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EVALUATION OF PHYTOCHEMICALS OF *AEGLE MARMELOS* L. AND ITS INCORPORATION IN SKIN CARE FORMULATION

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ABSTRACT:

Aegle marmelos L. is a plant belonging to the genus *Aegle* and family *Rutaceae*, which is widely present in the sub-Himalayan region. It is locally known as "Bael". It is considered as the promising and divine tree specially offered to Lord Shiva. The leaves of this tree are generally trifoliate--a leaf that's divided into three leaflets--which are believed to represent the 'trinetra' or three eyes of Lord Shiva.

According to Hindu mythology, the leaf also represents the holy trinity--Brahma, Vishnu and Mahesh i.e. Shiva.

The plant contains the significant amount of sattva component that absorbs and emits the negative frequencies and it has various therapeutic property.

The chemical composition of Bael leaves contains tannin, saponin, quinone, steroid, coumarin, alkaloid, protein, flavonoid, terpenoid, glycoside and has antioxidant property which gives value addition to Bael.

Considering the above points in view, the present study aims to conduct the phytochemical analysis of the aqueous extract of Bael leaf and its effect on skin.

Key Word: Antioxidant, Bael, Flavonoid, Skin care, Terpenoid.

INTRODUCTION

In recent years, there has been a growing effort among researchers to identify and validate plant-based substances for the treatment of various diseases. It was estimated that over 25% of modern medicines are either directly or indirectly derived from plants. Indian therapeutic plants, in particular, are considered a vast reservoir of pharmacologically active compounds and have been used traditionally in home remedies to treat multiple ailments. Some of the most popular plants include Neem, Turmeric, Tulsi, Tomato, and Lemon and etc., which are known for their therapeutic potential in cosmetic preparation. From these plants, numerous bioactive compounds have been extracted and used in various skin and hair care formulations.¹

Another important Indian therapeutic plant is Bael (*Aegle marmelos* L.), commonly known by different names such as Bilva, Shiva Patra, and Tri-patra. The name "Tri-patra" signifies the plant's symbolic association with the Hindu trinity of Brahma, Vishnu, and Mahesh (Shiva). Bael belongs to the *Rutaceae* family and is one of the three species in the genus *Aegle*, growing throughout the hills and plains of sub-Himalayan regions of India as well as in Thailand, Bangladesh, Pakistan, Sri Lanka^{2,3}. This plant holds immense significance in traditional Indian herb for its wide range of therapeutic properties.

Botanical description

In India, Bael flowers bloom in April and May after new leaves sprout, and the fruit takes 10 to 11 months to ripen, typically from March to June the following year⁴.

Bael, is a slow-growing, medium-sized tree that reaches 12-15 meters in height. It has a short trunk, thick flaking bark, and spreading, sometimes spiny branches. Young shoots often have stiff spines and exude a clear, gummy sap that solidifies when exposed to air. The tree has alternate, deciduous leaves that grow in clusters of 3 to 5 leaflets, each 4-10 cm long. The new leaves are glossy and pinkish-maroon, while mature leaves release a strong odor when crushed. Its fragrant flowers, found in clusters of 4 to 7, have fleshy petals that are green on the outside and yellowish on the inside.

The fruit is round, oval, or oblong, ranging from 5 to 20 cm in diameter, with a hard shell that turns yellowish when ripe. Inside, it contains 8 to 20 segments of aromatic, orange colored pulp, which is sweet but astringent. The fruit also contains 10 to 15 seeds, each encased in sticky mucilage that hardens when dried⁴.

Application of Bael leaves:

- Traditional uses: Bael fruits have been traditionally used in various folk medicines for their therapeutic properties. The fruit pulp is used to prepare dietary delicacies like murabba, puddings, and juice. In traditional medicine, Bael fruits are known for their effectiveness in treating chronic diarrhea, dysentery, and peptic ulcers. They are also used as a laxative and to aid in recovery from respiratory ailments. These traditional uses have been passed down through generations, highlighting its importance in natural remedies.

-Pharmaceutical uses: antidiabetic, anticancer, antifertility, anti-microbial, antimicrobial, immunogenic and insecticidal activity, make it a valuable ingredient pharmaceutical preparation.

-Cosmetic uses: Its extract is rich in vitamins, tannins, alkaloids and flavonoids, which help to reduce skin inflammation³, antimicrobial properties¹, anti-inflammatory, anti-acne and antioxidant properties of Bael help neutralize free radicals^{6,3}. It's incorporation into cosmetic products offers a natural and effective way to improve skin health

In this article, the moisturizing property of it were explored. The focus was on exploring the extract that contribute to skin hydration and improved texture.

By investigating this property and their effectiveness in formulations, the goal is to establishing the Bael leaf extract as a potential ingredient in herbal skincare products designed to enhance and rejuvenate skin health.

Chemical composition of Bael:

1. Alkaloids: Aegelenine, Aegeline, Dictamine, Ethyl Cinnamamide, Ethyl Cinnamate, and etc., are found in Fruits and Leaves.
2. Coumarins: Imperatorin, Marmelide, Marmesin, Marmin, Methyl Ether, Psoralen, Psoralen-A, Umbelliferon, Xanthotoxol, and Etc., Are Found in all Parts of Plant.
3. Terpenoids: Caryophyllene, Cineol, Cis-Linalool Oxide, Cubedol, Elemol, Limonene, Linalool, Elemol, Terpinolene, and Etc., are Found in Fruit, Leaves and Bark^{2,7,8}.

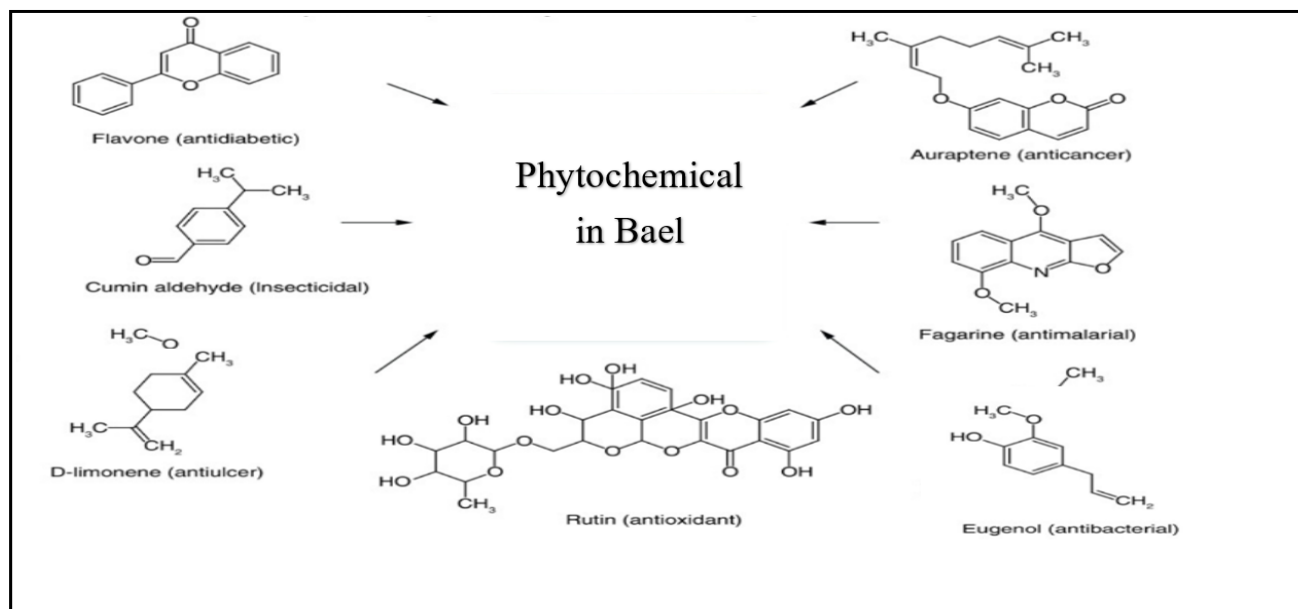


Figure 1: Phytochemicals present in Bael plant

MATERIAL AND METHODS

Big matured and un-infected by insects leaves of Bael are collected from same plant from residential area.



Figure 2: Bael Leaves



Figure 3: Bael Fruit

SELECTION OF SOLVENT

The solvents work on the principle that “like dissolve like”. The selection of solvents depends on the polarity of the compounds to be extracted, safety and compatibility with the plant material, boiling point, when selecting a solvent for extraction, as plant-derived active compounds may be sensitive to thermal degradation or volatility, and the efficiency of the solvent in preserving the integrity of the metabolites¹⁰.

Solvents are classified into three types of solvent

- 1) Polar solvents e.g., Water, methanol, ethanol, acetone and etc.,
- 2) Non polar solvents e.g., Hexane, benzene, chloroform, carbon tetra-chloride and etc.,
- 3) Semi- polar solvent e.g., Ethyl acetate, diethyl ether, acetonitrile, tetrahydrofuran and etc.

The solvents used for the extraction of Bael leaves include water, hydroalcoholic mixtures, and alcohol.

Water was used alone due to its high polarity, which effectively extracts highly polar soluble compounds from the leaves.

In contrast, hydroalcoholic mixtures enable the extraction of both polar and non-polar compounds.

Additionally, 95% alcohol is particularly effective in extracting non-polar compounds. As alcohol enable to penetrate in plant cell and cause the complete extraction.



Figure 4: Fresh Leaves



Figure 5: Shed dried leaves

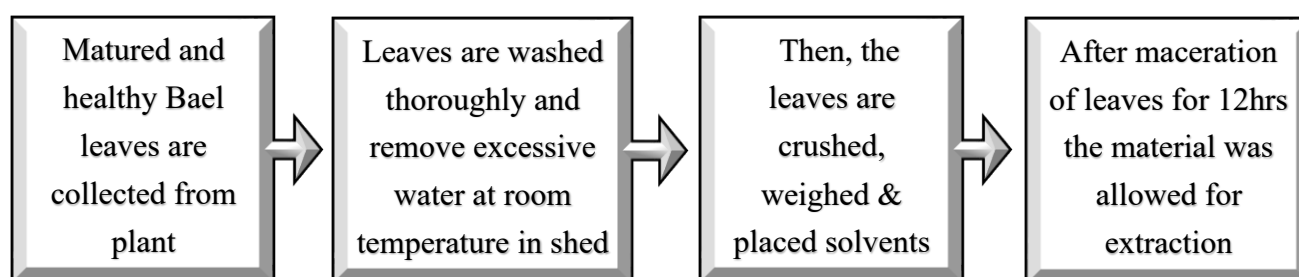


Figure 6: Pre-extraction technique of Bael leaves

EXTRACTION TECHNIQUE

Soxhlet Apparatus

The use of commercially available Soxhlet extractor is convenient way to prepare crude plant extract. This procedure is used mainly with pure solvent.

Compare to other method this method of extraction is selected due to following reason:

- Require relatively small amount of solvent,
- Direct heating to plant material is prevented,
- Extract containing solvent is placed in water bath during whole process to prevent direct heating and thermal degradation as possible,
- This method is useful for exhaustive extraction of plant material,
- Large amount of plant material is extracted by small amount of solvent,
- Does not require separation of plant material from solvent.

But this method also has some limitation like:

- Time consuming,
- Too long extraction may lead to thermal degradation of actives in solvent.

Soxhlet extraction is a powerful method for extracting a wide range of compounds from solid matrices. Its efficiency and reproducibility make it a preferred choice in laboratories.

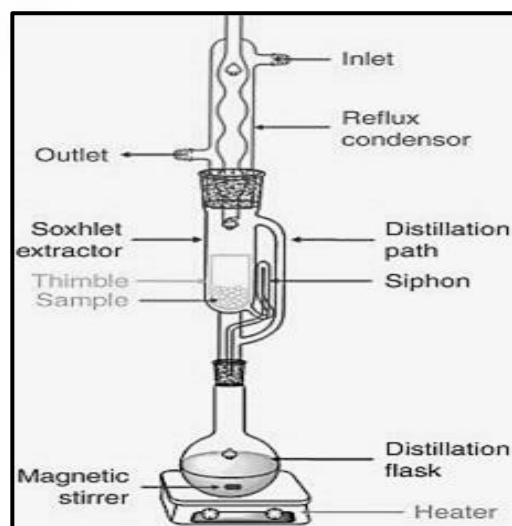


Figure 7 : SOXHLET APPARATUS

Soxhlet Extraction Procedure :

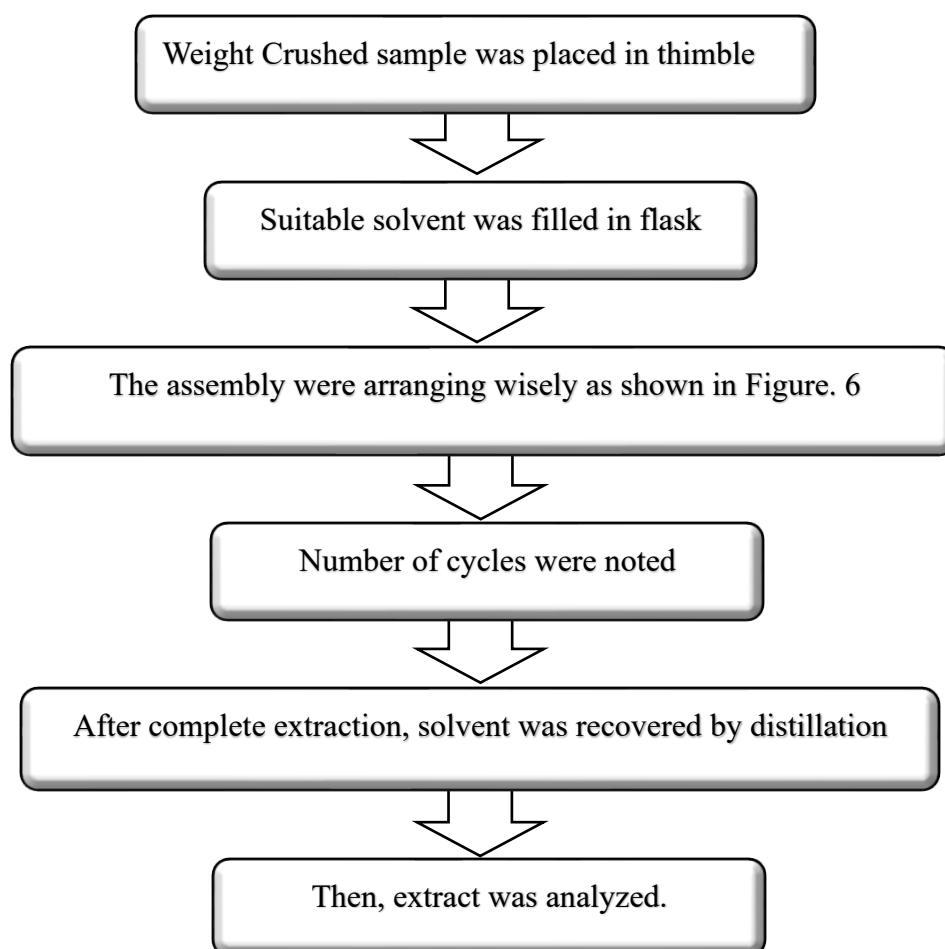


Figure 8 : Soxhlet Extraction Procedure

Note: The pure extract was placed in an oven at 28⁰-30⁰C to ensure complete removal of any solvent residues through evaporation or purification



Figure 9: Bael leaf extract

Table 1: Observation of extract (Figure: 9)

Sr. no.	Parameter	Observation
1	Colour	Dark brown
2	Odour	Characteristics
3	Texture	Smooth mucilaginous
4	pH	5.5-6.5

PHYTOCHEMICAL ANALYSIS

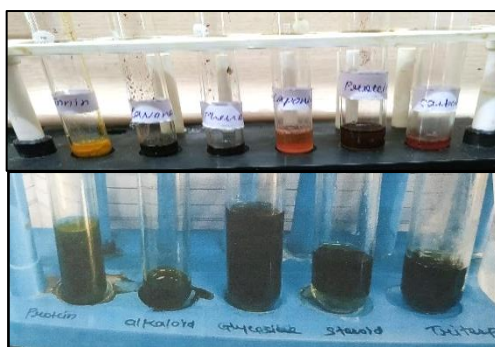


Figure 10: Phytochemical analysis of Aqueous Bael leaf extract

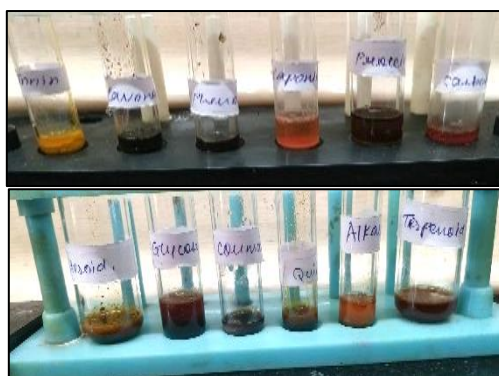


Figure 11: Phytochemical analysis of Hydroalcoholic Bael leaf extract

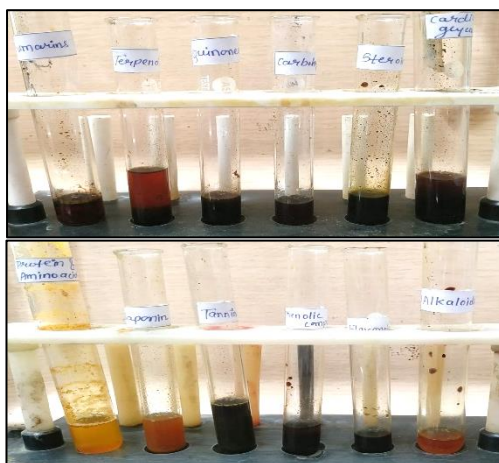


Figure 12: Phytochemical analysis of Alcoholic Bael leaf extract

Table 2: Observation of phytochemical in Aqueous, Hydroalcoholic and Alcoholic Extract

Sr. no.	Test name	Procedure	Observation		
1	Tannins	0.5ml extract+ Few drops of 10%lead acetate	+	+	-
2	Flavonoids	0.5ml extract+ Few drops 5% FeCl ₃ \20% NaOH	+	+	+
3	Alkaloids	0.5ml extract+ Few drops of 1% picric acid	+	+	+
4	Coumarin	0.5ml extract+ Few drops of 10% NaOH	-	-	-
5	Quinone	0.5ml extract+ 1ml H ₂ SO ₄	+	-	+
6	Phenols	0.5ml extract+ Few drops 10% FeCl ₃	+	-	+
7	Glycosides	0.5ml extract+ 2ml acetic acid +1ml H ₂ SO ₄	+	-	+
8	Terpenoid	0.5ml extract+ 1ml acetic acid +0.5ml H ₂ SO ₄	+	+	+
9	Steroids	0.5ml extract+ 2ml chloroform+1ml H ₂ SO ₄	-	-	+
10	Protein	0.5ml extract+ Few drops of 1% Copper sulphate and 4% NaOH	+	-	-
11	Carbohydrate	0.5ml extract+ Few drops of Molish reagent and H ₂ SO ₄	+	-	-

+ Present ; - Absent

Hydroalcoholic extract (+,-)

Alcoholic extract (+,-)

Aqueous/Water extract (+,-)

The phytochemical analysis of aqueous, hydroalcoholic, and alcoholic extracts showed varying compound presence (table: 5).

Quantitative analysis from the research articles consistently shows that while phytochemicals may be present across all types of extracts, their concentration is significantly higher in the aqueous extract.

Studies highlight the superior concentration of total phenolic content and antioxidant activity in aqueous extracts.

Due to the higher concentration of phytochemical compounds in the aqueous extract of Bael leaf, as supported by research studies, this extract is incorporated into skincare formulations.

INCORPORATION OF EXTRACT IN FACE SERUM :

The choice of a gel-based product is critical, as the gel itself does not contribute any moisturizing effects, thus serving as an inert medium. This ensures that the hydration and moisturizing properties of the Bael leaf extract can be studied independently, without interference from other components. Its richness in bioactive compounds, makes it more effective for therapeutic and cosmetic applications, promoting healthier skin.

FORMULATION

Table 3 : Formulation table for 100g Face Serum

Sr.no.	Ingredient	Uses	F1	F2
1	Water	Solvent	Up to 100	Up to 100
2	Glycerine	Humectant	3-6	3-6
3	Carbopol	Gelling Agent	0.8-1.4	0.8-1.4
4	Bael leaf extract	Active	-	0.5-1.2
5	Methyl paraben	Preservative	0.15-0.2	0.15-0.2
6	Perfume	Fragrance	QS	QS

F1- Serum without active

F2- Serum with active



Figure 13: Face Serum containing extract

Table 4 : Observation of Product(Figure: 13)

Sr. no.	Parameter	Observation
1	Colour	Pale orange
2	Odour	Floral
3	Texture	Gel like
4	pH	5.5-6.5

STABILITY OBSERVATION

Table 5 : Basic observation of stability of Product(Figure: 12) and extract(Figure: 11)

Sample	RT	O	F	For No. of days			
Serum	S	S	S	1D	1W	15D	1M
Extract	S	S	S	1D	1W	15D	1M

RT- Room Temperature;

O- Oven; F- Fridge; S- Stable; D- Day; W- Week

The table 8 represents a stability observation of two samples,

1) Serum & 2) Extract,

over different storage conditions and time periods.

1. Serum:

- Under all three conditions (RT, O, F), the serum remains stable (S).
- This stability was consistent across the different time periods: 1 day, 1 week, 15 days, and 1 month.

2. Extract:

- Similarly, the extract remains stable (S) under all three conditions (RT, O, F).
- The stability was observed over the same time periods: 1 day, 1 week, 15 days, and 1 month.

SUBJECT EVALUATION

Subjective evaluation was conducted to gather personal feedback and perception-based data from participants, which cannot be fully captured by objective measurements. This method allows for a more comprehensive understanding of user experiences and satisfaction levels. It helps in identifying subtle differences in product performance, particularly in areas like comfort, feel, or aesthetics. By incorporating human responses, subjective evaluation compliments technical data, providing a holistic view of the product's efficacy.

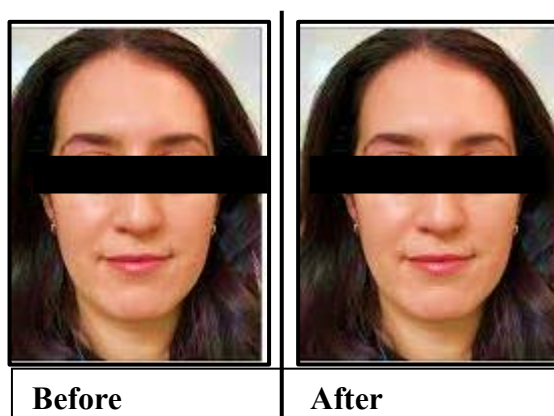
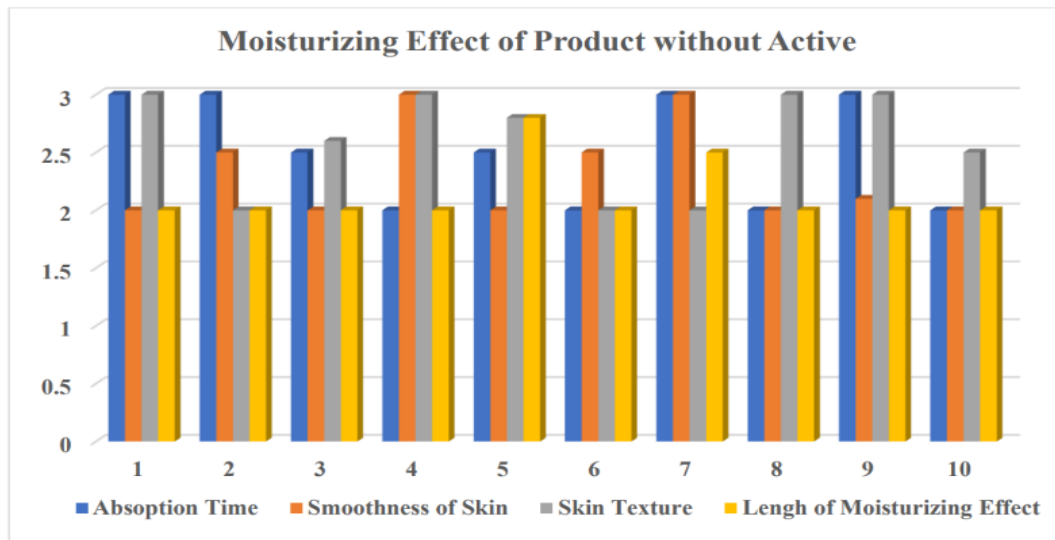


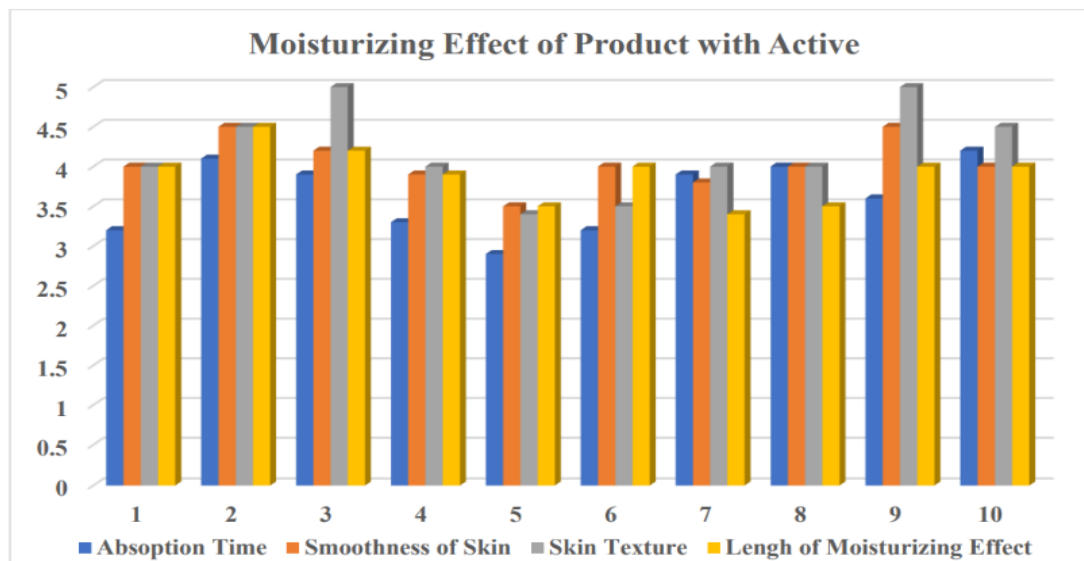
Figure 14: Before and After application of Face Serum

The graph comparing the moisturizing properties of the product without active (Graph:1) and with active (Graph:2) ingredients across different subjects and age groups (Graph: 3) is essential to visually demonstrate the product's effectiveness. By displaying results from various test groups, the graph highlights variations in skin hydration levels. It shows how the active ingredient may enhance the product's performance compared to the control (without active). This helps to draw clear, evidence-based conclusions about the product's moisturizing capabilities in different populations or skin types.

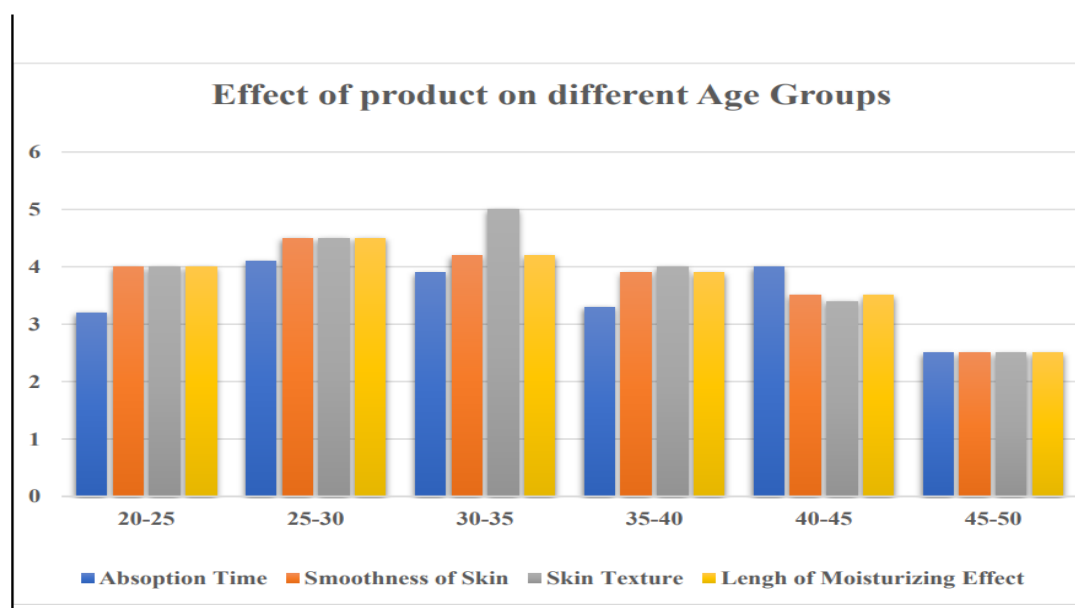
Graph 1: Moisturizing Effect of Product Without Active on Different Subjects



Graph 2: Moisturizing Effect of Product Active on Different Subjects



Graph 3: Moisturizing Effect of Product on Different Age Groups



Graph 1: Moisturizing Effect of Product Without Active on Different Subjects

Observations: - Absorption time shows consistent moderate values across most subjects (approximately 2.0 to 2.5).

- Smoothness of skin has more variability, ranging from around 1.0 to 2.5.
- Skin texture scores are relatively low, averaging around 1.0 to 2.0.
- Length of moisturizing effect tends to stay within 1.0 to 2.0 across the subjects, indicating that the product without active ingredients has a limited moisturizing duration.

Graph 2: Moisturizing Effect of Product with Active on Different Subjects*

Observations: -The product with active ingredients shows consistently higher values across all variables compared to the product without active ingredients.

- Absorption time values are higher, mostly between 3.0 to 4.0, showing better absorption efficiency.
- Smoothness of skin and skin texture have higher scores (between 3.0 to 4.5) for all subjects, indicating improved skin condition.
- The length of the moisturizing effect was significantly extended, with most subjects rating it between 3.5 to 4.5, suggesting prolonged effectiveness.

Graph 3: Moisturizing Effect of Product on Different Age Groups

Observations: --The younger age groups (20-25, 26-30) show higher values across all variables, indicating better product performance, particularly in absorption time, smoothness, and moisturizing duration.

- In the middle age groups (31-40 years), there was a slight decline in values, especially in the length of the moisturizing effect and smoothness of skin.

- The older age groups (41-50) show lower performance, particularly in the length of moisturizing effect and skin texture, indicating that age may impact the efficacy of the product.

RESULT AND DISCUSSION

Bael leaf (*Aegle marmelos* L.) has been selected as a potential ingredient in skincare cosmetics due to its rich cultural significance and remarkable therapeutic properties. Revered in Hindu mythology, it is associated with Lord Shiva, and its trifoliate leaves symbolize the divine trinity, enhancing its esteemed status.

Furthermore, the presence of sattva components in Bael leaves suggests that they may help absorb and emit negative frequencies, potentially promoting a calmness. Given these unique attributes, the study focuses on the phytochemical analysis of extract of Bael leaf and its effects on skin, highlighting its promise as an effective and holistic ingredient for skincare formulations.

From a scientific perspective, Bael leaves are known to contain a diverse range of beneficial bioactive compounds. These compounds not only provide protective benefits against oxidative stress but also possess anti-inflammatory and antimicrobial effects, making them valuable for skin health. And apart from those properties, the moisturizing property was explored, evaluated.

A detailed study through tables: 5 illustrates that although the presence of phytochemicals was observed in all three types of extracts (aqueous, hydroalcoholic, and alcoholic) by soxhlet method of extraction.

Based on a comprehensive review of various research articles, it was evident that the aqueous extract of Bael (*Aegle marmelos* L.) leaf was richer in phytochemical compounds when compared to both hydroalcoholic and alcoholic extracts. Multiple studies have demonstrated that the aqueous extraction method yields a higher quantity of bioactive compounds, such as flavonoids, tannins, alkaloids, phenols, and glycosides. This suggests that aqueous acts as a more efficient solvent for extracting these compounds from Bael leaves compared to alcohol-based solvents.

The incorporation of the aqueous Bael leaf extract into skincare products was largely attributed to its high concentration of these beneficial phytochemicals.

The skin care product(face serum; Figure:10) was prepared by incorporating Bael leaf extract. And their basic stability study was conducted. On stability it observes that, both serum and extract show stability across varying conditions (room temperature, oven, fridge) for **up to 1 month**, (table: 8)without any signs of instability within the observed time frames (Figure:9 & Figure: 10). It also reveals that the extract and other ingredients of serum base was compatible with each other.

Followed by stability observation, subject evaluation of face serum with active and without active on number of subject and different age groups was conducted.

On subject evaluation, it observes that:

- Product Without Active: Provides moderate moisturizing benefits, with limited absorption time and short-lasting effects, particularly regarding skin smoothness and texture(Graph: 1).
- Product With Active: Demonstrates significantly enhanced performance, with quicker absorption, better skin texture, smoother skin, and prolonged moisturizing effect across all subjects(Graph: 2).
- Age Group Influence: The product shows better performance in younger age groups, while the effects slightly decrease with increasing age, particularly for skin texture and moisturizing duration(Graph: 3).

This analysis provides insight into the differences between product formulations and how age groups respond differently to moisturizing products.

Face serum was prepared by incorporating Bael extract as an active ingredient and on subjective evaluation it was found that out of 10 subjects 9 subjects are satisfied with its moisturizing property.

The extract's potent composition makes it particularly valuable for therapeutic and cosmetic purposes, as it provides enhanced skin benefits compared.

It can be concluded that product with Bael leaf is stable, safe, convenient to use and gave satisfactory moisturizing property and is use a potential moisturizing agent in herbal cosmetics.

In the future, the use of aqueous Bael leaf extract in skincare formulations holds great potential for further exploration. Given its rich phytochemical profile and superior antioxidant properties, there is scope for developing more targeted cosmetic and therapeutic products, such as anti-aging creams, anti-inflammatory treatments, and so on.

Additionally, research can be expanded to isolate and study specific compounds within the aqueous extract to better understand their individual effects on skin health. Investigating the synergy between Bael leaf extract and other natural ingredients could also lead to innovative, multifunctional skincare solutions. Further studies may explore its application in various industries like nutraceuticals, and holistic wellness, broadening its use in cosmetics.

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