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PHYSICOCHEMICAL PROPERTIES OF HERBAL SOAP MADE FROM AVERRHOA BILIMBI LEAF AND FLOS LONICERAE

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Abstract: Malaysian herbs, *Averrhoa Bilimbi*, and *Flos Lonicerae*, are widely known in traditional Chinese medicine. Both are used as natural remedies for jaundice treatment in newborns. Formulating herbal bath soap by the saponification method is proposed because newborn babies are poor feeders. Today, a lot of chemicals are used in soap production. Users will have a wide range of issues due to chemicals like parabens and triclosan. As a result, soap is made from herbs without chemicals. This herbal soap uses two herbs, *Averrhoa Bilimbi* leaf and *Flos Lonicerae*. Paper journals examining these herbs' physicochemical properties are scarce in Malaysia. After the herbal soap has been successfully created, it will be examined for its physicochemical properties. The antioxidant activity and total phenolic content (TPC), of *Averrhoa Bilimbi* leaf extract and *Flos Lonicerae* extract were all tested. The antibacterial activity, heavy metal and pH tests were also validated the suitability of this herbal soap for skin application. According to the findings, *Averrhoa Bilimbi* leaf extract had a TPC value of 24.49 mg GAE/g compared to 4.96 mg GAE/g for *Flos Lonicerae* extract. *Averrhoa Bilimbi* leaf required a slightly higher concentration of 0.0588 g/mL extract to inhibit 50% of the DPPH assay compared to *Flos Lonicerae* plant extract, which required 0.0315 g/mL. Since there was no evidence of any bacterial growth, the herbal soap was effective against all the tested bacterial strains. The heavy metal test showed the soap does not contain any lead, mercury or arsenic and exhibits a pH value which close to the range for suitable skin application.

Keywords: *Physicochemical properties, herbal soap, averrhoa bilimbi leaf, flos lonicerae*

1. Introduction

The use of plants as lead compounds for drug development or refined herbal remedies has always played a critical role in the development of medicines and healthcare preparations (Ekpo et al., 2009). In China, the herbal treatment has been practiced for quite some time and is still very demanding. According to Ayobami et al. (2017), it is possible to formulate topical herbal remedies such as soaps, ointments, creams, lotions, gels or crude/solvent extracts from plants that contain bioactive (antimicrobial) principles for the care and treatment of skin infections as an alternative to synthetic antibiotics. Throughout history, people have taken baths in herbal waters and added other bathing additives that were believed to be beneficial. Nowadays, the use of natural product-based therapies such as herbal soap is very popular. In layman's terms, soap is any substance used with water. Often, it is used to clean skin, clothing, dishes, floors, and walls of soil or grease. According to Warra et al. (2011), from a scientific standpoint, soap is a mixture of sodium or potassium salts of various naturally occurring fatty acids. Unlike synthetic soaps, natural herbal soaps are prepared without artificial surfactants, and contain natural substances, essential oils, or plant extracts as functional ingredients.

As reviewed by Alhassan et al. (2016), *Averrhoa Bilimbi* is one of the important medicinal plants that has been widely used in the traditional system of medicines for various treatments. The tree belongs to the genus *Averrhoa* and the family Oxalidaceae, also known as "Bilimbi", "Cucumber Tree" or "Tree Sorrel" (Ismail et al., 2019; Sutrisna et al., 2019). It functions as a postpartum preventive medicine as well as an antibiotic, antiscorbutic, and astringent. Additionally, it can be used to treat syphilis, rheumatism, diabetes, boils, itches, mumps, and inflammation of the rectum. According to research done by Gogoi et al. (2010), the ripe fruit of *Averrhoa Carambola Linn*, which is related to *Averrhoa Bilimbi* and used to cure infective jaundice, including dyspepsia, is effective in treating this condition. *Lonicerae Flos* is a semi-evergreen climber with fragrant and paired flowers (Li et al., 2018). It has been used as a tea and traditional Chinese medicine remedy in China for more than 1,500 years. It was widely grown in Hunan Province and was a well-known traditional Chinese herbal medicine that was used as a heat-clearing medication and an alexipharmic agent (Zheng et al., 2022). *Lonicerae Flos* primarily contains biologically active substances such as terpenoids, flavonoids, iridoid glycosides, and derivatives

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of caffeic acid. Plant extracts have been shown to have a variety of biological properties, including anti-inflammatory, anti-cancer, antioxidant, antiallergenic, immunomodulating, and antibacterial action (Tang et al., 2018).

Antioxidants have gained recognition as scientifically fascinating compounds because of their many health benefits, such as their anti-inflammatory and anti-aging effects. Studies on antioxidants conducted in vivo or in vitro have shown a variety of findings about the prevention of illnesses. As a result, the use of antioxidants in pharmacology, cosmetics, and medicine gained significance (Zehiroglu & Ozturk, 2019). Antioxidants are helpful in extending the shelf life of consumer goods because they are effective bio-regulators, oxidation suppressors, and bio-regulators. Antioxidant-active components are present in manufactured natural herbal soaps, however, it is unclear how this affects shelf life (Adigun et al., 2019).

The microbes (bacteria, fungi, protozoa, archaea) that inhabit and exist in the human body have significant implications for health. According to the body places they dwell, these microorganisms are assumed to have co-evolved with humans. They serve a variety of tasks for the host, from digesting to immunological protection. Understanding how antibacterial products affect the microbial skin communities of people living in rural areas of developing countries is crucial as the availability and adoption of these products increase globally (Yu et al., 2018). Skin and wound bacterial infections can significantly reduce a patient's quality of life and even result in death in some cases. One of the main concerns in their management is the spread of resistant strains outside of hospitals and into the general population as a result of the increasing antibiotic resistance of bacterial pathogenic diseases. The discovery of innovative medicinal drugs that are effective and secure is encouraged by this pattern. The pharmaceutical industry frequently fails in its fight against germs because it primarily on libraries of synthetic compounds as a source for therapeutic discovery. Contrarily, numerous organic substances, as well as complete, complex plant extracts, are efficient in this area, inactivating resistant bacterial strains or reducing their pathogenicity. Natural products like herbs have not only antibacterial but also anti-inflammatory effects and may support tissue regeneration and wound healing (Bittner et al., 2021).

According to the American Cleaning Institute (2019), pH indicates whether a substance is acidic or alkaline. In soap preparation, soap quality is greatly influenced by pH. Warra (2013) claims that soaps with pH levels below five and significantly above 10 are harsh on the hands and skin. Additionally, by definition, soap is the salt of a weak acid (fatty acid) and a weak base, and because soap is generally alkaline in aqueous solutions, its pH is typically above 7. The statement is supported by Tokosh and Baig (1995), who state that all soap-based formulations should produce alkaline pH solutions. Incomplete alkaline hydrolysis has caused the pH to be high, indicating that a large percentage of matters are unclear and unresolvable during the saponification process.

Phytochemical compounds such as flavonoids and phenolic compounds have been well-known for their benefits for human health or for their ability to cure or prevent diseases for many years. Commonly known as secondary metabolites, these compounds have at least one hydroxyl group on their aromatic ring (Tungmunnithum et al., 2018). There are more than 8000 naturally occurring phenolic compounds present in plants. In addition to flavonoids and many other phenolic compounds found in herbs and nutrients. These phytochemical substances have been reported to act as effective antioxidants, anti-cancer agents, antibacterial agents, cardioprotective agents, anti-inflammation agents, immune system enhancing agents, and skin protection (Kumar et al., 2013; Chen et al., 2015; Dzialo et al., 2016; Ahmed et al., 2016; Andreu, 2018; Meng et al., 2018).

In this study, the Malaysian herbs *Averrhoa Bilimbi* and *Flos Lonicerae*, which are widely used in traditional Chinese medicine, were used to treat jaundice in newborn babies naturally. Because babies are not good eaters when they are newborn, herbal bath soap made from *Averrhoa Bilimbi* and *Flos Lonicerae* is made using saponification. Soap frequently contains many chemicals that may not suit babies' skin. Thus, this study aimed to produce herbal soap without using chemicals. Aside from that, studies on the antioxidant activity and total phenolic content of *Averrhoa Bilimbi* and *Flos Lonicerae* herbs are scarce in Malaysia. Thus, after the solvent extraction process, both *Averrhoa Bilimbi* and *Flos Lonicerae* extracts were quantified for their antioxidant activity and total phenolic content. The soap produced was further tested for antimicrobial activity, heavy metals, and pH to validate its suitability for skin application.

2. Methodology

Preparation of Herbal Soap

The tropical *Averrhoa Bilimbi* tree is a common species in Malaysia. The leaves of *Averrhoa Bilimbi* were washed and dried under the sun for one to two months, depending on the weather. Meanwhile, the dried *Flos Lonicerae* was bought from the store. Both dried *Averrhoa Bilimbi* and *Flos Lonicerae* were pounded into a fine powder. Virgin coconut and lavender essential oils were added to a glycerin-free SLS solution. An equal portion of both *Flos Lonicerae* and *Averrhoa Bilimbi* leaf powders were then added to the solution. After being poured into a silicone mold, the solution was dried at room temperature. The soap must be completely dry below room temperature to prevent fungus from growing on the surface of the soap.

Physicochemical Test

The extraction process of both dried *Averrhoa Bilimbi* and *Flos Lonicerae* is conducted before the antioxidant activity and total phenolic content of the herbs can be quantified. Physicochemical testing for the herbal soap has been carried out in the UiTM Pasir Gudang, Johor laboratory, with some tests being outsourced to independent labs.

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Extraction

The extraction process was done using a Soxhlet extractor and a rotary evaporator. The samples of *Averrhoa Bilimbi* and *Flos Lonicerae* were weighed. These samples were placed in an extraction thimble that was about ¾ full. A round bottom flask of the Soxhlet extractor was filled with 200 mL of methanol-water solvent. After being inserted into the extractor fitting, the thimbles were linked to the top of the flask with a round bottom. The condenser is then connected to the extractor fitting's top. For an 8-hour extraction period, the heater is put on to heat the methanol.

A round-bottom flask containing methanol and extraction solution was placed in a rotary evaporator after extraction to separate the oil further. The oil separation process was obtained to make sure no more solvent (methanol) was in the solution. The extraction of samples was transferred to bottles wrapped in aluminium foil and stored in a 2°C refrigerator.

Antioxidant Activity Test

The basic scavenging impact on the stable DPPH free radical activity was tested as part of the antioxidant activity test. The approach outlined by Veeru et al. (2009) was followed for the antioxidant activity procedure with some modifications. 1.0 mL of 0.002% DPPH in methanol was added into 1.0 mL of sample extract. A 30-minute storage period at room temperature was performed on the mixture in the dark. The absorbance of the mixture was measured at 517 nm by using methanol as the blank. The control was a solution containing 1.0 mL of methanol and 1.0 mL of 0.002% DPPH. The free-radical scavenging activities in terms of % inhibition can be calculated by the following equation:

$$\% \text{ inhibition} = [(A_0 - A_e) / A_0] \times 100\%$$

where A_0 is the absorbance value of the control while A_e is the absorbance of the extract. Tests were conducted on five concentrations of sample extracts to determine the % inhibition of each extract. To compare the antioxidant activity of the phenolic content, the IC_{50} value was used. This value represents the concentration of sample extract required to inhibit 50% of the free radical present in the DPPH solution.

Phenolic Contents Determination

Using Follin-Ciocalteu reagent, the Follin-Ciocalteu method is used to assess the phenolic acid concentration and total content of the sample extract. Based on Ibrahim et al. (2010), with slight adjustments, the following procedure was carried out: 1.0 mL of sample extract was combined with 3.0 mL of 20% sodium carbonate, 0.5 mL of the Follin-Ciocalteu reagent, and 10 mL of distilled water. The resulting mixture was kept at room temperature for a 2-hour reaction period. Following two hours, methanol was used as a blank in place of the sample extract to measure absorbance at 765 nm. The phenolic acid concentration is measured by comparing it to a constructed standard curve. On the other hand, the sample extract's total phenolic content was determined by applying the equation below:

$$C = cx (V/m)$$

where

c - concentration of gallic acid (mg GAE/mL) from a standard curve

C - total phenolic content (mg GAE/g) from the sample extract

m - dry weight of sample (g)

V - volume of the sample extract (mL)

Microbiology Test

Sample of the herbal soap was sent to Chemical Laboratory (Malaysia) Sdn Bhd for microbiology test.

pH Testing

The pH of the herbal soap was determined using pH meter model PH300 purchased from Eutech.

3. Findings

In this work, the saponification technique was used to turn *Averrhoa Bilimbi* leaf and the *Flos Lonicerae* plant into herbal soap product for easy application on the skin. Both extracts were tested for antioxidant activity and total phenolic content. The herbal soap was further tested for the antibacterial activity, toxicity and pH tests. This is to verify the heavy metal content and to ensure the pH falls within the suitable range for skin application.

Antioxidant Activity

Antioxidants are becoming increasingly popular as illness preventative and therapy options. Antioxidants are chemicals that interact with free radicals and neutralise them, preventing cellular harm. Many plants exhibit antioxidant activities that can be therapeutically beneficial, based on their therapeutic potential as an antioxidant in lowering such free radicals. Antioxidants' therapeutic potential in treating conditions including cancer, diabetes, and neurological disorders that are linked to oxidative stress has received a lot of attention recently. In this study, the antioxidant activity of both *Averrhoa Bilimbi* leaf and *Flos Lonicerae* plant extracts was examined in light of their ability to scavenge stable DPPH free radical activity. A percent inhibition versus concentration curve was constructed for each test solution, and the sample concentration necessary to achieve 50% inhibition was determined and given as an IC_{50} value. Figure 1 shows the amount of each *Flos Lonicerae* and *Averrhoa Bilimbi* leaf extract required for 50% inhibition of DPPH activity (IC_{50}). *Averrhoa Bilimbi* leaf required a slightly higher concentration of 0.0588 g/mL extract to inhibit 50% of DPPH assay compared to *Flos Lonicerae* plant extract which required 0.0315 g/mL. This suggest that *Flos Lonicerae* plant extract exhibited slightly higher antioxidant activity compared to *Averrhoa Bilimbi* leaf extract.

The findings of this investigation were in line with those of a previous study by Lan et al. (2007), who discovered that the aqueous, methanolic, and 70% ethanolic extracts of *Flos*

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Lonicerae have the ability to scavenge DPPH radicals and reduce Fe^{3+} to Fe^{2+}. As a significant contributor to this activity, this study also identified a crucial *Flos Lonicerae* ingredient termed chlorogenic acid, which was purified using HPLC from a 70% ethanolic extract. This implies that the extract of *Flos Lonicerae* might be employed as a natural source of antioxidants for the treatment of particular disorders (Lan et al., 2007). Rahman et al. (2014) investigation's into the antioxidant properties of the *Averrhoa Bilimbi* ethanolic extract revealed that the concentration of the extracts increased the reducing power of the crude ethanol extracts, and the *Averrhoa Bilimbi* extracts demonstrated moderate free radical scavenging activity against DPPH with an IC_{50} value of 635.066 ± 8.4102 g/mL. (Rahman et al., 2014). This suggest that both *Averrhoa Bilimbi* leaf and the *Flos Lonicerae* plant exhibit antioxidant activities that can be therapeutically beneficial, based on their therapeutic potential as an antioxidant in lowering such free radicals.

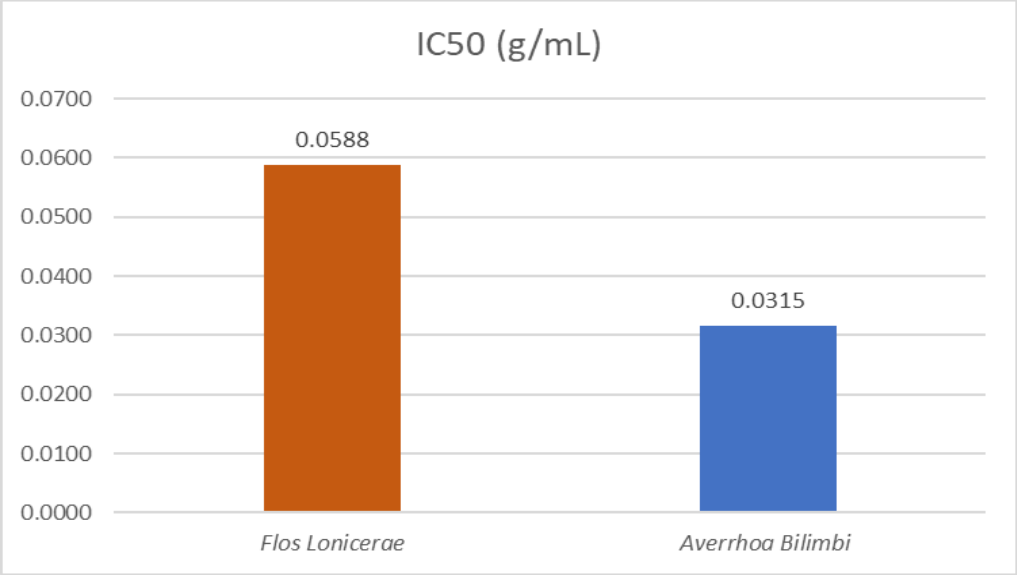

Figure 1. Free radical scavenging activity (IC_{50}) in *Flos Lonicerae* and *Averrhoa Bilimbi* leaf.

Phenolic Content

The total phenolic content (TPC) of both the methanolic extract of *Averrhoa Bilimbi* leaf and the *Flos Lonicerae* plant were investigated in this study. Phenolic compounds, which have low toxicity, are a form of secondary metabolite. These compounds can take the form of polyphenol structures, which include aromatic rings with hydroxyl groups, as well as simpler structures like phenolic acids and phenolic alcohols that consist of just one phenol ring. A phenolic molecule typically contains at least one phenyl ring, but a more reactive residue, such as methyl, hydroxyl, or acetyl, can replace it (Setyawan et al., 2021). TPC concentrations were calculated using the Follin Ciocalteu method. A spectrophotometer operating at a wavelength of 765 nm can be used to identify blue complexes, which are the foundation of the Follin Ciocalteu technique. Gallic acid equivalents were used to quantify the amounts of TPC in methanolic extracts (GAE). The results showed that *Flos Lonicerae* extract had a TPC value of 5.0 mg GAE/g, while *Averrhoa Bilimbi* leaf extract had a TPC value of 24.5 mg GAE/g (refer Figure 2). *Averrhoa Bilimbi* leaf ethanolic extract contained a greater value of TPC, 39.03 ± 0.25mg GAE/g, according to a study by Setyawan et al. (2021). The kind of solvent employed for the extraction had an impact on the value of TPC that was recovered. This is because the number of phenolic groups in a substance affects how it responds to the Folin-Ciocalteu reagent. The compatibility of phenolic compounds with the solvent system is closely related to a sample where its phenolic compounds are extracted, according to the "like-dissolves-like" idea. (Othman et al., 2014). The phytochemical screening of the *Averrhoa Bilimbi* extractives revealed the presence of flavonoid, tannin, and phenol, according to Hasanuzzaman et al. (2013). It has been demonstrated that plant-derived polyphenols such as flavonoids, tannins, and phenolic acids have a range of biological effects, including antioxidant activity. According to phytochemical screening, the flavonoids and tannins included in the plant extract from *Averrhoa Bilimbi* may be the cause of the extract's antioxidant action. According to the same study, the total phenolic content of several *Averrhoa Bilimbi* extracts ranges from 50.23 to 68.67 mg of GAE/g (Hasanuzzaman et al., 2013).

Flos Lonicerae, on the other hand, was found to contain 16 phenolic acids, the majority of which are caffeic acid derivatives. Neochlorogenic acid, caffeic acid, chlorogenic acid, isochlorogenic acid A-C, and cynarin are the primary phenolic acids found in *Flos Lonicerae* (Li et al., 2019). Among the naturally occurring substances with antioxidative strength are phenolic acids, which react with free radicals to form a new radical that is stabilized by the resonance action of the aromatic nucleus. The potential for antioxidant activity may be stronger in phenolic acids that have a second hydroxy group in the ortho or para position. Numerous

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Malaysian Journal of Science 43(1): 1-10 (March 2024)

investigations have shown that variations in phenolic acid concentration are always significantly correlated with changes in antioxidant intensity. Chlorogenic acid and caffeic acid, the two most researched elements in *Flos Lonicerae*, have both been demonstrated to have strong anti-inflammatory and antioxidant capabilities both in vitro and in vivo by removing harmful free radicals from the body (Li et al., 2019). Another study discovered that the chlorogenic acid in *Flos Lonicerae* shields mice from acute liver damage brought on by intraperitoneal injection of carbon tetrachloride as well as alcohol-induced chemical liver injury (CCl_4) (Li et al., 2019). It can be concluded that both *Averrhoa Bilimbi* leaf and the *Flos Lonicerae* plant are a good source of phytochemicals that exhibits many pharmacological activities.

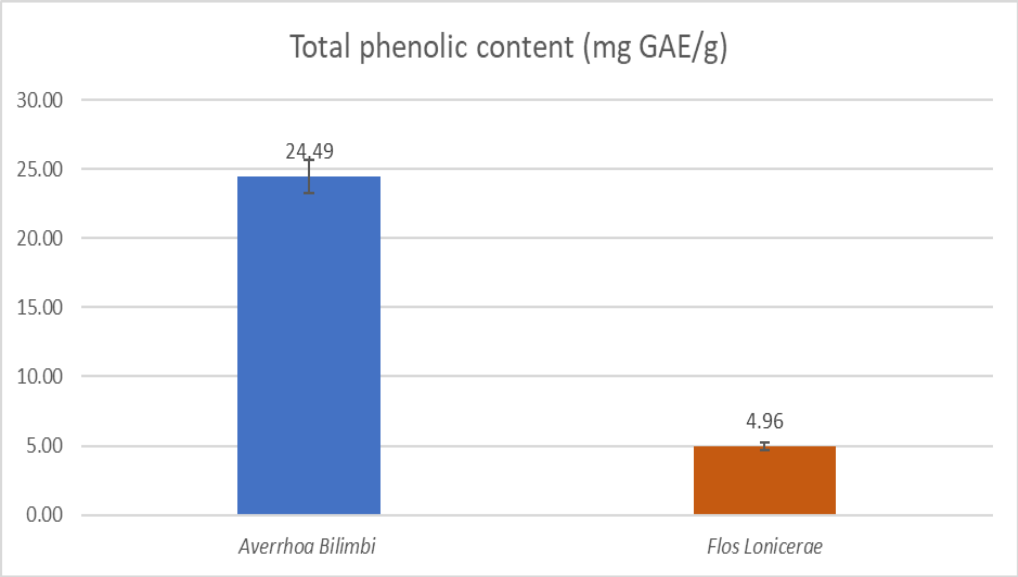

Figure 2. Total phenolic content (mg GAE/g) in *Flos Lonicerae* and *Averrhoa Bilimbi* leaf.

Microbiology Testing

The goal of the current study was to assess the antimicrobial activity of herbal soap against *Escherichia coli*, *Salmonella*, *Typhimurium*, *Staphylococcus aureus*, and *Enterococcus* sp. in vitro. The soap was made using the saponification process and contains extracts of *Averrhoa Bilimbi* leaf and *Flos Lonicerae*. According to Table 1, the herbal soap was efficient against all of the tested bacterial strains since no bacterial growth was seen. Compared to the National Pharmaceutical Regulatory Agency (NPRA) criteria, the total plate count test revealed relatively low bacteria growth of 30 cfu/g. The presence of hydrophilic and hydrophobic antibacterial components from extracts of *Averrhoa Bilimbi* leaf and *Flos Lonicerae* revealed that the herbal soap has antibacterial efficacy against some bacteria. In this study, the antibacterial activity of the herbal soap could be associated with the antioxidant activity and phenolic content of both the extracts of *Averrhoa Bilimbi* leaf and *Flos Lonicerae*. This conclusion was also consistent with prior research that suggested *Averrhoa Bilimbi's* antibacterial activity was linked to the presence of bioactive flavonoids such as apigenin and luteolin (Zakaria et al., 2007). In a different investigation, it was discovered that the ethanol extracts, water extracts with alcohol precipitating solution, and water extracts of *Flos Lonicerae* were highly inhibitory against a range of pathogens, including *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans, and Shigella dysenteriae* (Li et al., 2019).

Table 1. Antibacterial test on jaundice soap containing *Averrhoa Bilimbi* leaf and *Flos Lonicerae.*

Parameter Test	Results	NPRA Specification
Total plate count, cfu/g	30	≤ 5 x 10^4
Total Yeast & Mould Count, cfu/g	No growth <10	≤ 5 x 10^2
E. coli in 1g	Not present in 1g	Not present in 1g
Staphylococcus aureus in 1g	Not present in 1g	Not present in 1g
Salmonella in 10g	Not present in 10g	Not present in 10g
Enterobacteriaceae and certain other gram-negative bacteria	Absent	≤ 5 x 10^2

Heavy Metal Test and pH

The goal of this investigation was to find out how much heavy metals were in the herbal soap. There are concerns about the existence of hazardous compounds in herbal soap, which can be dangerous to children, particularly babies. It is noteworthy that babies have fragile skin and poor skin barriers, which makes them more vulnerable to skin damage and compels particular care (Kuo et al., 2020). Heavy metals such as mercury, lead and arsenic were

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not discovered in this product, as stated in Table 2. This is due to the ingredients used in the herbal soap where only glycerine, essential oil, and plant extract were used in the saponification procedure.

The acidic pH of the skin's surface is known to play a role in maintaining the homeostasis and permeability of the stratum corneum. Acidic pH seems to have the most important effects on the process of keratinocyte differentiation, the formation and function of epidermal lipids and the corneocyte lipid envelope, and the maintenance of the skin microbiome. Most people agree that products that go on the skin should be acidic and have a pH between 4 and 6 (Lukić et al., 2021). The pH of the herbal soap formulation has the pH of 7.82 (refer Figure 3). Skin products should have a pH that is as close to this range as feasible to reduce irritation. Even though the readings are not within the pH range, but the pH of this herbal soap is close to the recommended range.

Table 2. Heavy metal test on herbal soap containing *Averrhoa Bilimbi* leaf and *Flos Lonicerae*.

Parameter Test	Results	NPRA Specification
Lead as Pb, ppm	Not detected (<0.01)	≤ 10
Mercury as Hg, ppm	Not detected (<0.005)	≤ 0.5
Arsenic as As, ppm	Not detected (<0.005)	≤ 5.0

Figure 3. pH testing on herbal soap

4. Conclusion

As a conclusion, the herbal soap containing *Averrhoa Bilimbi* leaf and *Flos Lonicerae* by saponification method is successfully produced and its physicochemical properties have been investigated. The total phenolic content (TPC) value for *Averrhoa Bilimbi* leaf extract was 24.49 mg GAE/g as opposed to 4.96 mg GAE/g for *Flos Lonicerae* extract. Compared to *Flos Lonicerae* plant extract, which needed 0.0315 g/mL, *Averrhoa Bilimbi* leaf required a slightly greater quantity of 0.0588 g/mL extract to achieve 50% inhibition of the DPPH assay. The herbal soap proved efficient against all of the tested bacterial strains because there was no sign of bacterial development. Natural goodness from organic nature is present in herbal soaps, making them healthy for the skin. The created herbal soap contains no heavy metal and possesses a pH value which close to the recommended range.

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