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STUDY ON BIOACTIVE COMPOUNDS, CHARACTERIZATION AND PRODUCTION OF SOAPS FROM HERBAL SOURCE

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ABSTRACT

In this study, herbal extracts of *Glycyrrhiza glabra* (licorice), *Aegle marmelos* (bael), *Punica granatum* (pomegranate), and *Vitis vinifera* (grape) are used to produce and assess herbal soaps. The antioxidant and antibacterial qualities of these plants—which are abundant in bioactive substances such flavonoids, phenols, alkaloids, and terpenoids—led to their selection. All extracts contained necessary bioactive components, according to phytochemical study; licorice had the highest phenolic content (73.8 mg/g). The DPPH assay was used to evaluate the antioxidant activity, demonstrating the extracts' robust ability to scavenge free radicals. These samples were worn to produce herbal soaps, which demonstrated superior foam stability, moisturizing qualities, and a pH that was suitable for skin. Significant effectiveness against *Staphylococcus and Streptococcus* bacteria was demonstrated by antimicrobial tests, especially with licorice and pomegranate aqueous extracts. Key functional groups such as alcohols, carboxylic acids, and aromatic compounds were detected in the soap samples by FTIR analysis. From this outcome it speculated that the herbal soaps showed admirable quality, antibacterial efficacy, and aesthetic advantages, making them a viable substitute for manufactured the product. Scaling up production in the large scale and further refining the soaps' qualities for commercial usage should be the main goals of future study.

KEYWORDS: Herbal soap, antibacterial, antioxidant, *Glycyrrhiza glabra*, *Aegle marmelos*, *Punica granatum*, and *Vitis vinifera*.

1. INTRODUCTION

Medicinal plants have been an essential part of traditional healing for ages. Various plant parts such as leaves, stems, roots, and fruits are being used to treat a wide range of ailments. These herbal extracts are usually incorporated into creams, soaps, oils, and ointments to address skin conditions like eczema, acne, and wounds. While synthetic chemicals

have largely replaced traditional remedies, Ayurvedic and herbal medication are worn for their effectiveness, safety, and minimal side effects. In a world increasingly concerned about chemical toxicity and environmental pollution, herbal cosmetics, also known as Ayurvedic cosmetics, offer a natural alternative with both therapeutic and cosmetic benefits.[1]

Herbal soaps are renowned for their antibacterial and antifungal properties, making them highly efficient in treating infections, healing wounds, and supporting overall skin health.[2]

Plant extracts can help with skin conditions like fungal infections and acne while also softening the skin and speeding up healing, offering a natural alternative to synthetic skincare products.[2]

The pomegranate (Punica granatum), rich in antioxidants, flavonoids, and tannins, is commonly used in cosmetics for its antibacterial and anti-inflammatory effects. Pomegranate leaves, in particular, offer a more affordable and accessible option. Grapes (Vitis vinifera), rich in phytochemicals, help protect the skin from oxidative damage while promoting overall skin health. Additionally, plants like bael (Aegle marmelos) and licorice (Glycyrrhiza glabra) are renowned for their anti-inflammatory and antibacterial properties.[3]

This research focuses on creating herbal soaps from Aegle marmelos (bael), Glycyrrhiza glabra (licorice) and fruit peels of Punica granatum (pomegranate), Vitis vinifera (grape). The goal is to promote natural skincare while reducing agricultural waste and supporting sustainable practices.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction

Fruit peels and leaves were collected from a local market in Bangalore, washed with distilled water, and dried to get rid of moisture (Fig 1). The dried leaves and peels were milled into a fine powder. For extraction, 10g of each sample's powder was mixed with a solvent at a 1:10 ratio and sonicated for 1 hour. The extract was centrifuged at 5000 rpm for 10 minutes to eliminate any particulates. The supernatant was transferred to a test tube and preserved for subsequent examination.

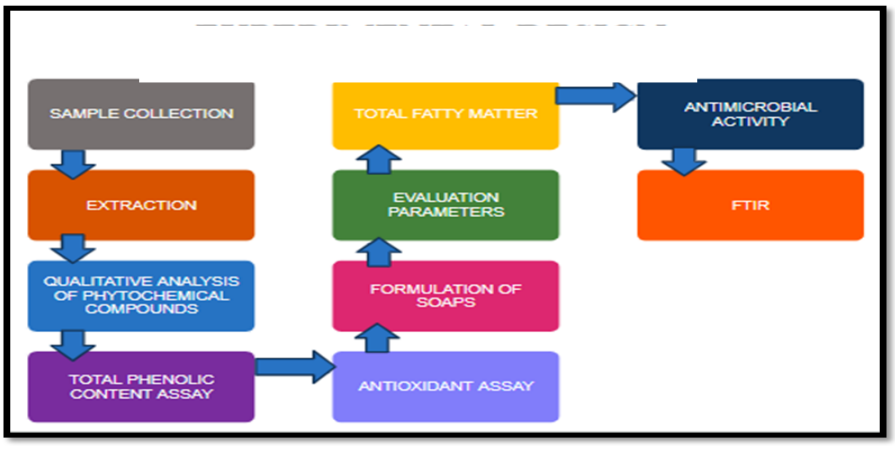


Fig. 1: Experimental design of the Research work.

2.2 Qualitative Analysis of Phytochemical Compounds

A phytochemical assessment, based on the method by Kumar et al. (2013) with some alterations, was conducted to analyze various substances, including alkaloids, flavonoids, steroids, tannins, anthocyanins, emodins, phenols, saponins, and terpenoids. Ethanol, methanol, and aqueous extracts were prepared and evaluated.[4]

2.3. ANTIOXIDANT ACTIVITY

2.3.1Total Phenolic Content (TPC)

The FC method was used to perform the antioxidant assay, with minor adjustments, in accordance with the methodology described by Singleton et al. (2017). A calibration curvature was prepared by mixing 1 ml aliquots of 20 - 100 µg/ml Gallic acid solutions with adding 0.5 ml of F-C reagent and sodium carbonate solution. After an hour, absorbance was calculated at 750 nm using a colorimeter and UV spectrometer. The TPC was expressed as milligrams of Gallic acid equivalents per sample.[5]

2.3.2 Free Radical Scavenging Activity – DPPH Method

Utilizing the DPPH (2,2-diphenylpicrylhydrazyl) assay (Katalinic et al., 2006), the extracts' ability to scavenge free radicals was assessed. After making methanolic DPPH, varying amounts of sample extracts (0.2–1.0 mg/ml) were combined with the DPPH solution. After half-hour incubation in the dark, the absorbance was calculated at 517 nm.[6]
The radical scavenging activity was calculated using the following formula - [(A0-A)/A0] × 100, where A is the sample's absorbance and A0 is the absorbance of the blank solution. Through linear regression, IC50 values which represent the extract concentration needed to inhibit 50% of DPPH radicals were ascertained.

2.4. FORMULATION OF SOAP

The Mak-Mensah et al. (2011) method was used, with minor modifications, to prepare the herbal soap. About 10g of palm wax was broken up and melted in an oven until it turned into a liquid in order to make the herbal soap. The melted wax was blended with 5 ml of *Aegle marmelos*, *Glycyrrhiza glabra*, *Vitis vinifera*, and *Punica granatum* extracts. Glycerin, fragrance, and five-six drops of coconut oil / Olive oil were added to the mixture. It was poured into a mold and allowed to solidify for 24 hours at room temperature.[7]

2.5. EVALUATION PARAMETERS

5.1 pH - The pH of the soap was measured using a digital pH meter for more accurate readings.[8]

2.5.2 Determination of Colour and Odour - The soap's colour was visually checked against a white background, and the odor was evaluated by smelling.[8]

2.5.3 Retention Time - Retention time was measured by shaking 25 ml of a 1% soap solution in a graduated cylinder and measuring the foam produced at intervals for up to 5 minutes.[8]

2.5.4 Foam Height - Foam height was measured by dissolving 0.5g of soap in 25 ml distilled water and adding 25 strokes. The foam height was confirmation after mixing.[8]

2.5.5 Moisture Content - Moisture content was estimated by heating 5g of soap in a crucible at 103°C for 2.5 hours, then weighing it before and after drying. The percentage of moisture content was calculated.[9]

2.5.6 High Temperature Stability - Liquid soap was kept at 50°C for a week, and its stability was assessed by noting any changes like crystallization or precipitation.[10]

2.5.7 Determination of Total Fatty Matter (TFM) - To estimate the total fatty matter, 5g of soap was dissolved in 100 ml of hot water, acidified, and fatty acids were separated and weighed after evaporation.[11]

2.5.8 Antimicrobial Activity – The agar well diffusion method was used to assess antimicrobial activity. A 100 µl aliquot of each extract (20% w/v) was added to wells on an agar plate inoculate with bacterial cultures. After 18 hours of incubation, the zone of inhibition was measured. The activity was determined by measuring the diameter of the zone of inhibition that appeared following the incubation period.[12]

2.6. FOURIER TRANSFORM INFRARED SPECTROMETRY (FTIR)

FTIR study was carrying out using an ATR-FTIR spectrometer to identify functional groups in the soap samples. The spectra were recorded in the range of 4000-400 cm^{-1}, and the results were used to find out the compound of the soap. The analysis was conducted at ambient temperature (25°C).[13]

3. RESULT AND DISCUSSION

3.1Phytochemical analysis

Numerous phytochemicals were identified by the phytochemical study. All samples extract contained flavonoids, phenols, anthocyanins, tannins, and alkaloids. Terpenoids and emodins were also present in grape peel extract. Glycosides and saponins were abundant in the licorice extract, and emodins, terpenoids, and saponins were abundant in the bael patre leaf extract.[14]

3.2 PRODUCTION OF SOAP

The modified Mak-Mensah et al. (2011) method was successfully used to prepare the herbal soap. Fig 2 shows the Herbal soap prepared from the different extracts of *Punica granatum*, *Vitis vinifera*, *Glycyrrhiza glabra* and *Aegle marmelos* leaf. A skin-friendly product with moisturizing, calming, and antioxidant qualities was produced by combining palm wax, plant extracts, glycerin, and oils. The soap successfully preserved its texture and structure, demonstrating that this process—with a few tweaks—is a workable way to produce herbal soaps. For possible commercialization, this herbal soap composition can undergo more testing to determine its stability, skin advantages, and general user approval.

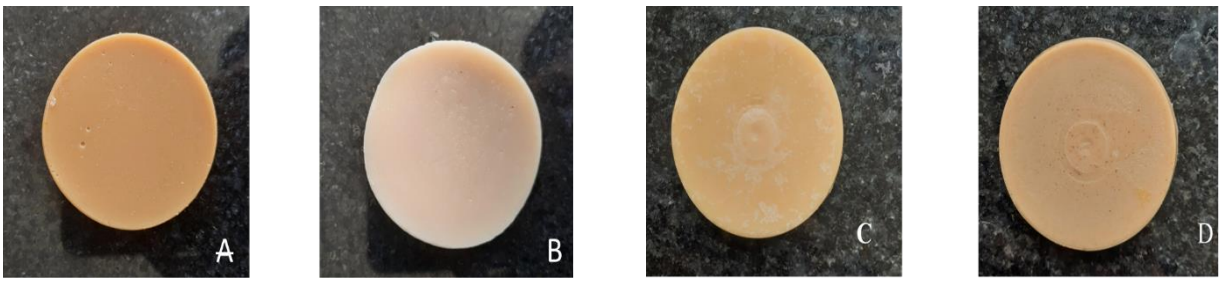
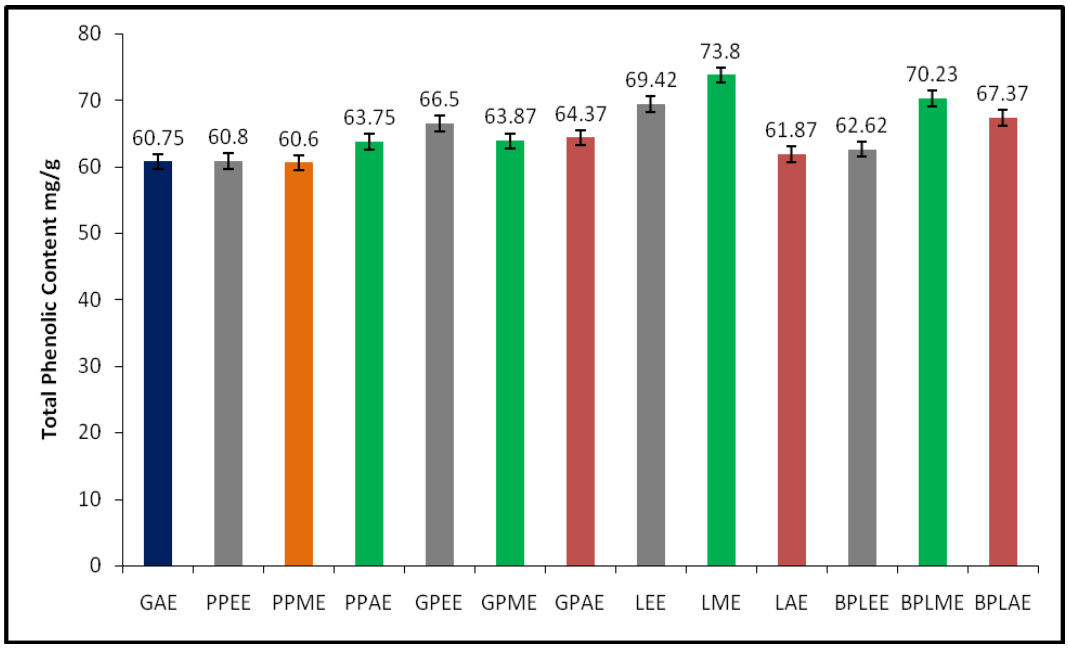


Fig 2: Herbal soap preparation from the different extracts of *Punica granatum* (A), *Vitis vinifera* (B), *Glycyrrhiza glabra* (C) and *Aegle marmelos* leaf (D).

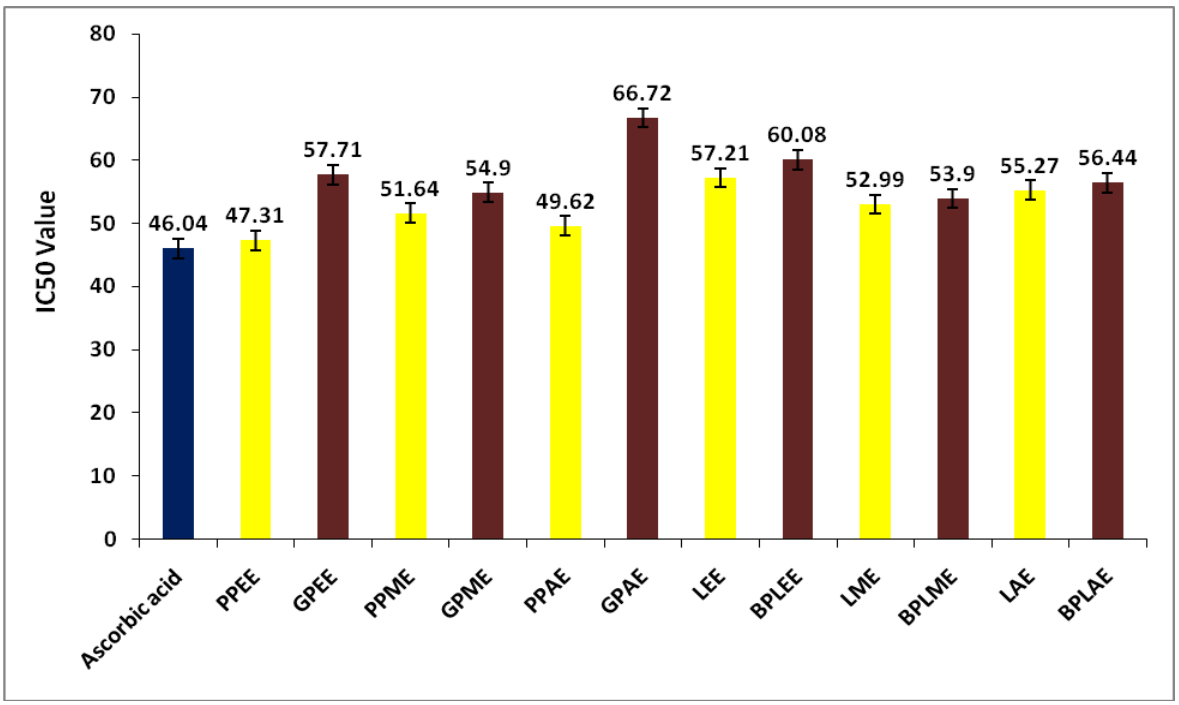
3.3 ANTIOXIDANT ACTIVITY

The FC method was used to measure the TPC content in the samples. The results were represented as mg of gallic acid equivalents per gram using a calibration curve (Y=0.0081x+0.1248, R2=0.9298, 60.75 mg/g). A significant predictor of antioxidant capacity is phenolic content. The Licorice methanolic extract has the greatest phenolic concentration (73.8 mg/g) among all the samples (Graph 1).



Graph 1: Total phenolic content of Gallic acids and sample Extracts.

The DPPH method was used to measure in vitro antioxidant activity, with ascorbic acid (100µg/ml, 89.54 mg/ml) serving as the standard. According to the calibration graph (y=0.8324x + 110672, R2=0.9413), the ascorbic acid IC50 value was 46.04. The pomegranate peel ethanolic and aqueous extracts showed the least degree of antioxidant activity (IC50 values of 47.31 and 49.62, respectively) among the samples (Graph 2). (In general, a lower IC50 value indicates that the medicine is more active and effective at blocking the biological process at lower doses).



Graph 2: IC50 value of Ascorbic acid and sample Extracts.

Chidambara MKN et al., showed the methanol extract of pomegranate peel have highest antioxidant activity that assess its effects on lipid peroxidation, hydroxyl radical scavenging, and human LDL oxidation.[16] Additionally, a previous study report showed the methanol extract exhibited 83% and 81% antioxidant activity at 50 ppm using the β-carotene-linoleate and DPPH model systems, respectively.[17]

3.4 EVALUATION PARAMETER

The evaluation parameters of herbal soap include foam height, temperature stability moisture content, pH, color, which are tabulated in Table 1.

Table 1: Evaluation parameters for *Punica granatum, Vitis vinifera, Glycyrrhiza glabra* and *Aegle marmelos based products.*

Samples	Evaluation Parameter						
	pH	Color	Foam Retention time	Foam height	Moisture content	High temperature stability	Irritation to skin
PPEE	7.5	Cream	4mins	2cm	15.7%	Soap melt at 40°c	No
PPME	7.8	Light cream	3.5 mins	2.5cm	14.1%	Soap melt at 45°c	No
PPAE	7.6	Brown	4mins	2cm	16%	Soap melt at 40°c	No
GPEE	7.3	Cream	3mins	1.8cm	17.5%	Soap melt at 45°c	No
GPME	7.4	Cream	3mins	1.5cm	13%	Soap melt at 40°c	No
GPAE	7.4	Cream	4mins	2cm	14.6%	Soap melt at 35°c	No
LEE	7.5	Cream	4mins	2.5cm	15.7%	Soap melt at 40°c	No
LME	7.8	Light cream	3.5 mins	3.5cm	14.1%	Soap melt at 45°c	No
LAE	7.3	Cream	4mins	2cm	16%	Soap melt at 40°c	No
BPLEE	7.2	Cream	3mins	2cm	17.5%	Soap melt at 45°c	No
BPLME	7.6	Cream	3mins	2.5cm	13%	Soap melt at 40°c	No
BPLAE	7.4	Light brown	4mins	1.8cm	14.6%	Soap melt at 35°c	No
CONTROL	7.5	Green	2 mins	1.3cm	20%	Soap melt at 40°c	No

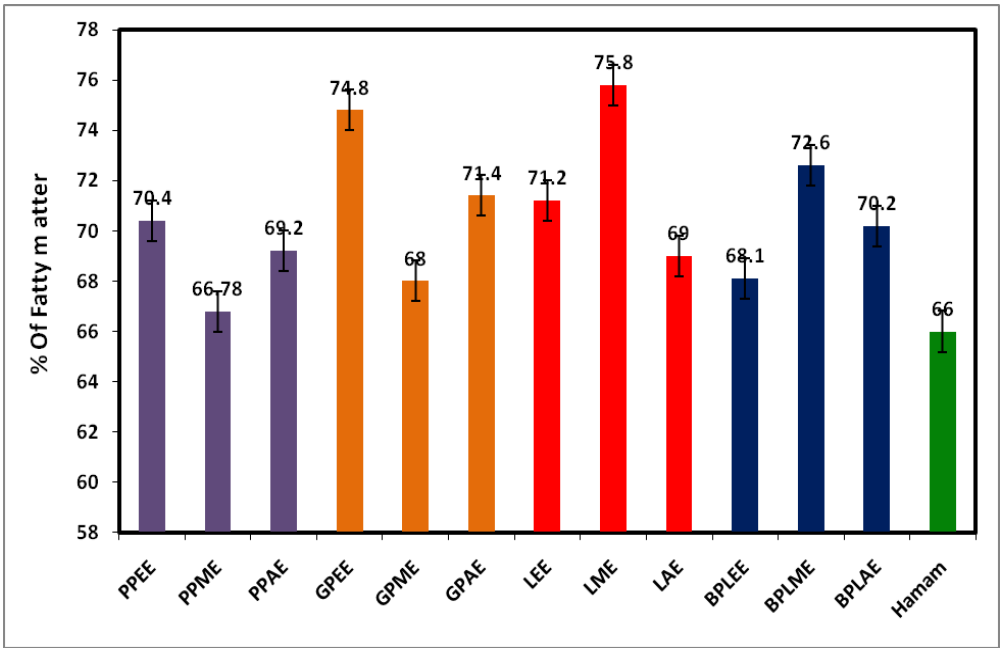
Herbal soaps made from pomegranate, grape peel, licorice, and bael patre leaf extracts have somewhat lower pH values (7.4–7.8) than other commercially available herbal soaps, which has a pH range of 8–9. The skin can be harmed by extremely high pH values. In contrast to Control, which utilizes olive oil, herbal soaps that contain coconut oil—such as those containing *G glabra, A marmelos, P granatum, and V vinifera*—have longer foam retention periods. Although it does not impair cleaning ability, foam formation is significant to customers. A high moisture concentration in soap can cause hydrolysis, which shortens its shelf life. After using the herbal soaps, there was no redness or itching on a skin irritation test.

3.5 TOTAL FATTY MATTER

The quality of soap is largely determined by its total fatty matter (TFM). According to the BIS, soaps are divided into three grades: Grade I (76%+ TFM) for excellent moisturization and cleaning, Grade II (70%-76% TFM) for efficient cleansing with less moisturization, and Grade III (60%+ TFM) for less expensive but lower-quality soaps [18 https://www.mdrcindia.com/blog/detail/total-fatty-matter-tfm-of-soaps-and-its-criteria]. A higher TFM is a sign of superior quality (Table 2 and Graph 3). GPEE, GPAE, PPEE LME, BPLME, LEE, and BPLAE are Grade II soaps according to TFM, but other soaps have lower TFM values.

Table 2: The products' total fatty matter was compared to Control soap in order to classify the grade.

Name of soap	% of fatty matter	Grade
PPEE	70.4	Grade II
PPME	66.78	Grade III
PPAE	69.2	Grade III
GPEE	74.8	Grade II
GPME	68	Grade III
GPAE	71.4	Grade II
LEE	71.2	Grade II
LME	75.8	Grade II
LAE	69	Grade III
BPLEE	68.1	Grade III
BPLME	72.6	Grade II
BPLAE	70.2	Grade II
CONTROL	66	Grade III



Graph 4: Total fatty matter of the products with Control.

The deviation in total fatty matter influences moisture content, with lower values resulting from the presence of untreated NaOH in the mixture. A higher concentration of fatty acids benefits the skin by promoting rehydration and improving overall cleansing properties.[18]

3.6 ANTIMICROBIAL ACTIVITY

The in vitro antimicrobial action of peel extracts in sample soaps was tested using the well diffusion method against Staphylococcus and Streptococcus (Table 3).

Table 3: Antibacterial activity of plant extracts against bacterial pathogens.

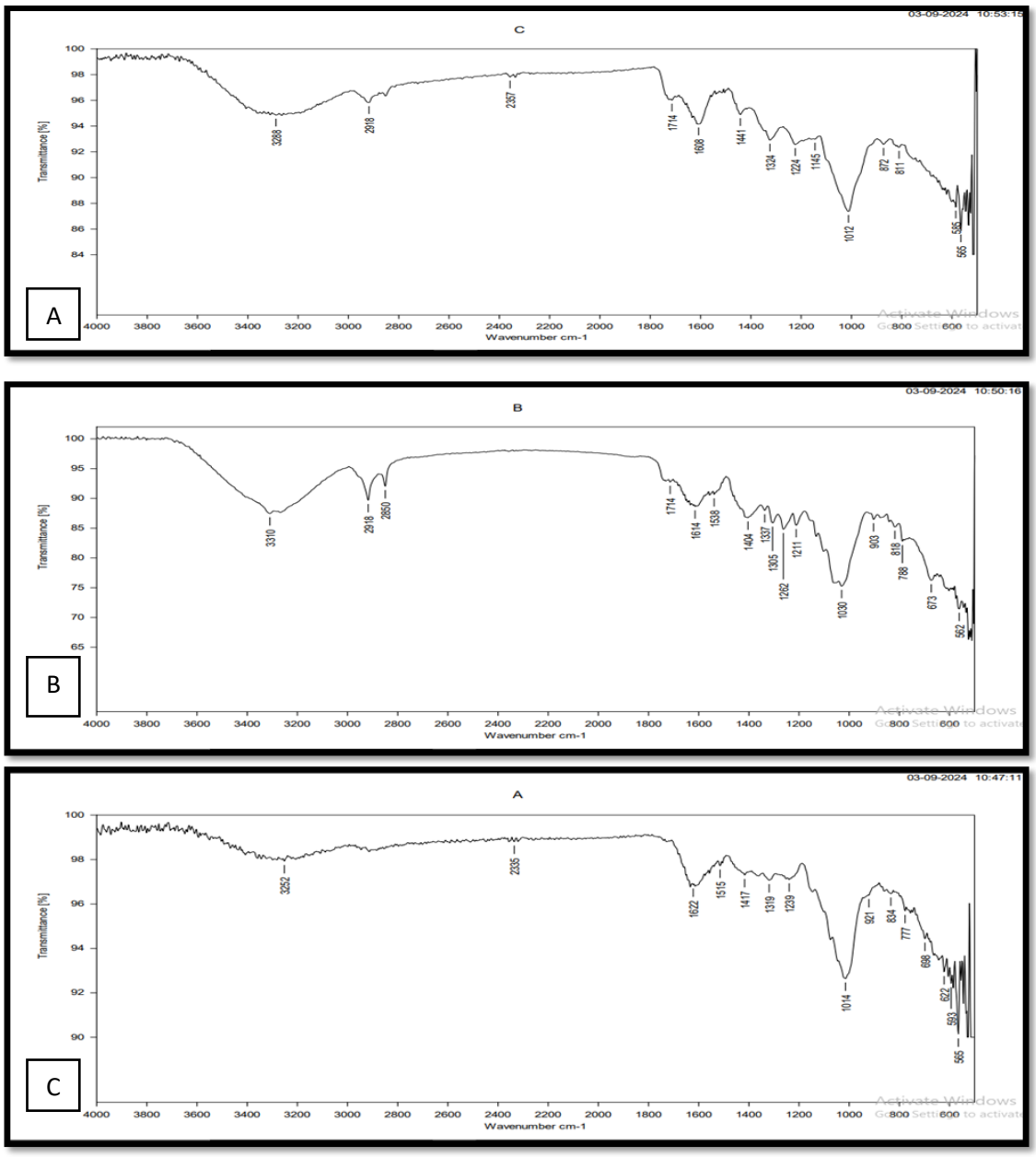
SAMPLE	DIAMETER ZONE OF INHIBITION (cm)	
	Staphylococcus	Streptococcus
Punica granatum	1.2±1.3	1.4±1.5
Vitis vinifera	0.6±0.5	1.2±0.5
Glycyrrhiza glabra	1.0±0.7	1.3±1.3
Aegle marmelos	0.5±0.4	1.0±0.8

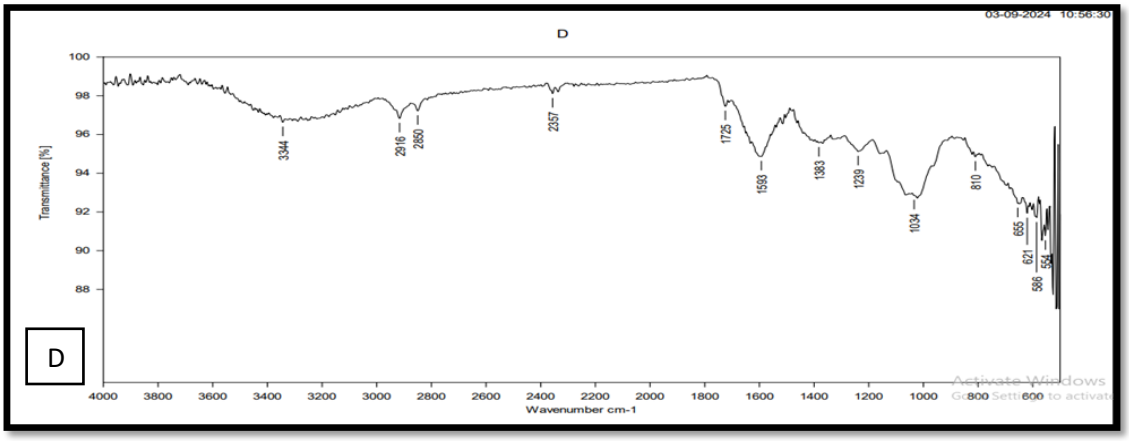
Traditional medicine has been using the root and rhizome of Glycyrrhiza species for millennium because of their many medicinal uses. According to research, licochalcone, a substance that is taken from the roots of Glycyrrhiza, has antibacterial qualities, particularly against gram-positive bacteria such as Bacillus species.[15]

Fukai et al. showed that the root extract of Glycyrrhiza glabra efficiently suppresses certain oral infections, especially those caused by Staphylococcus aureus.[19,20] Our findings suggest that extracts of Punica granatum, Vitis vinifera, Glycyrrhiza glabra, and leaf of Aegle marmelos exhibit antibacterial properties, specifically against Streptococcus rather than Staphylococcus.

3.7 FOURIER TRANSFORM INFRARED SPECTROSCOPY [FTIR]

FTIR helps to Verifies the existence of crucial chemical groups in herbal components, assuring correct composition. The analysis confirmed the occurrence of alcohols, aromatic compounds, alkenes, carboxylic acids, esters, amines, amides, aryl and alkyl compounds. The FTIR spectra are listed in the Figures 3A, B, C, D.





Figures 3: FTIR spectra of *Punica granatum* (A), *Vitis vinifera* (B), *Glycyrrhiza glabra* (C) and *Aegle marmelos* leaf (D).

CONCLUSION

The study on bioactive compounds, characterization, and production of herbal soaps from plant extracts like *G glabra (licorice), A marmelos (bel patra), P granatum (pomegranate), and V vinifera (grape)* demonstrates their therapeutic and cosmetic potential. These plants are rich in bioactive compounds of phenols, flavonoids, alkaloids, and terpenoids, offering antioxidant, antimicrobial, and anti-aging benefits. Licorice, with the maximum phenolic content (73.8 mg/g), provides strong antioxidant protect against oxidative stress and aging, alongside broad-spectrum antimicrobial activity. Herbal soaps with licorice extract show excellent foam retention, moisturizing properties, and no skin irritation. Pomegranate and grape peel extracts also offer significant antioxidant and antimicrobial effects, with grape peel extract showing higher phenolic content (66.5 mg/g) and antimicrobial strength. Herbal soaps from these extracts exhibit superior quality, foam stability, and a skin-friendly pH. Future research should focus on optimizing these soaps' properties and assessing their industrial-scale production.

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