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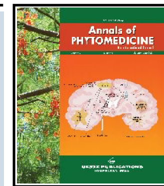

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Antiageing properties of *Plumeria pudica* Jacq. leaf extracts and development of antiageing cream for cosmetic applications

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Abstract

The process of skin ageing is intricate and affects both the epidermis and dermal layers. Research is being done on ways to protect and strengthen skin stem cells in order to slow down the ageing process of the skin. Science is looking for the best way to promote the growth of epidermal stem cells. The body's largest and most noticeable organ is the skin. Temperature control, microbial immunity, maintaining electrolyte balance, and defence against physical harm, chemical agents, and ultraviolet (UV) radiation are just a few of its many tasks. Every layer of the skin ages, mostly as a result of the breakdown of its constituent parts. Skin ageing is caused by the induction of oxidizing enzymes and the abundant production of reactive oxygen species. We can slow down the ageing process by comprehending the complexity of skin structure and the factors that contribute to skin ageing. Utilizing antiageing products is a common way to stop or fix the damage that causes ageing. Therefore, the present study concentrates on examining the antiageing properties of the *Plumeria pudica* Jacq. extracts. Qualitative and quantitative phytochemicals analysis was carried out. Antioxidant analysis like DPPH, FRAP, H_2O_2, phosphomolybdenum reduction were performed and antiageing lotion was developed. Biocompatibility of the developed lotion was examined by RBC hemolysis and skin irritation test. Phytochemical screening showed the presence of proteins, phenols, terpenoids, and flavonoids. The developed antiageing lotion showed significant levels of antioxidant activities. In addition, no toxicity signs were observed, *i.e.*, no hemolysis, skin irritation and red patches. Therefore, the developed antiageing lotion using *P. pudica* extracts can be used for cosmetic applications.

1. Introduction

The intricate process of skin ageing, which is influenced by intrinsic and extrinsic factors, affects both the epidermis and dermal layers. The ultraviolet (UV) radiation, which causes photoageing of the skin, is the most significant environmental component in skin ageing as it breaks down the lipids in the intercellular cement, resulting in holes in the skin's lipid barrier (leading to an increase in TEWL-trans-epidermal water loss), thereby causing modifications in the structure of fibrous proteins like elastin and collagen. Intrinsic factors that lead to the skin ageing include genetic, hormonal and biochemical processes which lead to epidermal-dermal interface flattening, skin thickness loss, dermal fibroblasts reduction, elastic tissue loss, *etc*., along with other irreversible changes to skin tissue. The primary internal variables affecting changes in skin structure include physiological disturbances in the rate of epidermal exfoliation, suppression of tissue regeneration and inhibition of tissue growth and differentiation processes. Lower stem cell activity, among other things, contributes to the skin's reduced capacity for regeneration.

Due to their efficiency and safety, natural materials are becoming increasingly popular in cosmetic goods today as interest in skin care grows (Ribeiro *et al.,* 2015; Hoang *et al*., 2021; Liu, 2022). According to their advantageous biological activities, different herbs have historically widely been used as food, medications and supplemental remedies. Natural herbal plant extracts have also been used in a range of sectors such as the food, chemical and pharmaceutical industries, *etc*.

Numerous biological activities have been assessed and recorded in various herbs as they possess diverse phytochemicals in them. Some of the herbal extracts have shown positive effects on topical treatments. Skin photoageing is largely caused by oxidative stress and, as some of the extracts can maintain the antioxidant/oxidant equilibrium (Petruk *et al.,* 2018; Papaccio *et al.,* 2022), those herbs which can externally deliver the endogenous antioxidants can be potential candidates against oxidative stress. According to reports, when used regularly, topical antioxidants can restore earlier photodamage by protecting the skin from damage caused by free radicals (Salavkar *et al*., 2011). In addition to oxidative stress, a steady decline in the functionality of skin tissue may serve to identify skin ageing (Calleja-Agius *et al*., 2007). Extracellular matrix, which is mostly made up of type I collagen fibrils and is primarily destroyed by the collagenase enzyme, specifically matrix metalloproteinases-1 (MMP-1), is essential for maintaining skin structural integrity and function (Fisher *et al*., 2009). Additionally, the elastase enzyme accelerates the degradation of elastin fibres, which is another factor contributing to the loss of skin elasticity (Weihermann *et al*., 2017). Therefore, a collagenase and elastase activity inhibitor would slow

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down collagen atrophy, the degradation of elastin networks, and skin ageing. Because the hyaluronidase-induced decrease in hyaluronic acid in the skin caused dryness and wrinkles, the hyaluronidase inhibitor was used for antiageing. (Abhijit *et al*., 2010). Furthermore, the most popular strategy for melanogenesis inhibitors that result in skin-whitening effects is the downregulation of tyrosinase, a crucial enzyme that catalyzes a rate-limiting phase of melanin formation (Pilliyar *et al*., 2017).

Human cell proliferation is constrained by two processes: replicative ageing, which is associated with changes in telomere structure and length, and premature ageing, which takes place without detectable telomere modifications. Cells with a high potential for proliferation are characterized by the first of the aforementioned mechanisms (*e.g.*, fibroblasts). The uncapping of the telomere loop structure precedes its onset, which is accompanied by a persistent loss of telomeric repeats. The end-replication problem, or the DNA polymerase's failure to fully synthesize a daughter strand on the matrix of the parental lagging strand, is thought to be the cause of this phenomenon (Ksiazek, 2010; Trehan *et al*., 2017). In addition to the end-replication issue, prolonged exposure of the cells to stimuli, particularly oxidative stress, may result in the progressive loss of telomeric DNA. Replication is instantly stopped as a result, telomeres shorten (Maestroni, *et al.,* 2017).

Research is being done on ways to protect and strengthen skin cells in order to slow down the ageing process of the skin. Regardless of the user's actual age, the goal of modern antiageing cosmetics is to improve the appearance of the skin by promoting and regenerating natural physiological processes that improve the skin's condition (Rhein *et al*., 2013). Consequently, antiageing cosmetics include ingredients that shield the skin from UV rays (UV filters). The second category of ingredients in these cosmetics includes antioxidants like coenzyme Q10, vitamins E and C, carotenoids, or polyphenols and flavonoids derived from plant extracts. Protein hydrolysates, nucleic acids, algal extracts rich in microelements, EFA plant oils and alpha hydroxy acids (AHA) with keratolytic and softening actions are among the substances that regenerate and postpone the ageing of the skin. Phytohormones, cytokines, and neuropeptides are other compounds that have become increasingly popular recently. Stem cells and their ability to promote the development of epidermal stem cells have received a lot of attention in recent years (Schmid *et al*., 2008; Moras *et al*., 2014)

The cosmetic industry is interested in plant resources because European law currently forbids the use of substances of human or animal origin (obtained against their welfare) due to ethical considerations. The total phenolic and flavonoid contents as well as biological properties relevant to skin applications, such as antioxidant, anti-tyrosinase, and antiageing properties, were also investigated in this study. The purpose of this research was to investigate the potential of the herbal extracts employed, notwithstanding the fact that they were derived from edible plants, for cosmetic or cosmeceutical uses when applied topically to the skin. The topical application not only has a local effect but also shields the active ingredient from first-pass metabolism and digestive tract damage. Therefore, the present study concentrates on examining the anti-ageing properties of the *P. pudica* extracts. Qualitative and quantitative phytochemicals analysis was carried out. Antioxidant analysis like DPPH, FRAP, H_2O_2, phosphomolybdenum reduction were performed and antiageing lotion was developed. Biocompatibility of the developed lotion was examined by RBC hemolysis and skin irritation test.

2. Methods and Materials

2.1 Collection of plant

The fresh plant of *P. pudica* was procured from local nursery garden (Figure 1). The washed leaves were dried under shade and stored in sterile containers to be used for extraction and analysis.

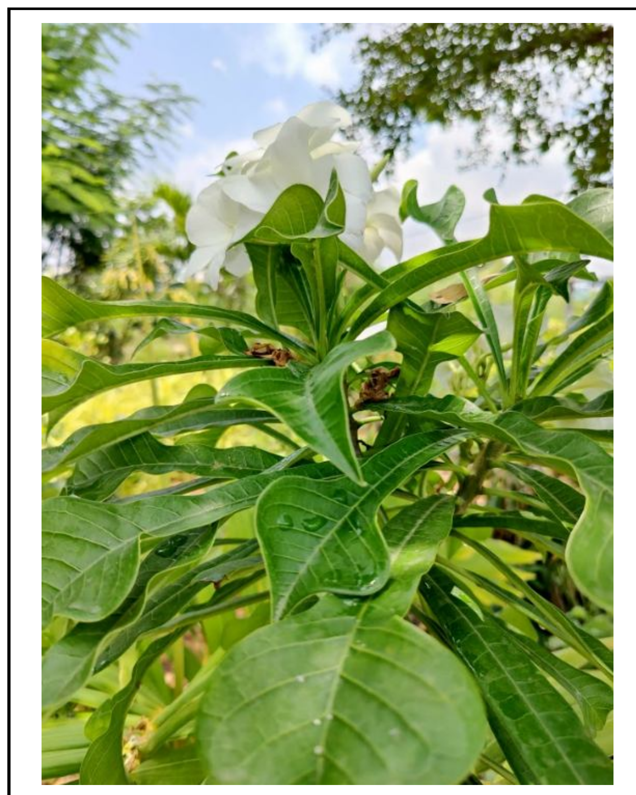

Figure 1: ***Plumeria pudica*** **Jacq.**

2.2 Extraction of bioactive compounds

In 10 cc of distilled water, methanol, acetone and ethyl acetate, one gram of sterilized leaves was dissolved. These were then pulverized using a mortar and pestle. The ground plant material was centrifuged for 10 min at 10,000 rpm. The supernatant was gathered and preserved for later studies.

The porous cellulose paper bag containing the powdered *P. pudica* leaf was placed in the Soxhlet apparatus thimble chamber. The extractor was filled with a methanol solvent solution, heated to 60°C, and left for 6 h. After gathering the extracts and evaporating the solvent, sterile containers were used to store them (Figure 2).

2.3 Phytochemical analysis

To identify the secondary metabolites, present in the various alcoholic and aqueous extracts of the leaf, a preliminary qualitative phytochemical study was carried out (Figure 3). After solvent extraction, the phytochemical test of leaf extract was examined.

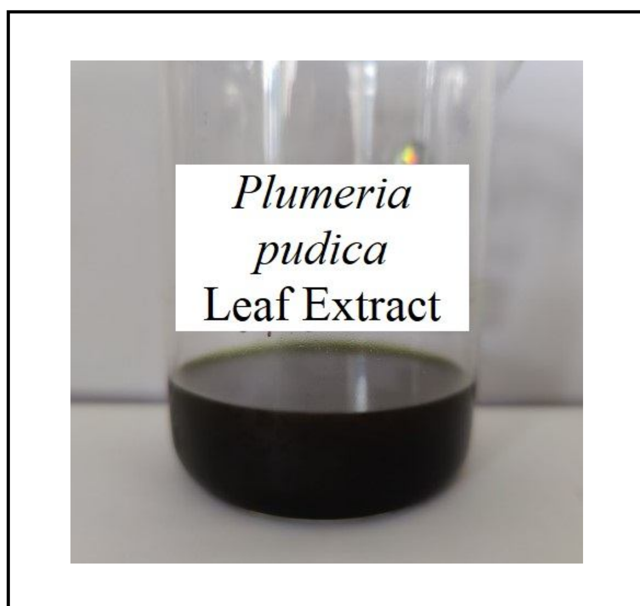

Figure 2: Leaf extract of *Plumeria pudica* Jacq. using Soxhlet apparatus.

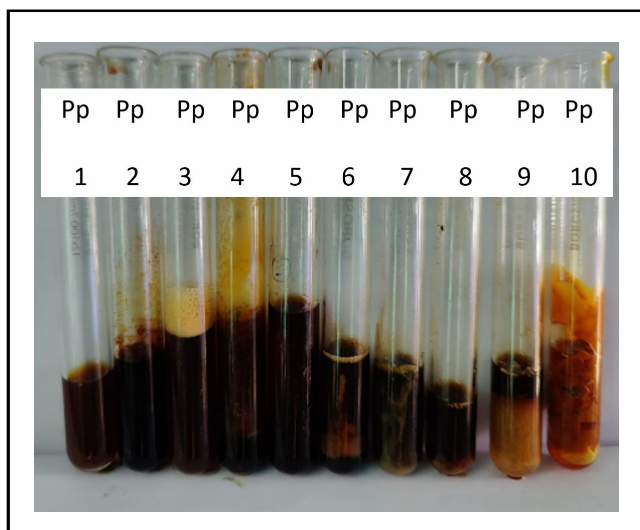

Figure 3: Phytochemical screening of bioactive compounds.

2.4 Identifying saponins

Test for foam: A small amount of the extract was shaken with 2 ml of water. The foam formed which would last for 10 min was considered as an indication of the presence of saponins.

2.5 Protein detection using a xanthoproteic assay

It involved treating the extracts with a little amount of concentrated nitric acid. Formation of a yellow color indicated the presence of proteins.

2.6 Test for steroid

After the crude extract had been mixed with 2 ml of chloroform, concentrated H_2SO_4 was applied through the sides of the test tube. A red color development in the lower chloroform layer was regarded as the presence of steroids. A second experiment involved by mixing crude extract with 2 ml of chloroform. The mixture was then added to 2 ml of concentrated H_2SO_4 and acetic acid, respectively. Presence of steroids were determined by observing a greenish coloration.

2.7 Test for terpenoids

In 2 ml of chloroform, the crude extract was dissolved, then evaporated to dryness. Then, for roughly 2 min, 2 ml of con. H_2SO_4 was heated with this. Terpenoids could be detected by their grey color.

2.8 Test for alkaloids

2 ml of 1% hydrochloric acid was added to the crude extract, which was then slowly heated. Later, the combination received Wagner's and Mayer's reagents. Alkaloids were assumed to be present based on the precipitate's turbidity.

2.9 Test for phenols and tannins

A 2% solution of $FeCl_3$ was dissolved in 2 ml of crude extract. A blue-green or black coloration was the indication of the presence of phenols.

2.10 Test for flavonoids

2.10.1 Shinoda test

A few pieces of a magnesium ribbon were combined with the crude extract, and then strong HCl was added drop by drop. After a little while, a scarlet color emerged, indicating flavonoids presence.

2.10.2 Alkaline reagent test

2 ml of crude extract was added with 2% NaOH solution and then dissolved. When a few drops of diluted acid were added, as a result of which, an intense yellow color that had formed turned colourless, indicating the presence of flavonoids.

2.10.3 Glycosides test

3 g of the was mixed with 3:7 technical ethanol, then refluxed for 10 min before being cooled and filtered. 20 ml of the filtrate from earlier was combined with 25 ml of distilled water and 25 ml of lead (II) acetate (0.4 M). The mixture is shaken, allowed to stand for 5 min, and then filtered. The resultant filtrate was extracted three times with 20 cc of a 2:3 solutions of isopropanol and chloroform. The collected water should be evaporated at a temperature no higher than 50°C. In 2 ml of methanol, dissolve the remaining material. Use the residual solution, which was placed in a test tube and evaporated on a water bath, for experiments. Add 2 ml of water and 5 drops of the Molisch's reagent to the remaining mixture. Once 2 ml of con. H_2SO_4 has been added gradually through the tube wall, check to see if a purple ring forms at the intersection of the two liquids, indicating the presence of a glucose bond.

2.11 Quantitative phytochemical analysis

2.11.1 Total phenolic content

The Folin-Ciocalteu reagent method was modified to determine the amount of phenol in the aqueous extract. To 1 ml of plant extract, 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 2% Na_2CO_3 solution were added. The resulting combination was allowed to sit at room temperature for 15 min. At 765 nm, the sample's absorbance was measured. The standard used was gallic acid (1 mg/ml). There were three copies of each test run. Gallic acid equivalent (mg/g of the isolated chemical) was used to express the results, which were calculated using the standard curve (Aiyegroro *et al*., 2010).

2.11.2 Total flavonoid content

A modified version of the $AlCl_3$ colorimetric method was used to calculate the flavonoid concentration. 5.6 ml of distilled water, 0.6 ml of CH_3OH, 0.2 ml of 10% $AlCl_3$, 0.2 ml of 1 M potassium acetate, and 1 ml of sample plant extract were combined with 1 ml of the extract and left at room temperature for 30 min. At 420 nm, the absorbance was measured. The standard utilized was 1 mg/ml of quercetin. There were three copies of each test run. The standard curve was used to calculate the flavonoid content, which was then represented as quercetin equivalent (mg/g of extracted molecule) (Aiyegroro *et al*., 2010).

2.11.3 Total alkaloids

With a few adjustments, the quantification method for determining alkaloids has been applied, as per Harborne (2005). 1 g of dry powdered plant was mixed with 100 cc of 10% acetic acid in ethanol, and the mixture was then covered and left to stand for 4 h. The extracts were then filtered and concentrated to 25 ml of their original volume on a water bath. After allowing the entire solution to settle, concentrated ammonium hydroxide droplets were added to the extract until precipitation formed. The precipitates were then washed with diluted ammonium hydroxide and filtered using Whattman filter paper. The residue was weighed after being dried in an oven set at 40°C. The following formula was used to calculate the alkaloid content:

% alkaloid = [final weight of the sample/initial weight of the extract] × 100.

2.12 Antioxidant assay

2.12.1 DPPH

Using the previously reported technique with a small modification, the antioxidant activity of the *P. pudica* leaf extract was assessed (Figure 4 a). In a nutshell, each well of the 96-well microplate received a mixture of 20 ml of five serial strengths of the diluted extract (12.5-100 g/ml) and 180 ml of DPPH (60 mol/l) in methanol. A microplate reader assessed the absorbance at 516 nm after 30 min in the dark (Versamac). Positive controls included gallic acid. The experiment was carried out three times. The following equation was used to calculate the DPPH radical scavenging activity:

% Inhibition activity = [(A control – A sample)/A control] × 100

where A control indicated the absorbance of the control and A sample was the absorbance of the sample. The IC_{50} value indicated the concentration of the sample that reduce 50% of the DPPH radical.

2.12.2 Ferric reducing antioxidant power (FRAP)

The extracts' reducing power was assessed using Oyaizuv's technique (Willet, 2002) (Figure 2). Briefly, 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%), along with a total volume of 1 ml, were added to 10-1000 l of cosmetic ethanol extract. For 30 min, the reaction mixture was incubated at 50°C. The reaction mixture was then gently mixed with 2.5 ml of trichloroacetic acid (10%) before being centrifuged at 3000 rpm for 10 min. Finally, 0.5 ml of 0.1% ferric chloride and 2.5 ml of distilled water were added to 2.5 ml of the upper layer from the reaction mixture. A 700 nm absorbance measurement was made. The increase in absorbance at 700 nm suggested that the extracts' reducing power had increased. The lotion extract's 50% inhibitory concentration (IC_{50}) was established.

2.12.3 Scavenging activity of H_2O_2 radical

Using the Ruch *et al.* (1989) technique, the H_2O_2 scavenging of the crude *P. pudica* extracts was evaluated. H_2O_2 (40 mM) solution in PBS (pH 7.4) was produced, and its concentration was assessed by spectrophotometry (Gene Quant 1300 UV-Vis) at 230 nm. Different amounts of this plant's stem and leaf extracts in distilled water were added to a 0.6 ml, 40 mM H_2O_2 solution, and after 10 min, the absorbance of H_2O_2 at 230 nm was measured in comparison to a blank solution made up of just the plant extracts without H_2O (Figure 4 c). The benchmark used was ascorbic acid. The following equation was used to calculate the percentage of H_2O_2 scavenging:

%scavenged [H_2O_2] = [(Abs control – Abs sample)/Abs control] ×100

2.12.4 Antioxidant activity using the reducing power (RP) method

The procedure outlined by was used to determine the reduction power of the plant extracts (Dorman *et al*., 2004) (Figure 4 d). A mixture of 2.5 ml of 0.2 mM phosphate buffer solution at pH 6.6 and 2.5 ml of 1% potassium ferrocyanide was added to aliquots of 1 ml of each sample's methanolic extract (at 8 different concentrations: 25, 50, 75, 100, 250, 500, 750, and 1000 mg/ml; two replicates per sample and concentration) for each sample.

The mixture was incubated in a water bath for 20 min at 50°C. Following the addition of 2.5 ml of 10% trichloroacetic acid, the solution was centrifuged at 3000 rpm for 10 min. Following centrifugation, 2.5 ml of the supernatant was combined with 0.5 ml of 0.1% $FeCl_3$ and 2.5 ml of distilled water. A UV-30 spectrophotometer was used to measure absorbance at 700 nm. The same amount of diluted extract was used to make the blank, but it was replaced with methanol.

2.12.5 Evaluation of antioxidant capacity by phosphomoly-bdenum method

The method described by Prieto *et al*. (1999) was used to assess the *P. pudica* leaves' overall antioxidant capability. 0.1 ml of the sample solution (1 mg/ml) and 1 ml of the reagent solution were mixed into an aliquot (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were sealed, and they were heated to 95°C (in a boiling water bath) for 90 min. The absorbance of the aqueous solution of each sample was measured at 695 nm against a blank after it had cooled to room temperature. A typical blank solution had 1 ml of reagent solution together with the necessary amount of the sample's solvent, and it was incubated in the same manner as the rest of the sample (Figure 4e). Water-soluble antioxidant capacity was measured in mg/g of extract for samples with unknown chemical compositions.

2.12.6 Preparation of antiageing cream

Oil-in-water lotion was made either with or without 0.05% PGLE. Glycerin, shea butter, 1,2-haxandiol, ethylhexylglycerin, cetearyl alcohol, mineral oil, and beeswax were among the main constituents in the lotion (Table 6). On the periorbital area, lotion (0.05%) with or without PGLE was used.

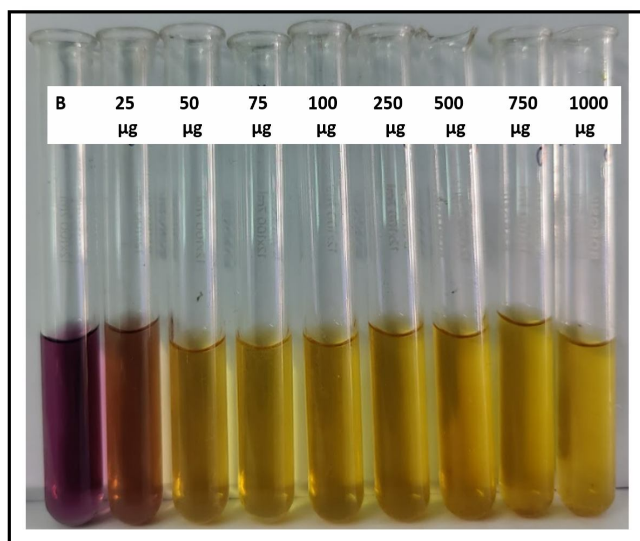

Figure 4(a): DPPH.

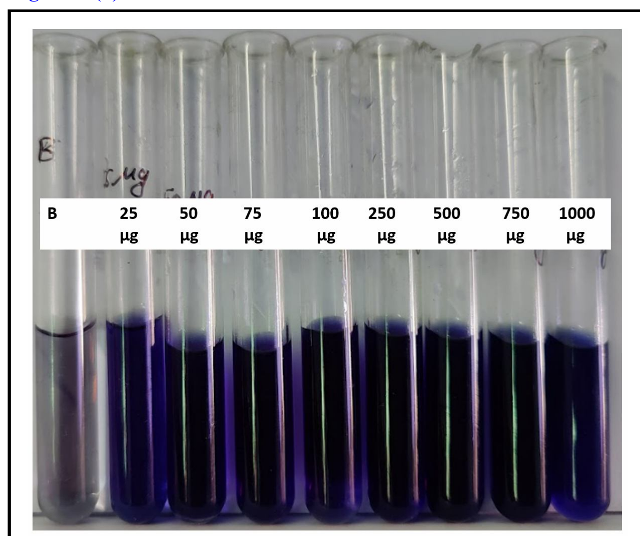

Figure 4(b): FRAP.

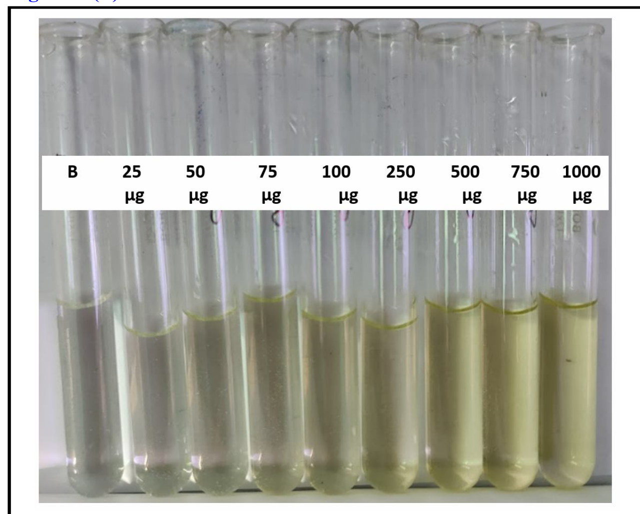

Figure 4(c): H_2O_2.

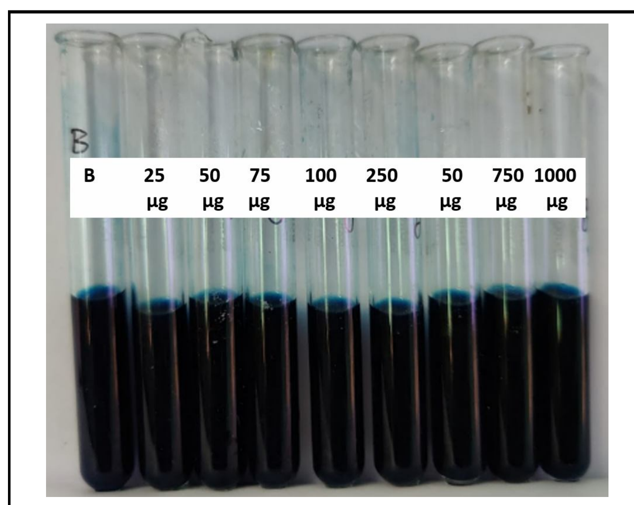

Figure 4(d): Reducing power.

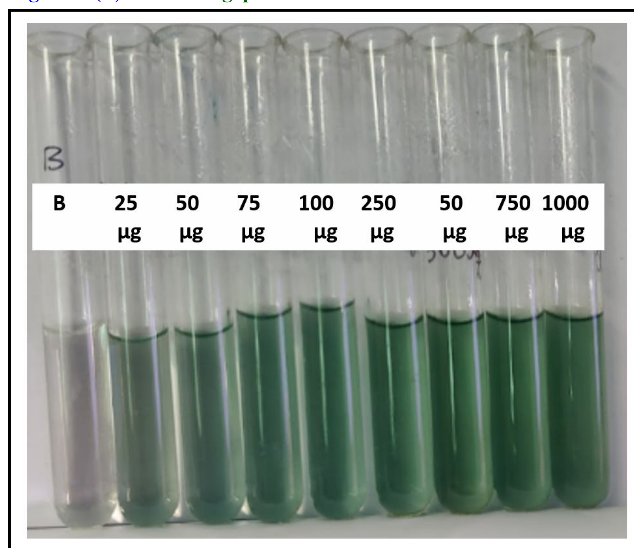

Figure 4(e): Phosphomolybdenum.

Figure 4 (a, b, c, d, e): Antioxidant activity of DPPH, FRAP, H_2O_2, reducing power, phosphomolybdenum.

2.13 RBC haemolysis assay

Fresh human blood obtained from a nearby pathology facility was used for the hemolytic assay, which was carried out as per standard protocols. 0.1% Triton X-100 was utilized in this experiment as a positive control. In a nutshell, different quantities of test samples were treated with fresh erythrocytes (10% in PBS pH 7.4) for 1 h at 37°C. To serve as positive and negative controls, respectively, Triton X-100 (0.1%) and PBS were employed. The erythrocytes were removed from each sample after the incubation period was over, and the supernatant was then put into brand-new Eppendorf tubes. The supernatant's absorbance was calculated at 540 nm. The following equation was used to calculate the hemolysis percentage:

$$\% \text{ Hemolysis} = [(AT - AC)/(A100\% \ AC)] \times 100$$

where, AC is the absorbance of the supernatant from controls (normal saline), and AT is the absorbance of samples that were incubated with the test samples. The absorbance of positive control supernatants after being treated with 0.1% Triton X-100, which completely lyses red blood cells, is equal to 100%.

3. Results

A new age in the creation of cosmetic products is currently underway. There are numerous variants of methods used to create cosmetic items that enhance skin appearance and postpone premature ageing. Skin physiology continues to be better understood. Topical treatments can have an impact on the skin, and new active ingredients and sophisticated formulations are now readily available, helping to create products that are more potent. There has been some scientific investigation into the antiageing properties of natural ingredients and organic cosmetics. Plants are known to contain secondary metabolites, which have been widely exploited in the cosmetics industry due to their considerable antiageing, skin-brightening, and sunscreen properties. *In vitro* research has demonstrated this. With the use of the latest cosmeceuticals manufacturing technology, wrinkles in the skin can be reduced, giving the appearance of a younger, healthier-looking face, radiant skin, and protection from skin ageing. This organic skin care product is typically hypoallergenic and quickly absorbs into the skin's outermost layers.

Figure 5(a): Subject 1 before applying the antiageing lotion.

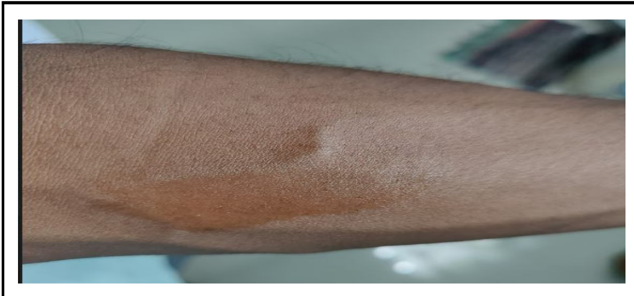

Figure 5(b): Subject 1 applying the antiageing lotion.

Figure 5(c): Subject 1 after applying the antiageing lotion.

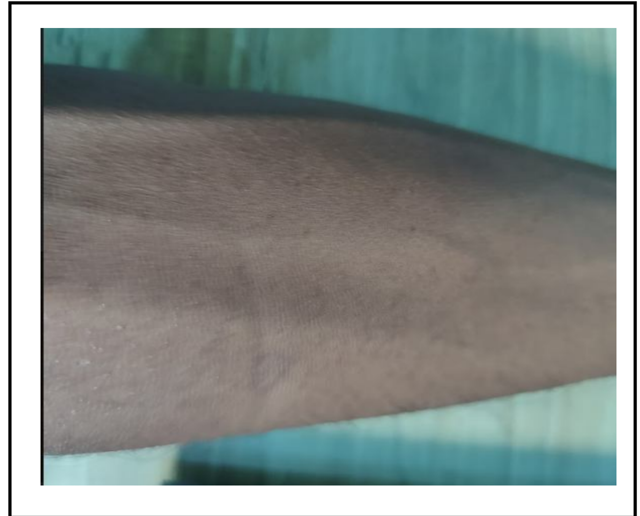

Figure 6(a): Subject 2 before applying the antiageing lotion.

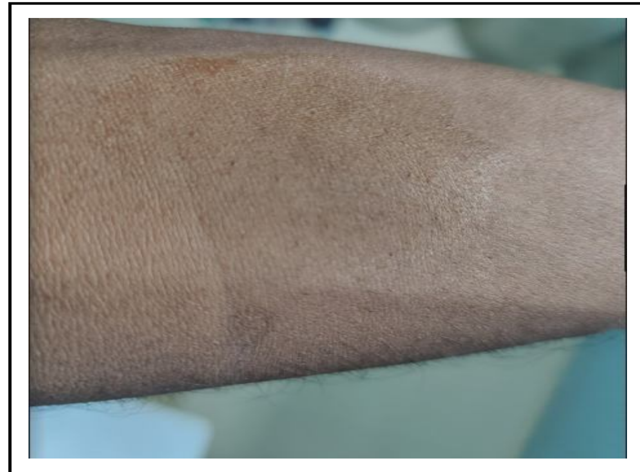

Figure 6(b): Subject 2 applying the antiageing lotion.

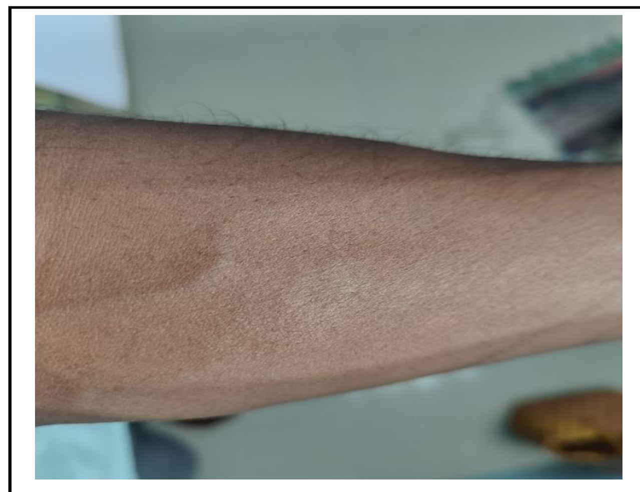

Figure 6(c): Subject 2 after applying the antiageing lotion.

Figure 5(a, b, c) and Figure 6(a, b, c): Subject 1 and 2 show no irritation on applying the antiageing lotion prepared from the extracts of *Plumeria pudica* Jacq.

3.1 Phytochemical screening

The results are shown in the table below, and they indicate the existence of biologically active substances like proteins, phenols, terpenoids, and flavonoids. There was no tannin, steroid, glyceroid, alkanoids, or quinone (Table 1).

Table 1: Phytochemical screening of the *P. pudica* extracts

S.No.	Phytochemicals	Results
1.	Tannin	Negative
2.	Flavanoids	Positive
3.	Saponin	Positive
4.	Steroid	Negative
5.	Terphenoid	Positive
6.	Glyceroid	Negative
7.	Phenol	Positive
8.	Alkanoid	Negative
9.	Quinone	Negative
10.	Protein	Positive

This table shows the presence and absence of phytochemicals in the leaf extracts.

3.2 Quantification of phenol, alkaloids and flavonoids

The OD values were measured for five different concentrations (g/ml), and the results were represented as gallic acid equivalents according to the conventional approach. 20 g of the substance had an OD value of 0.2836, 40 g had a value of 0.4218, 60 g had a value of 0.6173, 80 g had a value of 0.8242, and 100 g had a value of 1.0425 (Table 3).

Flavanoids were quantified using the accepted method using the quercetin standard. The OD values for 20, 40, 60, 80, and 100 in this study were measured to be, respectively, 0.0313, 0.0602, 0.1386, 0.1894, and 0.2417 (Table 2).

Table 2: Quantifications of flavonoids

Concentration (µg/ml)	OD value
20	0.0313
40	0.0602
60	0.1386
80	0.1894
100	0.2417

Quercetin standard values for the quantifications of flavonoids.

Table 3: Quantification of phenol

Concentration (µg/ml)	OD value
20	0.2836
40	0.4218
60	0.6173
80	0.8242
100	1.0425

Gallic acid standard for the quantification of phenol.

The quantification for the phenol test sample was measured to be 0.0381, 00910, 0.1618, 0.3526, and 0.5366 at five different concentrations, such as 20 g, 40 g, 60 g, 80 g, and 100 g. The OD values for the tested samples of flavonoids were measured as 0.0023, 0.0063, 0.0175, 0.0356, and 0.0718, respectively, for the concentrations of 20 g, 40 g, 60 g, 80 g, and 100 g. The table below contains the results that were displayed (Table 4).

Table 4: Quantification values of the sample for phenol and flavonoids

Concentration (µg/ml)	Phenol (OD)	Flavonoid (OD)
20	0.0381	0.0023
40	0.0910	0.0063
60	0.1618	0.0175
80	0.3526	0.0356
100	0.5366	0.0718

The quantification values of the sample for phenol and flavonoids for various concentrations.

3.3 Antioxidant activity

3.3.1 DPPH

The DPPH technique was used to assess the extracts' capacity to scavenge free radicals. The standard substrate for assessing the antioxidative activity of antioxidants is DPPH. The process is based on the reduction of methanolic DPPH-solution in the presence of an antioxidant that donates hydrogen, which occurs as a result of the reaction's production of the non-radical form of DPPH. The stable radical DPPH could be converted by the extracts to the yellow diphenylpicrylhydrazine. The results of the sample's DPPH activity were displayed as a percentage of eight distinct concentrations (mg/ml). The DPPH percentage was 12.38%, 18.05%, 21.19%, 34.81%, 46.87%, 51.51%, 65.29%, and 74.23% for doses of 25 mg, 50 mg, 75 mg, 100 mg, 250 mg, 500 mg, 750 mg, and 1000 mg. The results were tabulated below (Table 5, Figure 7).

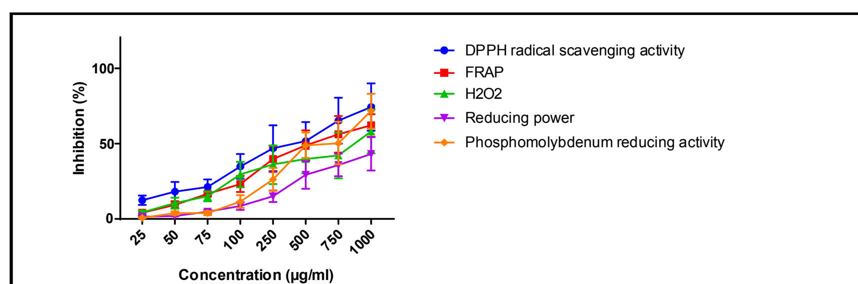

Figure 7: Graphical representation of the antioxidant activity of the *Plumeria pudica* Jacq. extract.

Table 5: Antioxidant activities of *P. pudica* extract

Concentration (µg/ml)	DPPH (%)	FRAP (%)	H_2O_2 (%)	Reducing power (%)	Phosphomolybdenum (%)
25	12.38 ± 3.2	3.9 ± 0.7	4.25 ± 1.1	1.48 ± 0.6	0.79 ± 0.2
50	18.05 ± 6.2	9.23 ± 2.3	10.46 ± 3.5	1.92 ± 0.3	3.73 ± 1.1
75	21.19 ± 4.9	16.57 ± 1.5	14.83 ± 3.2	4.72 ± 1.2	3.73 ± 1.5
100	34.81 ± 8.2	23.19 ± 5.4	29.30 ± 8.5	8.52 ± 2.6	11.42 ± 4.2
250	46.87 ± 15.3	39.73 ± 8.6	36.00 ± 12.9	14.96 ± 3.8	26.18 ± 7.6
500	51.51 ± 12.7	48.62 ± 10.1	39.61 ± 9.5	29.16 ± 9.2	48.86 ± 8.5
750	65.29 ± 15.3	56.1 ± 12.5	42.19 ± 15.3	35.79 ± 7.5	50.23 ± 14.2
1000	74.23 ± 12.7	62.08 ± 7.4	58.03 ± 14.6	43.15 ± 11.1	71.58 ± 11.5

This table shows the antioxidant activities; DPPH, FRAP, H_2O_2, Reducing power, Phosphomolybdenum for the leaf extract of *P. pudica*

Table 6: Skin irritation analysis

Subjects (oedema)	Irritation	Red patch
S 1	No	No
S2	No	No

The prepared antiageing lotion shows no redness and no irritation in both the subjects.

3.3.2 FRAP

The antioxidant activity of FRAP was found to be 3.90%, 9.23%, 16.57%, 23.19%, 39.73%, 48.62%, 56.1%, and 62.08% at various dosages, including 25 mg, 50 mg, 75 mg, 100 mg, 250 mg, 500 mg, 750 mg, and 1000 mg. The outcomes are listed above (Table 5, Figure 7).

3.3.3 H_2O_2

On the other hand, we used the H_2O_2 test in our study to further establish the antioxidant capacity of this plant. Because it may cross biological membranes, hydrogen peroxide is a significant reactive oxygen species. However, if it is transformed into a hydroxyl radical in the cell, it could be harmful (Gulcin *et al*., 2003). Plant extracts' ability to scavenge H_2O_2 is likely due to their phenolic compounds, which give H_2O_2 an electron and subsequently reduce it to water. H_2O_2 had antioxidant activity percentages of 4.25%, 10.46%, 14.83%, 29.30%, 36.00%, 39.61%, 42.19%, and 58.03% against various concentrations. Results are shown in the table above (Table 5, Figure 7).

3.3.4 Reduction potential

The tested sample's reduction potential percentage is 1.48%, 1.92%, 4.72%, 8.52%, 14.96%, 29.16%, 35.79%, and 43.15% for doses of 25 mg, 50 mg, 75 mg, 100 mg, and 250 mg, respectively (Table 5, Figure 7).

3.3.5 Phosphomolybdenum

The percentage of phosphomolybdenum activity for each concentration is shown in the table above (Table 5). For example, the percentage for 25 mg was 0.79%, for 50 mg and 75 mg; it was 3.73%, for 100 mg it was 11.42%, for 250 mg it was 26.18%, for 500 mg; it was 48.86%, for 750 mg it was 50.23%, and for 1000 mg; it was 71.58% (Table 5, Figure 7).

3.4 RBC hemolysis assay

An inexpensive and rapid way to assess the biosafety of compounds or formulations is to use the RBC hemolysis assay. This research aids in the screening of nontoxic formulations. The reaction mixture was incubated at 37°C for 1 h to assess the lotion extract's hemolytic behavior on human blood. Interesting results were obtained from the analysis of the lotion's ethanol extract on human RBC. The developed lotion showed no haemolysis (98% protection) of the red blood cells *ex vivo* analysis. Two people underwent the skin irritation test, and neither of the subjects showed any signs of irritationand red patches (Figure 5). The figure above shows the results (Table 6). The developed lotion was found to be safe and biocompatible for therapeutic purposes.

4. Discussion

The overexposure of human skin to external stressors like UV and pollution causes an increase in ROS production, which causes issues with man's skin like hyperpigmentation and premature ageing. Due to their effective skin protection capabilities, the biologically active chemicals found in plant extract pave the way for their usage as an active ingredient in the cosmetic industry. An antiageing cream could be made from the leaf extract's active ingredient. Plant extracts were subjected to phytochemical analysis, which identified constituents known to have physiological and medicinal effects. The phytochemicals tannins, saponins, phenols, steroids, flavonoids, terpenoids, glycosides and alkaloids were found in the plant extracts after analysis. One of the biggest and most common families of plant metabolites are phenolic chemicals (Singh *et al*., 2007). They have biological properties like those that prevent apoptosis, delay aging, fight cancer, reduce inflammation, prevent atherosclerosis, protect the heart, enhance endothelial function, and prevent angiogenesis and cell proliferation. The antioxidant qualities of medicinal plants that are high in phenolic compounds have been discussed in numerous studies.

The primary source of natural antioxidants is plant phenolic compounds such as phenolic acids, flavonoids, tocopherols, *etc*. (Ali *et al*., 2008). Tannins attach to proline-rich proteins and prevent the creation of new proteins. Plants produce flavonoids, which are hydroxylated phenolic compounds, in response to microbial infection. Flavonoids have been shown to have antimicrobial properties *in vitro* against a variety of microorganisms. Their capacity to interact with extracellular and soluble proteins as well as the bacterial cell wall is likely what causes them to be active. For centuries, alkaloids have been used medicinally, and one of their common biological characteristics is their cytotoxicity.

5. Conclusion

The study's findings so imply that the detected phytochemical compounds may be the bioactive components, and these plants are demonstrating their value as a source of bioactive substances with significant medical value. Antioxidants are chemicals that slow down the oxidation process by preventing the chain of polymerization that free radicals start and other oxidative reactions that follow. In this study, the lotion was made, and two subjects underwent a skin irritancy test. Applying the lotion did not cause any irritability in any of the subjects. Therefore, the lotion made from *P. pudica* leaf extract does not cause skin irritation.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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