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Assessment of the Total Flavonoid, Phenol, Alkaloid Content and Sun Protection Factor in *Grewia abutilifolia* Leaf Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A variety of secondary metabolites provide a valuable source of treatment for various pharmacological ailments. *Grewia abutilifolia* is a shrub found in the family Tiliaceae that is also investigated for its nutritional and medicinal benefits. Grewia is reported for its good antioxidant potential, which is applied to protect skin from UV rays. The main goal of this study is to determine the in-vitro sunscreen effect of *Grewia abutilifolia* based on phytochemical evidence of UV protection. Chemical treatments used to protect skin from the sun have several drawbacks, including numerous hazardous side effects on humans and the environment. The hydroalcoholic, ethyl acetate, and methanolic extracts were studied for their phytochemical, physicochemical, and sun protection activities. Total phenolic content, total alkaloid content, total flavonoid content, and SPF value were all highest in the methanolic leaf extract, with values of 99.20 0.12, 47.661.527, 96.02, and 14.50.01 for total phenolic content, total alkaloid content, total flavonoid content, and SPF value, respectively.

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Keywords: Grewia abutilifolia; sun protection factor; total phenolic content; total flavonoid contents; UV rays.

1. INTRODUCTION

Sunscreens have been quite popular on the market in recent years. However, the motive for their creation has changed. People desired a gorgeous sun tan quickly and effortlessly, without the risk of getting burned. Because of the need to protect against ultraviolet (UV) radiation, sunscreen products are now required for everyone. This review addresses all of the important features of herbs' potential as radioprotective agents, as well as their future possibilities.

1.1 UV (ultraviolet) Light

Infrared radiation (IR), visible light (VIS), and ultraviolet radiation (UV) are the three types of electromagnetic radiation. Heat is a type of infrared radiation that is invisible to the naked eye. The wavelength range of general light is referred to as VIS. UVA (320-400 nm), UVB (290-320 nm), and UVC (320-400 nm) are the three different bands of UV light, in order of decreasing wavelength and increasing intensity (200-290 nm). UV subdivision is divided into wavelengths and energy levels, which have diverse impacts on biological tissue [1].

1.2 Ultraviolet C Radiation

UVC, while having the highest energy and the greatest potential for biological harm, is well filtered by the ozone layer, and hence is not regarded a factor in human sun exposure [1] or of biological significance [2].

1.3 UVB (ultraviolet B) Rays

Latitude, height, season, time of day, cloudiness, and the ozone layer all influence the quantity of solar UVB and UVA reaching the earth's surface. The equator and higher elevations have the most irradiation. The ratio of UVA to UVB on the earth's surface is 20:1. Between 10 a.m. and 4 p.m., UV radiation is at its peak [3]. The UV spectrum that reaches the earth's surface on a summer day contains 3.5 percent UVB and 96.5 percent UVA. Erythema and sunburn are the most common side effects of UVB exposure. It has the potential to produce immunosuppression as well as photocarcinogenesis [1].

1.4 UVA (ultraviolet A)

UVA is less impacted by altitude or atmospheric conditions than UVB since it has a longer wavelength. UVA is more powerful than UVB, may pass through the skin more deeply [1,3] and is not filtered by window glass. In the shade, UVA radiation is thought to account for around half of all UVA exposure [3,4].

Unlike UVB, it is more effective at causing immediate and delayed pigment darkening as well as delayed tanning than it is at causing erythema. Immunosuppression, photoaging, eye damage, and skin cancer are among documented side effects of UVA exposure [5–9].

UVA rays are advantageous because they stimulate the formation of vitamin D3 by irradiating 7-dihydrocholesterol. They promote tanning by intensifying the darkening of produced melanin pigment. These rays, on the other hand, have been shown to cause photosensitivity, which can lead to a variety of allergic responses and actinic lesions [10].

1.4.1 UV radiation has a number of negative impacts

Erythema, edoema, and pigment darkening are the acute effects of UVB irradiation on human skin, followed by delayed tanning, thickening of the epidermis and dermis, and vitamin D production; longterm UVB effects include photoaging, immunosuppression, and photocarcinogenesis.

[3,11] UVB-induced erythema appears four hours after exposure, peaks between eight and twenty-four hours, and declines over a day or so; among fair-skinned and elderly people, UVB erythema may be chronic, occasionally latent [3,12]. With longer wavelengths, the efficiency of UV to induce erythema decreases rapidly; in comparison to UVB, roughly 1000 times more UVA exposure is required to elicit the same erythemal response. [2,3,13,14] UVA-induced erythema and tanning have biphasic temporal histories. Erythema is commonly seen just after the irradiation period ends, [3,15] but it disappears within a few hours, followed by a delayed erythema that begins at 6 hours and peaks at 24 hours. [3,15–18] UV-induced tanning and erythema have almost equal action

spectrums; however, UVA is more effective in causing tanning, whereas UVB is more effective in inducing erythema [3,19].

Grewia hirsuta, Grewia asiatica, Grewia optiva, Grewia nervosa, and *Grewia mesomischa* are only a few of the 673 species in the Grewia genus that have been scientifically confirmed to have anti-Diabetic properties, according to the plant list (2013) [5,6,7,8,9]. Shrub *Grewia abutilifolia* is belonging to the family *Tiliaceae,* additionally investigated for its diverse medicinal and nutritional benefits [10]. Ethnobotany studies reveal its various uses like a cooling agent, refreshing drink, Anti-inflammatory, Anti-rheumatism, demulcent and anti-diabetic [11]. With time, different therapeutic effects of the concerned genus are being established apart from its traditional utilization as fruit and cooling drink. Grewia bicolor seed oil is a promising cosmetic ingredient because of the ability of linoleic acid to encourage skin regeneration and moisturization [12]. Grewia is a good source of vitamins, minerals, flavonoid, and phenolic compounds, among other things, according to the literature, making this plant very beneficial to people. Grewia has a lot of flavonoids and phenols, hence it has a lot of antioxidant action.

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction Procedure

The *Grewia abutilifolia* leaves were collected during July-August of 2018, from in and around Pune, Maharashtra. Plant material washed properly with the help of Tap water was shade-dried. Anhydrous plant material, pulverized and screened through mesh size 100 mm was authenticated from National Institute of Science Communication and Information Resources (voucher no Ref No: NISCAIR/RHMD 3290/91). The hot Extraction method was used to extract Hydro alcoholic, ethyl acetate, and methanolic leaf extracts and concentrated under reduced pressure and temperature so that we can integrate the therapeutic effects. Apparatus used in analysis was LAB INDIA 3200 spectrometer.

2.2 Physicochemical Evaluation

2.2.1 Determination of extractive values

Coarsely powdered drug weighted in the quantity of 4.0 g, hot percolation was carried in a round bottom flask having 100mL of solvent. After six successive rounds, allow standing extract for 18 hours. Remove any remaining foreign elements from the extract. Take 25ml of extract and evaporate to dryness by rotary evaporator. At 105°Cfor 6 h residue was dried and cooled for 30min and weighted [13].

2.2.2 Determination of percentage loss on drying

*Grewia abutilifolia*is an edible plant and water encourage microbial, fungal and other chemical change is the physical state of coarse drug. 5gm of material was used to test for loss while drying. The sample was dried in an oven at 100°C–105°C for 2 hours or until the weight difference between two subsequent weighing was greater than 5 mg.

2.2.3 Determination of Total cash value

In a silica crucible, coarse powdered (4.0 gm) was placed (pre ignited and tared). Silica crucible placed in a preheated muffle furnace having temperature 500-600°C.Heat until drug converted into white ash (indication for absence of carbon). After cooling in desiccators, weigh it for total ash content.

2.3 Determination of Acid-Insoluble Ash

Add 25 mL of hydrochloric acid to the silica crucible having carbonless ash. Boiled for 5 minutes gently (covered with watch glass). 5mL of hot water is used to rinse watch glass and add this 5mL to the crucible. Filter insoluble matter with the help of ash less filter paper and neutralize filtrate with hot water. The dry insoluble matter with the help of a hot plate and ignite until finding constant weight. After cooling in a desiccator, weight acid insoluble content.

2.4 Estimation of Water-Soluble Ash

Boiled 25 mL of water with total ash, transfer soluble matter in a crucible, after ignition weight insoluble matter. Calculated the percentage as per the given formula.

% Water soluble ash =TA-IA/IA*100

TA= wt. of Total ash
IA=Wt. of Insoluble ash

2.4.1 Determination of sulphated ash content

The test was used to estimate the inorganic content present in the drug. To carry the test, take 1 gm coarse powdered drug and incinerate

in silica crucible (previously tarred and ignited). Without causing ignition sample was charred with 1 ml of concentrated sulphuric acid until white fumes no more given off. Put the charred sample in a muffle furnace (at 800±250C), until the sample is carbonless. Crucible then cooled and the difference was calculated sulfated ash content.

2.4.2 Determination of loss on drying

'Moisture' is the amount of water present on or within solid. This affects the quality and durability of the product. In medical science, all products used to determine the better health of human beings, where this small water amount can cause microbial as well as physical changes in the state of the medicinal plant. Moisture content or loss on drying was determined using a drying oven (Gravimetric method), in which a glass stopper shallow bottle was filled with 1gm of sample and put in the drying chamber (stopper was removed while process). The sample was dried until it reached a constant weight. In comparison to air-dried drugs, the percentage loss on drying was determined.

2.4.3 Phytochemical evaluation

The extracts were screened for alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins, amino acids, lipids/fats, as well as other phytochemicals. As mentioned in numerous publications Diverse polarity solvents plant extracts of *G. abutilifolia* were preliminary tested for the active components [14].

2.4.4 Quantitative determination of total phenolic content (TPC)

As per Dutta S and Ray S, 2020 Folin-ciocalteau reagent method was used to analyze Total Phenolic Content [15, 16]; for total phenolic content test, 02 ml leaf extracts were blended with Folin Ciocalteu reagent (1ml). A mixture of extracts and Folin Ciocalteu reagent was incubated for 5 minutes and 10 % Na_2CO_3 (5ml) was added. It was allowed to sit for 30 minutes (at room temperature) before even being measured for absorbance at λ_{max}725 nm. Using Gallic acid as a norm, and the observations were calculated as mg Gallic acid equivalent (GAE) per gram of extract (mg GAE/g DW). The LAB INDIA 3200 spectrometer was used to measure the absorbance.

2.4.5 Quantitative determination of total flavonoid content (TFC)

As per Dutta S and Ray S, 2020 TFC was estimated by Aluminium chloride (Colourimetric assay) "[15]". 0.5 ml of all extracts (1mg/ml) were put down in test tubes containing 1 ml distilled water with 1.5ml of 5% Sodium nitrite (5gm in 100ml distilled water) and 0.15 ml of 10% aqueous Aluminum chloride. Incubate for 6 minutes. After incubation, 1 ml of Sodium hydroxide (1M) was added, and distilled water was used to up to 5ml. Using Quercetin as a standard, determine the absorbance at 510 nm. The flavonoid concentration was measured in milligrams of Quercetin equivalent (QE) per gram of extract.

2.4.6 Quantitative determination of Total alkaloid content (TAC)

A 100 ppm solution of the generic alkaloid is made by diluting atropine (1 mg in 10 ml DW). Then, 0.5, 1, 1.5, 2, and 2.5 mL atropine add 5 mL of phosphate buffer (pH 4.7) and 5 ml of BCG solution in shaker. 5 ml of chloroform was added to each of them. The extracts were collected in a 10-ml volumetric flask and then diluted to the volume with chloroform. Absorption of each flask was measured at 470 nm read before a witness was prepared to absorb. After reading the absorption observed, calibration graphs were plotted. Extract (1mg/ml) was deliquesced in 2 N HCl strained later. With the help of 0.1 N NaOH, the pH of the PBS was adjusted. Add 5mL of Bromocresol green solution to One ml of solution separated along with 5mL of phosphate buffer. Shake the mixture and extracted it with chloroform with vigorous shaking. Collect extract in 10mL volumetric flask and make up the volume with chloroform. Absorbance was measured at 470nm [17].

2.4.7 Sample preparation for SPF

1 gram of sample from each extract was weighed separately and an ethanolic solution was made. It was sonicated for few minutes for aid in dissolving the extract completely; 5 ml from this stock solution was taken and made up to 50 ml ethanol. Similarly, 25 ml ethanolic samples were made with all extracts. Calibration was carried out in the range of 290 to 480 nm. Mansur equation was used to calculate Sun protection factor (SPF) values [6].

Sun Protection Factor = CF x $^{320}\sum 290$ EER x I x Abs Where

CF stands for Correction factor (10)

EER (λ) stands for Erythmogenic effect of radiation with wavelength λ Abs (λ) stands for Spectrophotometric absorbance (at wavel ength λ)

3. RESULTS AND DISCUSSION

3.1 Physicochemical Evaluation

Table 1. Physicochemical evaluation of *Grewia abutilifolia*

S. No.	**Tests**	**Wt. % (*Grewia abutilifolia*)**
1.	Total Ash	16.5±0.3 w/w
2.	Wt. of total acid insoluble ash	4±0.04%w/w
3.	Water-soluble Ash	85±0.36% w/w
4.	Sulfated Ash	3.5±0.02% w/w
5.	Loss on drying	12% w/w
6.	pH (In Water)	6.63

3.2 Extractive Value

Table 2. Extractive value of *Grewia abutilifolia*

S. No.	**Tests**	**Wt. % (*Grewia abutilifolia*)**
1.	n-Hexane extractive	0.713% w/w
2.	Ethyl acetate extractive	1.93%w/w
3.	Methanol extractive	7.58% w/w
4.	Hydroalcoholic extractive	6.88% w/w

3.2.1 Organoleptic behavior of powdered drug

Table 3. Organoleptic nature of drug *Grewia abutilifolia*

S. No.	**Treatment**	**Observation**
1.	HCl	The Powder settles down slowly Color: greenish-black
2.	Conc. HN03	Powder settles down slowly Color: greenish-brown
3.	Conc. H2SO4	Immediately give black color Powder settles down slowly Color: light green
4.	5% aq. NaOH	Powder settles down immediately Color: greenish Brown
5.	Iodine Solution	Powder float on the surface Color: light Green
6.	5% aq. KOH	Powder settles down slowly Color: green
7.	Glacial Acetic Acid	Powder float on the surface Color: greenish-brown

3.2.2 Fluorescence behavior of drug

Table 4. Fluorescence behavior of powder drug *Grewia abutilifolia*

S. No.	**Chemical Treatment**	**Under ordinary light**	**Under UV light 254 nm**	**Under UV light 366 nm**
1	Drug green powder as such	Green	Light Green	Greenish Black
2	Drug green powder treated with 1 N NaOH in Methanol	Dark green	Light Green	Greenish Black
3	Drug green powder treated with 50% HNO3	Green	Light Green	Greenish Black
4	g green powder treated with 50% H2SO4	Dull Green	Light Green	Greenish Black

3.2.3 Erythmogenic effect of radiation

Table 5. Erythmogenic effect value of radiation with wavelength λ and spectrophotometric absorbance values at the wavelength (I)

λ (nm)	EE×I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

3.2.4 Preliminary phytochemical analysis of *Grewia abutilifolia*

Preliminary Phytochemical analysis for the phytoconstituents present in a plant species forms the basis for its Pharmacological activity. Phenols and Flavanoids are responsible for the antioxidant property of plants which further contribute to curing Diabetes, Osteoarthritis, and various types of cancer. The plant *Grewia abutilifolia* consist of Tannins, triterpenoids, alkaloids, cardiac glycosides, flavonoids, and steroids, etc. as showed in Table 6.

3.2.5 Determination of total phenolic, flavonoid, and alkaloid contents

Naturally occurring secondary metabolites in plants which are aromatic in nature containing hydroxyl group come under Phenolic group category. Phenolic Groups have flavonoid as a subcategory. Both Flavonoid and other metabolites of Phenolic compound can be helpful in various Pharmacological activities i.e. inflammation, diabetes, cell proliferation, and many more [15, 16, 18]. The total phenolic compound of all leaf extract (Table 7) of *Grewia abutilifolia*, were calculated from the Calibration curve (Fig: 1) and were 89.06 ± 0.611, 99.00 ± 0.230 and 95.00 ± 0.529 for ethyl acetate, methanol, and hydro alcoholic extract and the total flavonoid contents were 92.33 ± 2.309, 97.66 ± 3.214 and 93.33 ± 0.577 for ethyl acetate, methanol and hydro alcoholic extract respectively (Table 8). Phenolic and hydroxyl group-containing compounds have redox properties, which permit them to act as good antioxidants. The total alkaloid compound of all leaf extract (Table 9) of *Grewia abutilifolia*, were calculated from the calibration curve (Fig: 3) and were 37.33 ± 1.527,47. 66 ± 1.527, and 33.00± 1.00 for ethyl acetate, methanol and hydro alcoholic extract.

3.2.6 Determination of the *in-vitro* sun protection factor

The sun protection factor of hydro-alcoholic, ethyl acetate, and methanolic leaf extract of *Grewia abutilifolia* were found out to be 8.45 ±0.001, 4.05 ±0.012, and 14.5 ±0.01, respectively (Table 10). The methanolic extract result showed a high SPF value whereas ethyl acetate extract showed the least SPF value among all. Highest SPF value indicated that the methanolic extract can be used as a potent sunscreen agent"[18]".

Table 6. Phytochemicals present in different extracts of leaves of *Grewia abutilifolia*

Phytoconstituents	ALC	MET	ETA
Alkaloids	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Terpenoids	-	-	-
Cardiac glycosides	-	-	-
Carbohydrates	+	-	+
Glycosides	+	+	+
Phenols	-	+	+

*** ALC- Hydroalcoholic Extract (Hot Extraction), MET-Methanolic Extract (Hot Extraction), ETA- Ethyl Acetate Extract (Hot Extraction) + Present, - Absent*

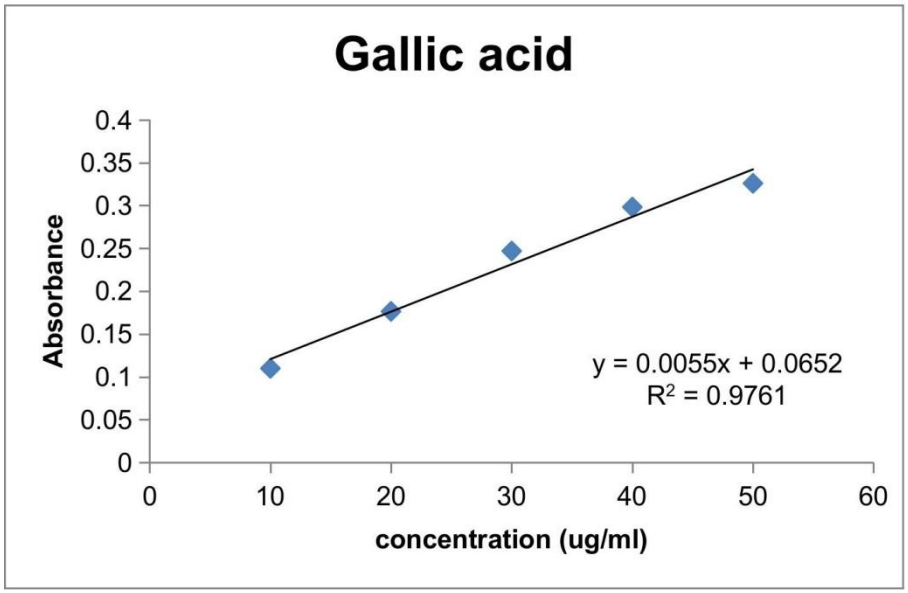

Fig. 1. The Standard curve for Gallic acid in total phenolic content table

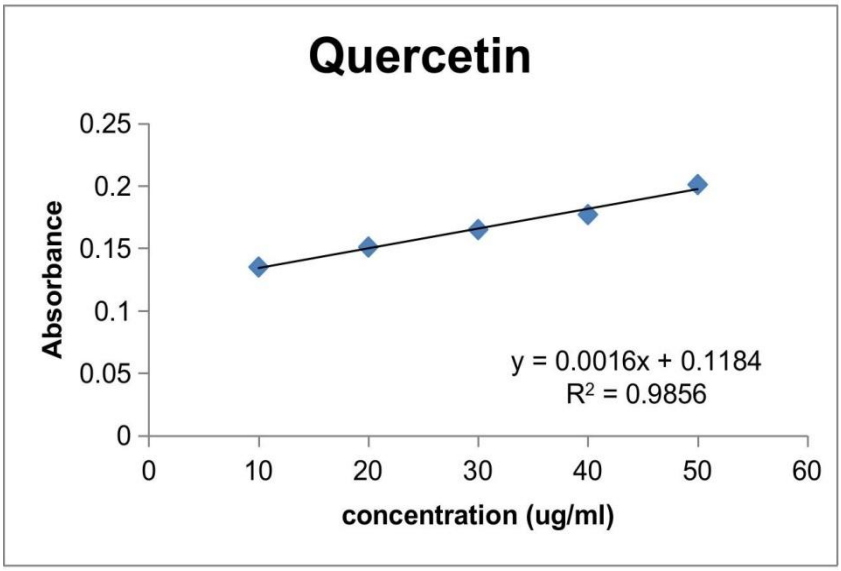

Fig. 2. The Standard curve for Quercetin in total flavonoid content

Table 7. Total phenol contents of all leaf extracts of *Grewiaabutilifolia*.

Extracts	Total Phenolic content (mg/gm. equivalent of Gallic acid)
Ethyl acetate	89.06 ± 0.611
Methanol	99.00 ±0.230
Hydro alcoholic	95.00±0.529

Table 8. Total flavonoid contents of all leaf extracts of *Grewiaabutilifolia*.

Extracts	Total Flavonoid content (mg/gm. equivalent Quercetin
Ethyl acetate	92.33±2.309
Methanol	97.66±3.214
Hydro alcoholic	93.33 ±0.577

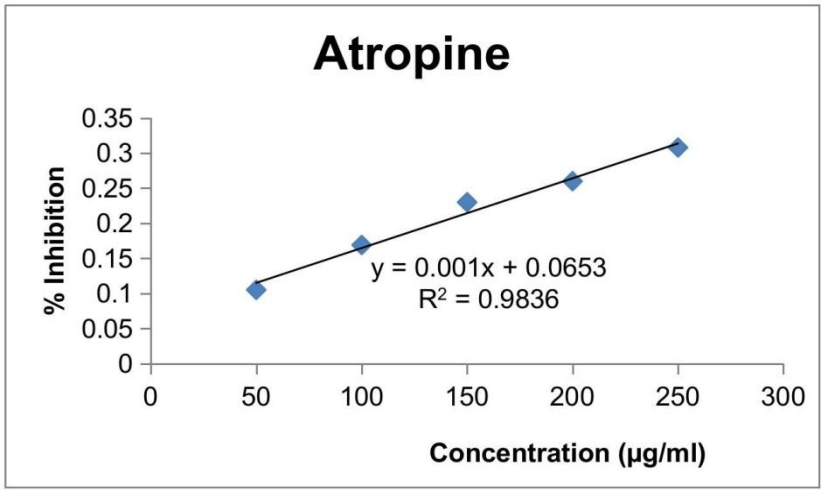

Fig. 3. Standard curve for atropine in total alkaloid content

Table 9. Total alkaloid contents of all leaf extracts of *Grewia abutilifolia*

Extracts	Total Alkaloid content in mg/g equivalent of Atropine
Ethyl acetate	37.33±1.527
Methanol	47.66±1.527
Hydro alcoholic	33.00 ±1.00

Table 10. SPF value of all leaf extracts of *Grewia abutilifolia*

S. No.	Type of extract	Sun protection factor (SPF)
1	Alcoholic	8.45 ±0.001
2	Ethyl acetate	4.05 ±0.012
3	Methanolic	14.5 ±0.01

4. CONCLUSIONS

Several active components were detected in leaf extracts of Grewia abutilifolia at various dosages in this investigation. The physicochemical parameters for Grewia abutilifolia leaves were identified, which are beneficial for determining the plant's legitimacy as well as ensuring the purity and uniformity of the coarse material. The plant's capacity to be employed as a medicinal plant is determined by its physicochemical qualities. Grewia abutilifolia's legitimacy as a cosmeceutical and medicinal plant was further proven by these studies. There will be many opportunities in the future to extract responsible bioactives from Grewia abutilifolia in order to justify the plant's potential use.

The total flavonoid content was calculated using milligrammes of quercetin equivalents (mg QE) (QE). Tables 7&8 reveal the total phenolic and total flavonoid content in the plant extracts investigated. By comparing spectral data (UV) with conventional atropine, the alkaloids were calculated and described. This chemical's standard values were quite near to the reported value. The UV Spectrometric technique may be used to evaluate the in-vitro SPF value of cosmetic formulations and is easy, rapid, and economical. In terms of time and cost, as well as ethical issues, using an in-vitro SPF test technique would be preferable. Natural alternatives are less toxic and have a less negative impact on the environment. According to the research, the extracts may absorb UV radiation and have UV shielding characteristics. We will be able to collect and compare data from various areas of the plant in the future. This illustrates the plant's value and preventative utility in the development of sun protection. Natural sunscreens will be a superior, competitive, and practical alternative to the currently available toxic chemical sunscreens. Chemical-based cosmetics are equally harmful and detrimental to the environment. It is economically feasible for both sun and skin care because of its sun-protective qualities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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