100	71.06	0.75
Aceclofenac	76.7	0.72



Graph No.1. The anti-inflammatory activities of *Psidium guajava* L.

CONCLUSION

From the study, it was concluded that the ethanolic leaf extract of *P. guajava* L. possesses marked *In-vitro* anti-inflammatory activity and this is a novel finding.

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