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Formulation and Evaluation of Transdermal Patches from Peperomia Pellucida for Anti-Inflammatory Activity

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ABSTRACT
Peperomia pellucida is one of the significant plant found through out India, and has a wide range of ethno medical uses. It is widely used in Siddha, Ayurveda, Unani, and other traditional medicinal systems. Many secondary metabolites found in this plants can be effectively employed to treat diseases, and infections. Peperomia pellucida plant and its extract is used to treat head ache, stomach pain, gout, and bite from insects. Major phytoconstituents present in Peperomia pellucida are alkaloids, flavonoids, terpenoids, glycosides, carbohydrates .Phytosomes of the Peperomia pellucida hexane extract was prepared by using rotary evaporator method. In vitro anti-inflammatory activity of phytosomes were performed by protein denaturation method. Which revealed that the plant is having significant anti-inflammatory activity. Transdermal patch was prepared using phytosomes of Peperomia pellucida and evaluation of the patch confirmed that it is of good quality.
Keywords: Peperomia pellucida, Phytosomes, anti-inflammatory, Protein denaturation test, Transdermal patch,

I. INTRODUCTION

Arthropods are the largest animal phylum, encompassing diverse groups like insects, spiders, crustaceans. Bites and stings of this species can cause swelling, redness, itching, and other reactions on the skin. It is possible to lessen the inflammation of these stings by using anti-inflammatory medications. [1] There are many medicinal plants which possess anti –inflammatory action, Peperomia pellucida is one of them. Peperomia pellucida is a herb which belonging to piperaceae family. Ethanobotanical survey reveals that it is used to treat insect bites and stings, wounds, fever. An adhesive patch that has been medicated and applied to the skin to administer a predetermined dosage form of medication is called a transdermal patch. An anti-inflammatory patch of phyto origin will be more convenient, because of its less side effects.

Collection of plant material

The plant of peperomia pellucida was collected in the month of November from Thrissur district .The Plant was authenticated by Dr.Ranjusha A. P, a botanist from the Department of Botany at NSS college ottapalam. The plant is standardised as per WHO guidelines.

Extraction

The coarsely powderedPeperomia pellucida plant leaves were extracted with n-hexane using maceration, at room temperature for seven days with occasional shaking. At the end of the extraction micelle is separated from the menstrum by filtration and transferred into a china dish for evaporation.The dried extract collected and stored for further studies.[3]

Determination of Total Flavonoid Content (Aluminium Chloride Colorimetric Method)

Stock solution: 100µl of test sample was pipetted out and the volume in each tube was made up to 1.0 ml with distilled water. Total flavonoid content was determined using aluminum chloride colorimetric method. The first step was the addition of 0.3 ml of 5% sodium nitrite followed by 5 min later addition of 0.3 ml of 10% aluminium chloride After 5 min later, 2 ml of 1 M sodium hydroxide was added. To the above mixture, distilled water was added to bring the total volume of the solution to 10 ml. Reference standard solutions of Quercetin (20, 40, 60, 80 and 100 µg/ml) were also prepared from the above solvent by same method. The absorbance of the test and standard solution was noted with reagent blank against the standard solution of known concentration at 510 nm

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UV/Visible spectrophotometer. Total flavonoid content was expressed as microgram of QE per milligram of extract.[4]

Determination of Total Phenolic Content(Folin Ciocalteu's Method)

1ml of sample (1 mg/ml) was mixed with I ml of Folin-Ciocalteu's phenol regent. Then 10 ml Sodium carbonate solution was added to the mixture followed by the addition of 13 ml of deionizes water and water and mixed thoroughly. The mixture was kept in the dark for 90 min after which absorbance was read at 760 nm, Standard Gallic acid solutions (20, 40, 60, 80, 100µg) were prepared . Total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution, total [phenolic content was expressed as µg/mg of extract.[4]

TLC of flavonoid

The plant extract and the biofractions applied on pre coated TLC plate(TLC silica gel 60 Fax) by using capillary tubes. Drawing a light line on the plate and dots to know the place of each extract applied on the plate. After using each mobile phase the TLC plate were air dried and observed under ultra violet light (254nm) for the development of the separated bands, the movement was expressed by its retention factor (Rf) values were calculated for different sample.[5,6]
Solvent phase: Toluene: Ethyl acetate: Formic acid [5:4:1]

Formulation of phytosomes
Rotary evaporation Technique

The isolated plant extract and soya lecithin were dissolved in dichloromethane at ratio of 1:1, using a rotary round bottom flask .The mixture was stirred for one hour, ensuring the temperature did not exceed 40c.Thin film of the sample was formed, to which n-hexane was added and stirred continuously until a monomer layer was achieved. Following this, phosphate buffer at pH 6.8 was incorporated and stirred to ensure thorough mixing .The final sample was stored in an amber bottle at room temperature the main source of the temperature The final sample was stored in an amber bottle at room temperature. The phytosome with molar ratio of 1:1, of the isolated plant extract and soya lecithin, were prepared.[7]

Sl.No	PhytosomalFormulationCode:	Ratioof Drug:soya lecithin	Dichloromethane[ml]	n-hexane[ml]	Phosphatebuffer solution[ml]
1	P1	1:1	20ml	15ml	5ml

Evaluation of phytosomes

The behaviour of phytosomes is influenced by factors like size, shape, stability, and distribution. Therefore the phytosomes are characterized by the following evaluation parameters :

- Measurement of Zeta potential (ZP)
- Measurement of particle size (PS)

Measurement of Zeta potential (ZP)

Zeta potential measurement of the optimized phytosome was measured using the Zetasizer Nano ZS90. The sample was diluted with water to 10 mL; a further 5 mL of this diluted sample was placed in a cuvette to determine the Zeta potential.[8]

Measurement of particle size (PS)

The particle size of phytosomes was determined using a particle size analyzer. Particle size is measured by dynamic light scattering (DLS). Particles suspended in a liquid are constantly undergoing random motion, and the speed of this motion depends on the size of the particles: smaller particles move faster than larger ones. In DLS, light is scattered by the sample, and the scattering is then detected and recorded many times. Comparison of those records with each other reveals how much the particles have moved in the time between each record (and therefore how fast they are moving). From this information, the average size of the particles can be calculated, as, the size distribution.[8]

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In-vitro anti-inflammatory test
Inhibition of albumin Denaturation
The following procedure was followed by Saleem. for evaluating the percentage of inhibition of protein denaturation.[9]

Control solution (50 ml)
2 ml of egg albumin, 28 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.

Standard drug (50 ml)
2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of standard drug (Aspirin) concentration of I, 2, 4, 8, and I ug/m.

Test solution (50 ml)
2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of plant extract (Peperomia pellucida hexane plant extract) concentration of 100, 200, 400, 800, and 1000 ug/ml. All of the above solutions were adjusted to pH using a small amount of IN HCI, The samples were incubated at 37° C for I5 minutes and heated at70o C for 5 minutes. After cooling the absorbance (at 23O nm) of the above solutions Percentage inhibition of protein denaturation was calculated using the following formula.

Calculation
Percentage inhibition = [Vt/Vc- 1] x 100 Where,
Vt= Absorbance of test sample
Vc = Absorbance of control

Preparation Of Transdermal Patch Of Peperomia pellucida n hexane extract
- Add chloroform (7.5 ml) and ethanol (7.5 ml) in the ratio 1:1.
- HPMC (180 mg) is added and dissolved by stirring.
- Glycerin (0.1 ml) and PEG 400 (0.1 ml).
- Phytosomes of Peperomia pellucida plant extract (15 mg) is diluted in Few drops of ethanol, add to the above solution and placed in magnetic stirrer for 30 minutes.
- Add few drops of Menthol.
- Pour in to a Petri dish and kept overnight for drying .[10]

Evaluation Test for Transdermal Patches
Organoleptic Characteristics
The physical appearance of developed patch was evaluated by using a naked-eye examination for its appearance, colour, clarity, flexibility,and smoothness.

Uniformity of Weight
Three matrix systems were taken and they were weighed individually. The readings obtained were recorded and average weight was determined.

Folding Endurance
A strip of specific area is to be cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be fold at the same place without breaking gave the value of folding endurance.

Percentage Elongation Break Test
The Percentage Elongation Break is to be determined by noting the length just before the break point, the percentage elongation can be determined from the below mentioned formula.
Elongation Percentage = [L1 – L2 / L2] × 100
Where,
L1 = Final length of each strip L2 = Initial length of each strip

Skin Irritation Test
The prepared patch of Peperomia pellucida was placed on the skin and tapped on the place. The patches are placed on for 20-30 minutes. The area of skin that was tested will be evaluated after the patches are removed.[10]

II. RESULTS

The hexane extract is collected after maceration.
Determination of Total flavonoid content

SL.NO	Concentration of Quercetin(mg/ml)	Absorbance at 510nm
1.	20	0.481
2.	40	0.484
3.	60	0.487
4.	80	0.490
5.	100	0.493
6.	Hexane extract of Peperomia pellucida leaf	0.488

Table no: 1 Absorbance of Standard Quercetin and extract at 510nm

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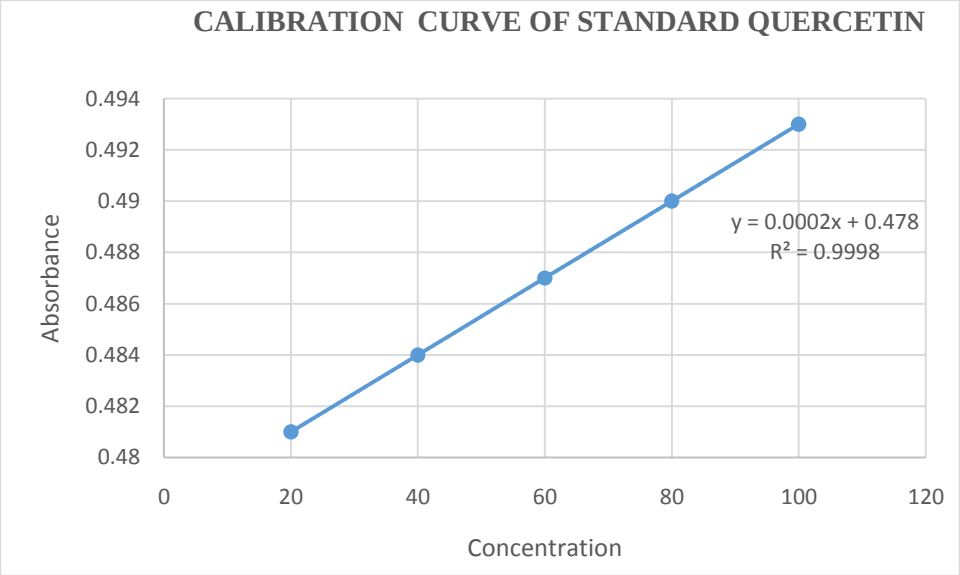

Figure no: 1 Calibration curve of standard Quercetin

SL.NO	Extract(100µg/ml)	Concentration of flavanoid content in µg/ml of sample
1	Hexane extract (100µg/ml)	63.12

Table no: 2 Total Flavanoid Content Hexane extract of peperomia pellucida

Determination of Total Phenolic Content

SL.NO	Concentration of Gallic acid	Absorbance at 510nm
1.	20	0.109
2.	40	0.311
3.	60	0.506
4.	80	0.686
5.	100	0.870
6	Hexane extract of peperomia pellucida plant	0.308

Table no: 3 Absorbance of Standard Gallic acid and extract at 510nm

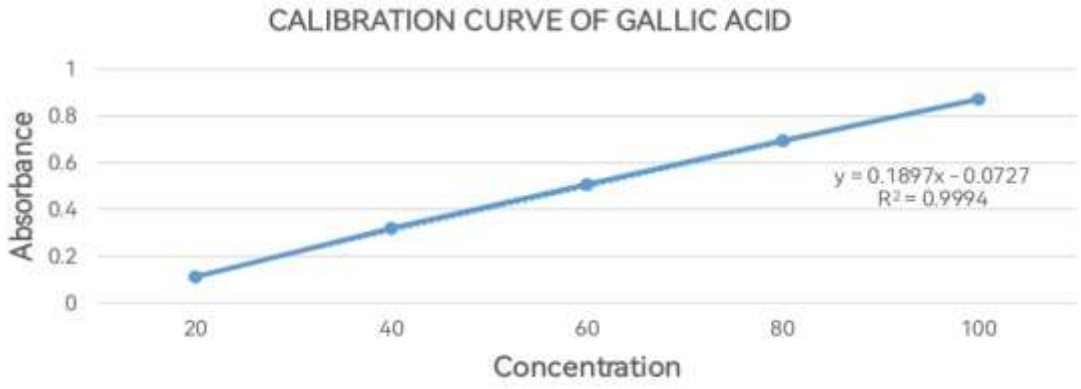

Figure no: 2. Calibration curve of standard Gallic acid

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SL.NO	Extract(100µg/ml)	Concentration of flavanoid content in µg/ml of sample
1	Hexane extract (100µg/ml)	40.90

Table no: 4 Total Phenolic content Hexane extract of peperomia pellucida

TLC of Flavanoid

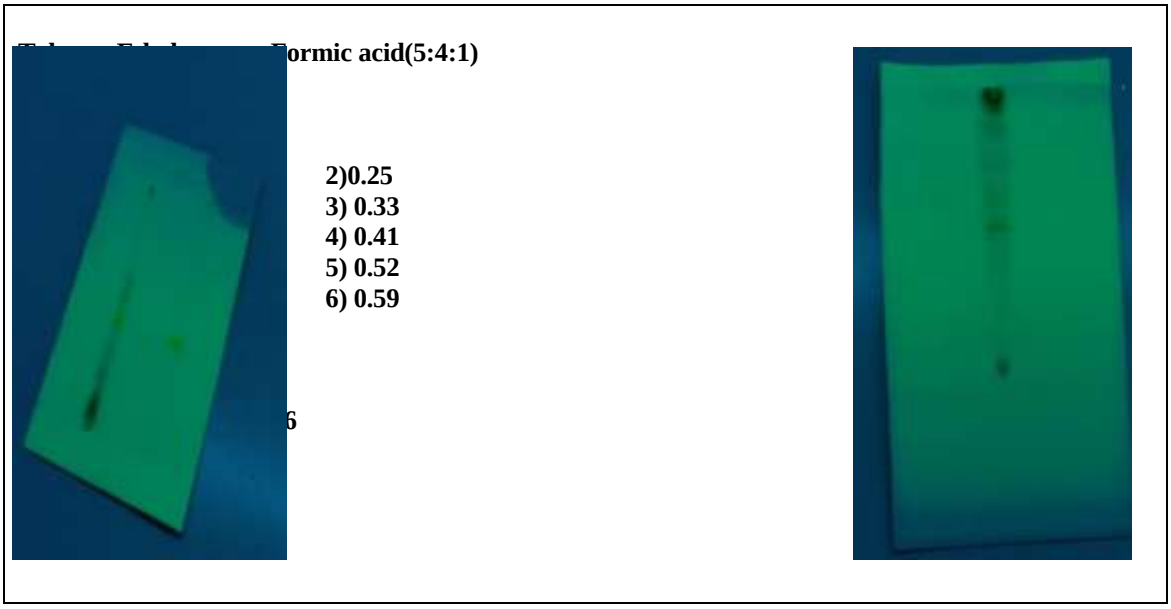

Figure no: 3 TLC Profile-Flavanoid(Toluene:Ethyl acetate:Formic acid)

Formulation of Phytosomes

Measurement of Zeta potential (ZP)

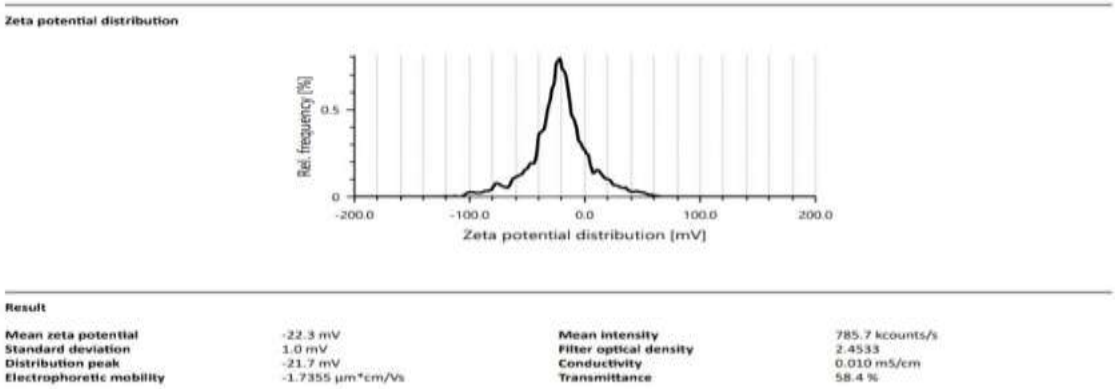

Figure no:4 Zeta potential (ZP)

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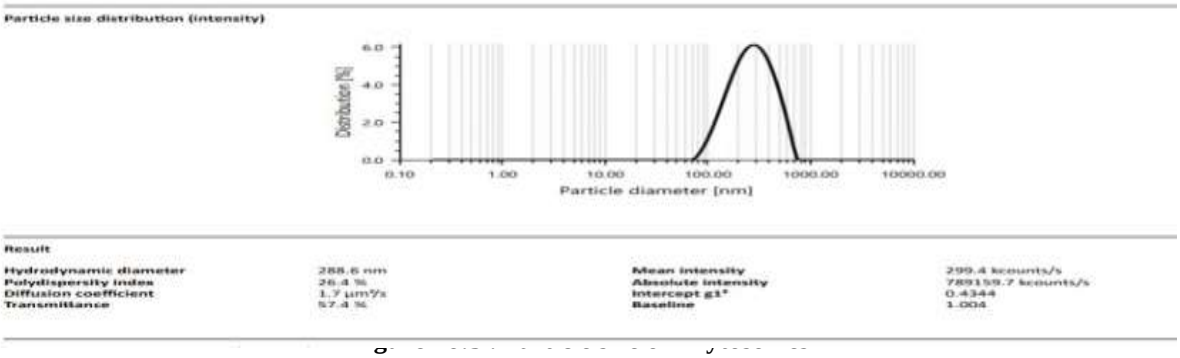
Measurement of Particle size:



In-vitro anti-inflammatory test

Sl.NO	Sample	Concentration (µg/ml)	Absorbance at 230 nm	Percentage inhibition (%)
1	Control		2.568	
2	Peperomia pellucida	100 200 400 800 1000	2.102 2.110 2.122 2.136 2.142	134.05 134.5 135.33 136.22 136.60
3	Aspirin	100 200 400 800 1000	2.227 2.329 2.373 2.425 2.448	142.02 148.53 151.33 154.6 156.1

Table no:5 Comparison of percentage inhibition of Aspirin and Peperomia pellucida extract

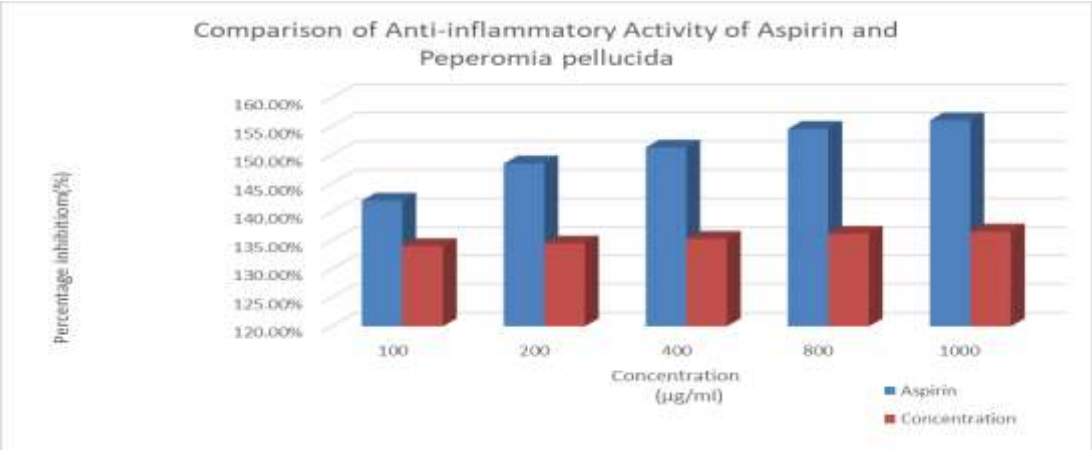


Figure no:6 Comparison of Percentage inhibition of Aspirin and extracts of Peperomia pellucida

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Evaluation test for transdermal patches

- **Organoleptic Characteristics**

The physical appearance of developed patch was evaluated by using a naked-eye examination for its appearance, colour, clarity, flexibility, and smoothness.

SL.NO	PHYSICAL APPEARANCE	OBSERVATION
1	Appearance	Jellified Preparation
2	Colour	Yellowish green
3	Clarity	Opaque
4	Flexibility	Yes
5	Smoothness	Good

Table no:6 Organoleptic characteristics

- **Uniformity of weight**

Weight of individual patch (g)	Average weight of patch (g)
0.250	0.249
0.251	
0.250	
0.249	
0.248	

Table no:7 Uniformity of weight

- **Folding endurance**

The number of times the film was folded at the same place without breaking is equal to value of folding endurance. So, number of times the film folded at the same place without breaking was found to be 130 times.

- **Percentage elongation break test**

Initial length of strip (LI) = 4cm
Final length of strip (L2) = 4.3cm
Therefore, percentage elongation = 7.5%

- **Skin irritation test**

The skin irritation test for patches of Peperomia pellucida was performed.Itchiness, Redness, Rashes, Burning were not observed.

III. CONCLUSION

The transdermal patches of Peperomia pellucida extract was prepared by using phytosomes of n hexane plant extract. plant leaves were extracted with n hexane using maceration. Quantitative estimation of phytoconstituents revealed that the extract having 63.12 µg/ml of flavonoid and 40.90 µg/ml of phenol. It shows good quantity of flavonoid and phenol.It may be responsible for anti-inflammatory activity.TLC profiling of the n hexane extract shown the presence of six flavonoids, out of which one was identified as Quercetin.The phytosomes of the hexane extract was prepared by using rotary evaporator method and evaluation was done by using particle size analyser (Hydrodynamic diameter = 288.6nm) and zeta potential (Mean zeta potential = -22.3Mv). so it is of good quality phytosomes and it helps in the penetration of active phytoconstituents in to the blood stream. Transdermal patch of Peperomia pellucida phytosomes were prepared. Evaluation test for patch were performed and concluded that the patch passes the evaluation parameter.In ethanobotanical survey revealed that the peperomia pellucida plant is used for treatment of insect bite, and from this research we concluded that the plant have significant anti-inflammatory activity which will be useful for treatment of inflammation caused by insect bite.

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