# INVESTIGATION OF ANTI SKIN AGING PROPERTIES OF GRAPE PEEL HYDRO-ALCOHOLIC EXTRACT

**[1]Roopa C, [2]Bhuvaneshwari J**

Department of pharmacognosy
Al-Ameen college of pharmacy
Bengaluru 560027

***Abstract*- The present study aimed at the investigation of skin aging properties using *Vitis vinifera* peel extracts. Soxheleted hydro-methanolic extracts were tested for their phytochemical composition, Polyphenol, flavonoid and procyanidin contents of the extract were determined. The extract containing varying quantities of total Polyphenols were subjected for TLC studies and comparatively studied for their antioxidant potential by in-vitro DPPH and Ferric ion reducing power assay method.**
**The grape peel extract were evaluated for elastase inhibition and tyrosinase inhibitory activity.**
**Polyphenol content, flavonoid content and procyanidin contents of GPE was found to be (18.93, 10.4, 5.99% respectively); GPE showed good antioxidant activity and elastase and tyrosinase inhibition, indicating grape peel extract is a good ingredient for skin firming, anti-aging, skin lightening and sun protecting properties, it may be a suitable ingredient for anti-aging topical formulation.**

***Keywords*: Grape peel extract; antioxidant activity; Sun protection factor; elastase inhibition; Tyrosinase inhibition.**

## INTRODUCTION:

Skin, our largest organ by weight and extent, can be viewed as a sensor of the body's periphery, a veritable 'brain on the outside'.(1) Skin serves a variety of purposes. It serves as a barrier against water loss, infections and other types of stress, including thermal, chemical and UV radiation. Through a variety of nerve endings, skin keeps us connected to our surroundings, controls body temperature, improves metabolic processes, and synthesizes vitamin D.(2)
Skin aging is characterized by features such as wrinkling, loss of elasticity, laxity, and rough-textured appearance. Along with phenotypic changes in skin cells, extracellular matrix elements like collagen and elastin also undergo structural and functional changes as a result of ageing.(3)
*Vitis vinifera* (Grapes) is the most known of the about 900 species present in the Vitaceae family.(4) According to Ayurveda grape is framed as Drakshaa Phalottamma, meaning 'the best of all the fruits.' About 71% of the entire grape production is used to make wine, 27% to make fresh fruit, and the remaining 2% to make dried fruit. It is reported to improve vision, aids in digestion, appetizer, diuretic, purgative, laxative, cures jaundice, asthma, thirst, blood disease and heals diseases of vata, pitta and rakta.(5) Grape peel/skin, the outer layer of the grape (*Vitis vinifera*), is either green, red, or purplish-black in color. grape skins contain an array of bioflavonoids (quercetin, catechins, flavonols, and anthocyanidins) and non-bioflavonoid polyphenols (acid derivatives). One important non bioflavonoid in grape skin is called resveratrol. Extracts of grape peels are reported to promote cardiovascular health; prevent degenerative diseases; having antioxidant; antimicrobial; anti-inflammatory; antiaging activities.(6)(7)

## MATERIALS AND METHODS:

Grape peels were procured from MVM Agro tech Bengaluru, Karnataka; Gallic acid: Sigma, Germany; Methanol: 99.5% purity, Thomas baker(Chemicals) pvt. limited, Mumbai; Phenol reagent (Folin & Ciocalteu): s d fine-chem Limited, Mumbai; Sodium carbonate: Finar limited, Ahmedabad; Rutin: John baker Inc. Colorado, U.S.A; Potassium acetate: Thomas baker(Chemicals) pvt. limited, Mumbai; Aluminium chloride: Finar limited, Ahmedabad; Acetic acid: 99.8% Spectrochem pvt. ltd, Mumbai; Catechin: yucca enterprises; Mumbai; Concentrated Sulphuric acid solution: (98%) Finar chemicals; Vanillin: 98% Titan biotech limited; Twin trough chamber, CAMAG Reprostar 3 -version 1.3.3; Precoated TLC plate- Merk Precoated silica gel G 254, 10X10 cm; HPLC grade Methanol, Acetone, Acetic acid, Toluene, AR grade Formic acid.; DPPH: purity 85%, Himedia, Mumbai; Ascorbic acid: AR Grade, Titan Biotech Ltd; Potassium; Dihydrogen phosphate: AR Grade, purity 99,5%, Himedia, Mumbai; Disodium hydrogen phosphate: purity 99.5%, Qualigens; Trichloro acetic acid: purity 99.0%, Finar chemicals Limited, Ahmedabad; Potassium ferricyanide: purity 98.0%, Thomas Baker (chemical) pvt. Limited, Mumbai; Ferric chloride: purity 96.0%, Finar chemicals Limited,

Ahmedabad; Ethanol (Merck®) analytical grade; TrisHCL 100mM, SucAla3-*P*na (N-Succinyl-Ala- Ala- Ala-p-nitroanilide), pancreatic elastase 3.33mg/ml in sterile water, Epigallocatechin gallate (EGCG), L-DOPA 20mM, Phosphate buffer (0.1M) pH 6.2, DMSO 1.25%, Mushroom tyrosinase (100 U/ml), Kojic acid.

**COLLECTION AND AUTHENTICTION OF SAMPLES:**

Peels of *Vitis vinifera*. L (Vitaceae) used for this project were procured from MVM Agro tech Bengaluru, Karnataka. The sample drug have been identified and Authenticated by Central Ayurvedic Research Institute, Bengaluru, Karnataka.

**PREPARATION OF EXTRACTS.**

Grape peel powder of mesh size 16 was extracted by soxhlation with 70% methanol for 12 hours; and concentrated using a rotary vacuum evaporator at $60^0$C.

**PHYTOCHEMICAL STUDIES**

Qualitative phytochemical test and TLC was performed for identification of phytoconstituents.
Results are shown in table no 1

**ESTIMATION OF TOTAL POLYPHENOL CONTENT BY FOLIN CIOCALTEU COLORIMETRIC METHOD (8)**

The total polyphenol content of GPE (Grape peel extract) was determined using gallic acid as standard. Gallic acid of different concentration (0.8- 4 µg/ml) were reacted with 1:1 diluted FCR reagent and saturated sodium carbonate solution. Sample containing polyphenol were treated similarly, blank was maintained. The reaction mixture was incubated for 30 min at RT and absorbance of the color was measured at 670nm. Results are show in table no 2

**Estimation of total Flavonoid content by Aluminium chloride colorimetric method (9) (10)**

The total Flavonoid content of GPE was determined using Rutin as standard. A series of Rutin of 200µg/ml concentration were prepared and diluted suitably with methanol. Each of these test tubes were reacted with 10% AlCl2, 1M potassium acetate solution, followed by distilled water. Sample solution was treated similarly, blank was maintained. The reaction mixture were allowed to stand at room temperature for 30 min and absorbance of the reaction mixture was measured at 415nm. Results are show in table no 2

**Determination of procyanidin content by Vanillin sulphuric acid method (11)**

The procyanidin content of GPE was determined using Catechin as standard.
Standard solutions containing 0, 0.1, 0.2, 0.3, 0.4 and 0.5mg mL−1 were prepared. Aliquots of
0.5mL were transferred to five different test tubes, then mixed with 2.5mL of 30% H2SO4 methanol solution, 2.5mL of 4% vanillin methanol solution and the mixture was kept at $30^0$C for 20min. Sample was trated similarly and blank was maintained. The absorbance of reaction mixture was measured at 500nm (as per Lianfu Zhang et al with slight modifications) Results are show in table no 2

**TLC ANALYSIS OF EXTRACTS (12)**

**Solvent system:** Acetone**:** Acetic acid: Toluene: Formic acid (3:1:6:0.1)
**Stationary phase:** Silica gel GF 254
**Chamber saturation time:** 20 minutes
**Plate saturation time:** 5 minutes
**Development time:** 45mins.
**Detection:** UV (254nm, 366nm) and visible light.
**Procedure:** The sample (20mg/ml)and standards (1mg/ml) were applied as 5ml each as bands. The mobile phase was allowed to rise up to 8cms above the line of application. The plate was dried in air and observed under UV at 254nm and 366nm: and also, under visible light. The number of bands and Rf value were calculated and recorded. Photograph of the chromatogram at 254nm, 366nm and under visible light was captured. Results are
represented in Table no: 03; TLC chromatograms are shown in Photo no: 01

**IN-VITRO SCREENING OF ANTIOXIDANT ACTIVITY**

**In-vitro DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging assay (13)(14)(15)**

The method described by Brand-Williams et al was used with slight modifications to study the antioxidant activity. The assay was standardized using ascorbic acid as standard. The reaction mixture 4 ml contained, 0.1 ml of 25- 500 µg/ml of ascorbic acid in methanol were treated with 3.9 ml of freshly prepared DPPH (0.034 mg/ml) solution. The reaction mixture was kept in dark at room temperature for 30 min. The sample was treated similarly. The optical density of the

blank prepared in a similar way omitting sample /standard solution was measured at 517nm and compared with standard ascorbic acid. Percentage inhibition of DPPH was calculated as per formula given below. Results are show in table no: 04

**Calculation:**

**% Percent inhibition** = $\frac{A-B}{A}$ X 100

Where,
A= Absorbance of control
B= Absorbance of test sample / Standard

**DETERMINATION OF FERRIC (FE) ION REDUCING POWER (14)(16)**

The method described by vinayakaks et al was used to study the antioxidant activity Tannic acid was used as standard. The reaction mixture 9 ml contained 1 ml of 25-500 mg/ml of tannic acid in methanol were treated with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated for 20 minutes at $50^0$C, cooled rapidly, and mixed with 2.5 ml of 10% trichloroacetic acid and 0.5 ml of 0.1% ferric chloride. The sample was treated similarly. The blank was maintained. The intensity of iron (II) - ferricyanide complex was determined by measuring the formation of Perl's Prussian blue color at 700 nm after 10 min. Results are show in table no: 05

**DETERMINATION OF SPF (SUN PROTECTING FACTOR) (17)**

**Apparatus**

Shimadzu 1700 UV/Visible spectrophotometer,
equipped with 1 cm quartz cell.

**Procedure:**

1.0g of extract was weighed, transferred to a 100 mL volumetric flask, diluted to volume with ethanol, followed by ultrasonication for 5 min and then filtered through Whattman filter paper. The absorption spectra of samples in solution were obtained in the range of 290 to 400 nm using 1 cm quartz cell, and ethanol as a blank. The absorption data were obtained in the range of 290 to 320nm, every 5 nm, and 3 determinations were made at each point, followed by the application of Mansur equation.
(Mansur *et al.*, 1986).
equation: SPF = $CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$

Where: EE (l) – erythemal effect spectrum
I (l) – solar intensity spectrum;
Abs (l)- absorbance of sunscreen product ;
CF – correction factor (= 10)
The values of EE x I are constants. They were determined by Sayre *et al.* (1979), and are showed below
The values of EE X I :

| **Wavelength ($\lambda$ $nm$)** | **EE X I** |
|---|---|
| 290 | 0.0150 |
| 295 | 0.0817 |
| 300 | 0.2874 |
| 305 | 0.3278 |
| 310 | 0.1864 |
| 315 | 0.0839 |
| 320 | 0.0180 |
| **Total** | **1** |

Results are show in table no: 06

**DETERMINATION OF ANTI-ELASTASE ACTIVITY**

Different concentrations of extract were assayed for elastase inhibition by measuring their effect on elastase activity. The reaction mixture consisted of 2.6 ml of 100mM TrisHCL buffer, 0.2ml of substrate 4.4 mM SucAla3-*p*NA Solution and mix gently maintained at 25ºC. To this mixture 0.1ml of different concentration of extract (1-6mg) was added, followed by the addition of 0.1ml of pancreatic elastase the reaction mixture was mixed gently. The optical density was measured at 405 nm using UV-Vis thermofisher double beam spectrophotometer at 0 min after addition of all reactants

and after 30 min after incubation for all tubes at 25ºC. A blank contained TrisHCL buffer and the positive control contained TrisHCL buffer with Epigallocatechin gallate (EGCG). The percent inhibition of elastase was calculated with reference to positive control. The % inhibition of absorbance was plotted against the concentration to obtain the amount of extract necessary for enzyme inhibition to 50% (IC50). Results are show in table no: 07

**Calculation:**
Enzyme activity of extract was calculated according to the equation.

Units of Enzyme/ml = OD of Test – OD of Blank X3.00XDF8.8X0.10

where: 3.00=Total volume (mL) of assay
df = Dilution factor
8.8 = Millimolar extinction coefficient of *p*-Nitroaniline at 405 nm at pH 8.0
0.1 = Volume (mL) of Enzyme Solution used.
Percentage inhibition was calculated according to the equation:
% Inhibition = $(A_0\text{-}A_1/A_0)\times100$
Where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the sample extract.
Anti-elastase activity of GPE is recorded in table no:07

**DETERMINATION OF ANTI-TYROSINASE ACTIVITY**
Different concentration of extract (20-100µg) were dissolved in 1.25% DMSO were maintained at 37ºC temperature for 5 minutes. The reaction mixture consisted of 2 ml Phosphate buffer (0.1M) pH 6.2, 0.5 ml L-DOPA 20mM. To this add 0.5ml Mushroom tyrosinase (100 U/ml) and incubated for 10min at 37ºC. The optical density was measured at 490 nm using UV-Vis Thermofisher double beam spectrophotometer. A blank was maintained. IC 50 value was calculated using Graphpad prism software.
A blank contained Phosphate Buffer (0.1 M pH 6.2), L-DOPA and DMSO. Control contained Phosphate buffer, DMSO, L-DOPA with enzyme solution. Test contained Phosphate buffer, L-DOPA, extract and enzyme solution. Results are show in table no: 08

**RESULTS:**
**PHYTOCHEMICAL EVALUATION**

**Table no 01: Phytochemical screening of Grape peel extract (GPE)**

| PHYTOCHEMICALS | | GSE | GPE |
|---|---|---|---|
| Alkaloids | Mayer's test | -ve | -ve |
| | Wagner's test | - ve | -ve |
| | Dragendroff's test | - ve | - ve |
| | Hager's test | -ve | ve |
| Carbohydrates | Molish's test | + ve | + ve |
| | Benedict's test | + ve | + ve |
| | Fehling's test | + ve | + ve |
| Glycosides | Borntrager's test | + ve | + ve |
| | Modified Borntrager's test | + ve | + ve |
| | Liebermann Burchard's test | + ve | + ve |
| Saponins | Foam test | -ve | -ve |
| Triterpenes and phytosterols | Salkowski's test | + ve | + ve |
| | Liebermann burchard's test | + ve | + ve |
| Fixed oils and fats | Stain test | + ve | + ve |
| Resins | Acetone water test | -ve | + ve |
| Phenols | Ferric chloride test | + ve | + ve |
| | Lead acetate test | + ve | + ve |
| | Iodine test | + ve | + ve |
| Flavonoids | Alkaline reagent test | + ve | + ve |

| | | | |
|---|---|---|---|
| | Lead acetate test | + ve | + ve |
| | Shinoda test | + ve | + ve |
| Proteins and amino acids | Xanthoproteic test | - ve | - ve |
| | Ninhydrin test | - ve | - ve |
| | Biuret test | - ve | - ve |
| | Millon's test | - ve | - ve |
| Mucilage | Ruthenium red test | - ve | + ve |
| | Swelling test | - ve | + ve |
| Anthocyanins | Hydrochloride test | + ve | + ve |

**PERCENTAGE POLYPHENOL, FLAVONOID AND PROCYANIDIN CONTENT OF GRAPE PEEL EXTRACT**

**Table no 02:**

| Sample | % polyphenol content | % flavonoid content | % procyanidin content |
|---|---|---|---|
| **GPE** | 18.93 | 10.4 | 5.99 |

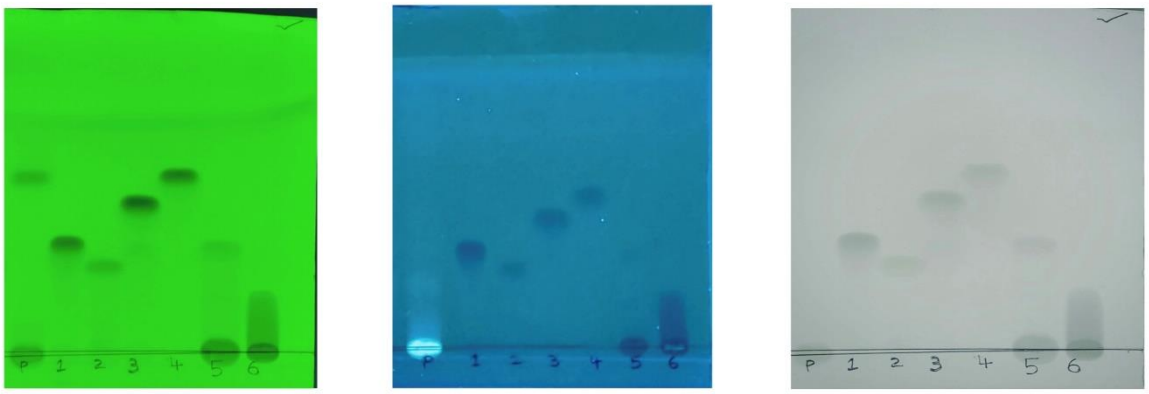


**Photo no 01: TLC of grape peel extract & standard polyphenols at 254nm, 366nm and visible light.**
Track 1: Grape peel extract
Track 2: Gallic acid
Track 3: Catechin
Track 4: Methyl gallate
Track 5: Propyl gallate
Track 6: Tannic acid
Track 7: Ellagic acid

**Table no 03: TLC data for identification of various polyphenols in grape peel extract:**

| SL.NO | CONTENT | UV VISUALISATION | | | | VISIBLELIGHT | |
|---|---|---|---|---|---|---|---|
| | | 254nm | | 366nm | | | |
| | | No. of spots | Rf value | No. of spots | Rf value | No. of spots | Rf value |
| 01 | Track 1 | 02 | 0.05<br>0.60 | 02 | 0.30<br>0.05 | 01 | 0.30 |
| 02 | Track 2 (Gallic acid) | 01 | 0.36 | 01 | 0.36 | 01 | 0.36 |
| 03 | Track 3 (Catechin) | 01 | 0.30 | 01 | 0.30 | 01 | 0.30 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **04** | **Track 4 (Methyl gallate)** | **01** | **0.48** | **01** | **0.48** | **01** | **0.48** |
| **05** | **Track 5 (Propyl gallate)** | **01** | **0.60** | **01** | **0.60** | **01** | **0.60** |
| **06** | **Track 6 (Tannic acid)** | **01** | **0.31** | **01** | **0.31** | **01** | **0.31** |
| **07** | **Track 7 (Ellagic acid)** | **01** | **0.17** | **01** | **0.17** | **01** | **0.17** |

**INHIBITION OF FREE RADICAL FORMATION (DPPH) OF GRAPE PEEL EXTRACT AND STANDARD ASCORBIC ACID**

Table no 04:

| **Concentration mg/ml** | **Absorbance at 517nm** | |
|---|---|---|
| | **Standard ascorbic acid (% inhibition)** | **Grape peel extract (% inhibition)** |
| 25 | 10.81 | 11.81 |
| 50 | 18.0 | 17.12 |
| 100 | 33.47 | 19.74 |
| 150 | 56.53 | 23.07 |
| 200 | 64.77 | 26.72 |
| 250 | 91.46 | 29.26 |
| 300 | 94.74 | 40.99 |
| 400 | 95.77 | 50.35 |
| 500 | 97.11 | 65.26 |
| **$IC_{50}$ in mg/ml** | **133** | **400** |

**FERRIC ION REDUCING POWER OF GRAPE PEEL EXTRACT AND STANDARD TANNIC ACID BY FRAP ASSAY.**

**Table no 05:**

| **Concentration mg/ml** | **Absorbance at 700 nm** | |
|---|---|---|
| | **Standard tannic acid** | **Grape peel extract** |
| 25 | 0.056 | 0.020 |
| 50 | 0.541 | 0.024 |
| 100 | 1.225 | 0.049 |
| 150 | 1.658 | 0.079 |
| 200 | 1.738 | 0.272 |
| 250 | 1.747 | 0.350 |
| 300 | 1.755 | 0.762 |
| 400 | 1.787 | 1.048 |
| 500 | 1.827 | 1.130 |

**DETERMINATION OF ANTI-ELASTASE ACTICITY**

**Table no 06:**

| Concentration of GPE / Epigallocatechin gallate (mg) | Optical density at 405 nm | Enzyme activity | Percentage of inhibition |
|---|---|---|---|
| Positive control | 0.119 | 0.405 | ----- |
| 1mg | 0.082 | 0.279 | 31.09 |
| 2mg | 0.076 | 0.259 | 36.13 |
| 3mg | 0.068 | 0.231 | 42.85 |
| 4mg | 0.052 | 0.177 | 56.30 |
| 5mg | 0.041 | 0.139 | 65.54 |
| 6mg | 0.040 | 0.136 | 65.10 |

**DETERMINATION OF ANTI-TYROSINASE ACTIVITY**

**Table no 07:**

| Concentration mg | Optical density at 490 nm | | Percentage of inhibition | |
|---|---|---|---|---|
| | Std (Kojic acid) | GPE | Std (Kojic acid) | GPE |
| Control | 0.913 | 0.812 | ---- | ---- |
| 10 | 0.726 | ---- | 20.48 | ---- |
| 20 | 0.508 | ---- | 44.35 | ---- |
| 30 | 0.392 | ---- | 57.09 | ---- |
| 40 | 0.335 | ---- | 63.30 | ---- |
| 50 | 0.327 | ---- | 64.18 | ---- |
| 20 | ---- | 0.713 | ---- | 12.19 |
| 40 | ---- | 0.643 | ---- | 20.81 |
| 60 | ---- | 0.514 | ---- | 36.69 |
| 80 | ---- | 0.452 | ---- | 44.33 |
| 100 | ---- | 0.417 | ---- | 48.64 |

**DISCUSSION:**

Phytochemical screening of GPE showed presence of polyphenols hence, we have quantified polyphenols, flavonoids and procyanidins in GPE. Phenolic compounds are secondary constituents distributed widely in leaves, seeds, bark and flowers of the plants offering protection against UV rays and pathogens. Polyphenols are reported to inhibit several enzymes such as cyclo-oxygenase (COX), glycosidase, amylase. (18) These phenolics are also important constituents of Human diet as they are found in fruits, vegetables as well as tea and wine. There are a variety of spectrophotometric and colorimetric methods which can be used for, quantification of various phenolic groups. Variety of methods are available of which the most popular one was Folin Ciocalteu colorimetric method is widely used.

Flavonoids are significant group of natural products: They are specifically a group of secondary plant metabolites with a polyphenolic structure that are commonly found in fruits, vegetables, and some beverages. Flavonoids are essential in a wide range of nutraceutical, pharmacological, medical, and cosmetic uses because they have a wide range of health-promoting benefits. This is due to their ability to regulate important cellular enzyme activities as well as their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties. Additionally, they have been found to be powerful inhibitors of a number of enzymes, including cyclo-oxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase. Flavonoid content was determined by aluminium chloride colorimetric method.(19)

Proanthocyanidins are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers and bark. Proanthocyanidins belong to the category known as condensed tannins, one of the two main categories of plant tannins. These are high molecular weight polymers comprised of the monomeric unit flavan-3-ol ((+) catechin and (-) epicatechin) which are linked through C4 C or C-Cs units. Approximately 15% of total proanthocyanidins are stored in grape skin, it has been shown by various in vitro and in vivo studies that grape seed proanthocyanidins seem to have pharmacological effects. These include properties that are cardio- and eye-protective, anti-oxidant, anti-microbial, anti-obesity, anti-diabetic, anti-neurodegenerative, anti-cancer, and anti-osteoarthritis. (20) procyanidin content was determined by Vanillin Sulphuric acid method.

Grape peel extract contains higher content of polyphenol compared to flavonoid and procyanidin content, polyphenols are the main components of grape peel extract and flavonoid content was found to be higher than the procyanidin content.

GPE showed the presence of polyphenols, hence TLC analysis of GPE was performed to develop fingerprint of the extracts for its polyphenols. The plate was spotted with few of the known polyphenolic compounds, made available in

our institution, viz. Gallic acid, catechin, methyl gallate, propyl gallate, tannic and ellagic acid. The GPE showed the presence of Catechin and Propyl gallate. No other spots were detected in the chromatogram indicating that the identified polyphenols are the major components of the extract.

Reactive oxygen species (ROS) are typically formed during cellular metabolism, but, when the balance between free radicals and antioxidants favours the former, they can also contribute to the pathological condition known as oxidative stress. Oxidative stress may cause cell damage, which could result in the emergence of numerous diseases. Reactive oxygen species effect on the skin causes skin sagging, melasma and aging. Several secondary plant metabolites are endowed with antioxidant activity and have been studied to fight against free radicals.(21) Rutin, Lutein, Arbutin and proanthocyanidins which are phenolic substances obtained from many plant species are potent free radical scavenger.(22) Arbutin is also reported for sun protection and skin brightening (23)

In vitro antioxidant activity of the extracts was done by 2 different assays namely DPPH free radical scavenging assay and ferric ion reducing power assay. GPE showed DPPH radical scavenging potential. however, it was lower than that of ascorbic acid used as standard. IC50 value of GPE was found to be 212 mg/ml.

Results of the ferric ion reducing power showed a concentration dependent increase in ferric ion reducing power for GPE; however, the ferric ion reducing potential of GPE were lower compared to standard tannic acid.

In-vitro evaluation of anti-aging potential was carried out for the GPE by two different bioassays namely elastase inhibition assay and tyrosinase inhibition assay.

Elastase is a protease enzyme which causes degeneration of elastin, which is an important component of the extracellular matrix of skin. Inhibitors of elastase can be useful to prevent loss of skin elasticity and thus prevent skin sagging. GPE shows concentration dependent elastase inhibitory activity, IC 50 value of GPE was found to be 3.5mg.

Tyrosinase is an oxidase enzyme that is the rate-limiting enzyme for controlling the production of melaninin skin . Since tyrosinase is a crucial enzyme in synthesizing melanin through melanogenesis, making it the most popular and effective target for inhibitors of melanogenesis that specifically block tyrosinase catalytic activity. Tyrosinase inhibitors such as hydroquinone, kojic acid, and L-ascorbic acid have been used as skin-whitening agents and as sun screen ingredients to protect against sun-uv rays damage to skin. (24)

We have employed tyrosinase inhibition assay to evaluate skin lightening, anti-blemish and sun protection effects of grape peel extract. The extract showed concentration dependent tyrosinase inhibition, showing IC 50 value of GPE was found to be 96.45mg. The SPF values for grape peel extract was determined by spectrophotometric method using mansur equation. GPE showed SPF of 19.44.

The enzyme inhibitory potential of the extract may be attributed to its phenolic compounds.

Thus, GPE is a suitable ingredient as anti-aging agent with protection from skin sagging and wrinkling due to their elastase inhibitory potential. Beside GPE exhibit anti-tyrosinase activity; which indicates their utility for sun protection.

## CONCLUSION:

Our investigation reveal that grape peel extract is a good ingredient as antiaging, skin lightening and skin firming components as evidenced by their antioxidant, anti-elastase and anti-tyrosinase activities. Besides, these ingredients are also reported for their anti-bacterial and anti-fungal properties thus, it may be suitable ingredient for anti-aging topical formulation.

## BIBLIOGRAPHY:


1. Tobin DJ. Introduction to skin aging. Journal of tissue viability. 2017 Feb 1;26(1):37-46.
2. Abdo JM, Sopko NA, Milner SM. The applied anatomy of human skin: A model for regeneration. Wound Medicine. 2020 Mar 1;28:100179.
3. Zhang S, Duan E. Fighting against skin aging: the way from bench to bedside. Cell transplantation. 2018 May;27(5):729-38.
4. Morazzoni P, Vanzani P, Santinello S, Gucciardi A, Zennaro L, Miotto G, Ursini F. Grape seeds proanthocyanidins: Advanced technological preparation and analytical characterization. Antioxidants. 2021 Mar 9;10(3):418.
5. Chopra V, Kaur A, Kalsi V. "An insight of *Vitis vinifera* (Draksha ): A Review." 2018;5(11):293-300.
6. "Grape Skin ." Gale Encyclopedia of Alternative Medicine. . *Encyclopedia.com.* 28 Oct. 2022 <https://www.encyclopedia.com>.
7. Iuga M, Mironeasa S. Use of Grape Peels By-Product for Wheat Pasta Manufacturing. Plants. 2021 May;10(5):926.
8. Heinonen IM, Lehtonen PJ, Hopia AI. Antioxidant activity of berry and fruit wines and liquors. Journal of agricultural and food chemistry. 1998 Jan 19;46(1):25-31.
9. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharm Sci. 2009 Jul 1;22(3):277-81.
10. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002 Jul 1;10(3).

11. Zhang L, Mou D, Du Y. Procyanidins: extraction and micro-encapsulation. Journal of the Science of Food and Agriculture. 2007 Sep;87(12):2192-7.
12. Villani TS, Reichert W, Ferruzzi MG, Pasinetti GM, Simon JE, Wu Q. Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration. Food chemistry. 2015 Mar 1;170:271-80.
13. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995 Jan 1;28(1):25-30.
14. Vinayakak S, Prashith Kekuda T.Rm, Raghavandra H.L., Sandeep M, Otari, Swarnalatha S.P, Surabhi K.S, Preethi H.R. Antidermatophyte and Antioxidant Potential of Remisatia Vivipara(Roxb) Schoot(Araceae). International Journal of Drug Development and Research, 2010; 2(1): 96-101.
15. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture. 1998 Feb;76(2):270-6.
16. Pandey S, Sah SP, Sah ML, Mishra D. An antioxidant potential of hydromethanolic extract of urtica parviflora roxb. Journal of basic and clinical pharmacy. 2010 Jun;1(3):191.
17. Sudhahar V, Balasubramanian V. Sun production factor (SPF) determination of marketed sunscreen formulation by In-Vitro method using UV-VIS spectrophotometer. Archives of Applied Science Research. 2013;5(6):119-22.
18. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity. 2009 Nov 1;2(5):270-8.
19. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. Journal of nutritional science. 2016;5.
20. Unusan N. Proanthocyanidins in grape seeds: An updated review of their health benefits and potential uses in the food industry. Journal of Functional Foods. 2020 Apr 1;67:103861.
21. Petruk G, Del Giudice R, Rigano MM, Monti DM. Antioxidants from plants protect against skin photoaging. Oxidative medicine and cellular longevity. 2018 Aug 2;2018.
22. Pavlović RD, Lakušićć B, Došlov-Kokoruš Z, Kovačević N. Arbutin content and antioxidant activity of some Ericaceae species. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2009 Oct 1;64(10):656-9.
23. Chandorkar NI, Tambe SR, Amin PU, Madankar CS. Alpha arbutin as a skin lightening agent: a review. Int J Pharm Res. 2021 Apr;13(2):3502-10.
24. Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. Journal of enzyme inhibition and medicinal chemistry. 2017 Jan 1;32(1):403-25.