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Screening of Active Compounds of *Baeckea frutescens* Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Its Saponification Process

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Bar soap is a widely used cleansing and lubricating product. It primarily comprises fatty acid salts and surfactants that help remove dirt and impurities from the skin. This research innovates the production of soap that can serve as both a cleaning agent and a medication. *Baeckea frutescens* is a wild herb with a historical track record for its medicinal applications, such as treating fever and sunstroke. This investigation involved extracting the oil for the saponification process, followed by an analysis using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR). The validation of C-H v and C=C aromatic signals at 2923.04 cm^{-1} and 1609.12 cm^{-1}, respectively, in the FTIR data has been corroborated through Gas Chromatography-Mass Spectrometry (GCMS) analysis. The result illustrates the mass spectrum, confirming the presence of 1-ethyl-3-methylbenzene with a molecular ion [M^+] at *m/z* 120. This observation is consistent with the mass spectrometric properties of the genuine standard, 1-ethyl-3-methylbenzene, thereby strengthening the reliability of our analytical findings. The soap's unique spherical design not only acts as a barrier against microbial growth but also highlights the oil's potential aesthetic and health benefits. This research highlights the potential of *Baeckea frutescens* soap as an eco-friendly and therapeutic alternative that combines natural healing characteristics with contemporary skincare requirements.

Keywords: Phenolic acids; antibacterial; bar soap; anti-inflammatory

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Soap has been used for thousands of years and is essential for personal hygiene, cleanliness, and a wide range of cleaning applications [1]. It can be used to remove dirt, oils, and impurities from both skin and surfaces [2], and it is also critical in preventing the spread of harmful microorganisms, which promotes public health. Modern soap formulations contain moisturizing ingredients that contribute to skin care [3], effectively eliminate odor-causing sub-stances, assist in stain removal, and can be used as a multipurpose tool for household cleaning activities.

Maintaining cleanliness is essential for a healthy lifestyle, especially during the current spread of diseases such as COVID-19, influenza, and conjunctivitis. Pathogens and viruses can easily spread through surfaces. Therefore, practicing good hygiene is crucial to prevent the transmission of such illnesses. Additionally, a clean and organized environment can promote both physical and mental well-being [4].

The primary objective of this research is to develop soap from *Baeckea frutescent* oil and screen its active compound using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) with validation from Gas Chromatography-Mass Spectrometry (GCMS). *Baeckea frutescens*, also known as the "Common Heath-leaved Myrtle," is an aromatic evergreen shrub native to Southeast Asia and Australia. It is valued for its traditional medicinal uses, including treating coughs, colds, skin infections, and respiratory ailments by indigenous communities. *Baeckea frutescens* is recognized for its aromatic leaves, which release a pleasant citrusy scent when crushed [5]. Note that recent research has highlighted its potent antibacterial, antifungal, and anti-inflammatory properties, particularly its potential in treating skin infections [6]. This plant's rich history of traditional use and emerging scientific interest underscore its relevance as a botanical ingredient. This makes it an attractive candidate for personal care products such as soap, which potentially benefit skin health and hygiene.

These innovative soap bars are intended to provide a portable and space-efficient solution for travelers to maintain their personal hygiene needs while on the move. *Baeckea frutescens*, known for its

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medicinal properties and antibacterial attributes, is incorporated to enhance the soap's effectiveness in cleansing and safeguarding against microbial threats. The small ball shape of these soap bars offers an additional advantage by facilitating a hygienic and controlled application, effectively preventing bacteria from spreading. By harnessing the unique properties of *Baeckea frutescens* and the practical design of the soap bars, this research aims to create travel-sized hygiene products that prioritize cleanliness and contribute to travellers' overall well-being and health in diverse environments.

EXPERIMENTAL

Sample Collection

The plant *Baeckea frutescens* was first discovered within the recreational area of Kolej PERMATA Insan at Universiti Sains Islam Malaysia. To ensure accurate measurements and analyses, the collected sample underwent a 48-hour drying process at 60°C. This drying procedure served the dual purpose of removing excess moisture, which could have otherwise interfered with precision. It also preserves the structural integrity of the sample, preventing any decomposition. Following the drying phase, the dried *Baeckea frutescens* were further mechanically ground, which effectively increased the overall surface area of the sample in preparation for the subsequent oil extraction process.

Solid-liquid Extraction

The sample was extracted using ethyl acetate solvent at room temperature to isolate compounds, including oils, pigments, and phytochemicals, potentially trapped within the plant tissue. For each 10-gram sample, 100 ml of ethyl acetate solution was used for extraction at room temperature for 48 hours. Following extraction, filtration with Whatman filter paper No.1 separated the solute from the solvent. Subsequently, the solution was separated and concentrated using the rotary evaporator at 50℃, resulting in a highly concentrated extract solution suitable for ATR-FTIR analysis.

Saponification Method

Saponification is a chemical process that occurs between fats and alkalis, resulting in soap formation. In this experiment, a solution was created by combining 50 cm^3 of 5 mol dm^{-3} sodium hydroxide solution with the oil extracted from *Baeckea frutescens*. The mixture was then heated to 100°C while being continuously stirred until the oil layer became indistinguishable. Following this, 50 cm^3 of distilled water and three spatulas of sodium chloride were introduced into the solution to induce precipitation, and the mixture was boiled for 10 minutes. To eliminate residual salt and alkali from the soap, the mixture was purified using Ultrapure water and filtered through filter paper. Finally, the soap was dried and shaped into small spherical balls.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) Analysis

This method was utilized to detect the presence of functional groups in the samples, regardless of whether the samples were in solid or liquid form, as the outcomes remained consistent. Subsequently, the soap underwent characterization through ATR-FTIR analysis, with frequency measurements recorded as wave numbers spanning the range of 4000 to 650 cm^{-1}. In summary, the samples were positioned onto the pristine surface of an Agilent Cary 630 instrument equipped with a diamond Attenuated Total Reflectance (ATR) accessory. Subsequently, the pressure clamp was securely closed until an audible click was heard, enabling real-time analysis via Micro-Lab software.

Gas Chromatography-Mass Spectrometry (GCMS) Analysis

The GC-MS investigation was conducted by Agilent using a GC-MS 5977B equipped with a pulsed split injector. The separation was achieved employing the DB-WAX Ultra Inert column (30 m × 0.18 mm × 0.18 μm film thickness), and helium gas was used as the carrier at flow rates of 2.1 mL/min with a 5:1 split ratio. The injector temperature was maintained at 250°C. The oven temperature was programmed to initiate at 50°C with a 1-minute holding time, followed by a ramp of 25°C/min up to 200°C, then a gradual increase to 230°C at 3°C/min, maintaining the final temperature for 23 minutes. Note that GC-MS operations were controlled using the Intuvo MS program. Mass Spectrometry (MS) spectra were obtained within the *m/z* range of 46-500 u, with a transfer line temperature of 250°C, source temperature of 230°C, quadrupole temperature of 150°C, and a 3-minute solvent cut time. The analysis was conducted in triplicate following the methodology outlined by [7].

RESULTS AND DISCUSSION

ATR-FTIR is a remarkable and exceptional technique for conducting quantitative and qualitative chemical analyses. Moreover, ATR-FTIR spectroscopy offers a range of key advantages compared to unconventional or alternative analytical methods. These advantages encompass precision, rapid identification, simplicity, cost-effectiveness in terms of acquisition, non-destructiveness, minimal sample preparation requirements, and the imposition of minimal burden on samples [8]. In **Figure 1**, the spectrum of *Baeckea frutescens* reveals the presence of various chemical bonds and functional groups. Notably, these include O-H bonds at 3308.23 cm^{-1}, C-H v at 2923.04 cm^{-1}, and C=N bonds at 1690.35 cm^{-1}. Furthermore, the spectrum exhibits distinctive peaks associated with C=O bonds at 1720.40 cm^{-1}, C-O bonds at 1313.80 cm^{-1}, and C=C aromatic bonds at 1609.12 cm^{-1} and 1448.62 cm^{-1}. These three types of carbon atoms are prominently featured in the spectrum.

Based on the results gained, flavonoids, phenolic acids, and oxazole were observed in the oil. Flavonoids, phenolic acids, and oxazole compounds have undergone thorough investigation due to their diverse biological effects and possible advantages for health. Certain phenolic acids, including ferulic and caffeic acids, have demonstrated robust antioxidant and anti-inflammatory characteristics. In contrast, flavonoids, a varied group of compounds, display a broad spectrum of biological activities encompassing antioxidative, anti-inflammatory, anti-cancer, and neuroprotective effects. When employed in combination with oxazole compounds, these naturally occurring substances have exhibited cooperative interactions, resulting in heightened therapeutic possibilities [9].

Flavonoids constitute a diverse class of polyphenolic compounds that enrich the plant world with vibrant colors prominently displayed in fruits and flowers. Renowned for their trifecta of attributes of antioxidant, anti-inflammatory, and immune-boosting properties, flavonoids have garnered attention in both scientific research and health-conscious circles [10]. Varieties like quercetin and catechins, abundant in foods like apples, tea, and berries, are recognized for their potential health benefits. Beyond their roles as nature's pigments, flavonoids play pivotal rolesin both plant physiology and human nutrition, promoting overall well-being and bolstering immune functions. In addition, they offer numerous health advantages, encompassing the reduction of inflammation, enhanced blood vessel function, protection against heart disease and cancer, even cognitive enhancement, and lowering the risk of age-related memory decline, all complemented by their antioxidant prowess [11].

Phenolic acids, characterized by their distinctive phenolic ring structure adorned with hydroxyl(-OH) groups [12], are abundant in the plant kingdom, prominently in fruits, vegetables, and grains. Aside from adding vibrant colors to nature, these compounds have garnered attention for their remarkable health benefits. This includes potent antioxidant properties that mitigate oxidative stress and alleviate inflammation [13], significantly contributing to human well-being. Additionally, phenolic acids play vital roles in various natural processes and find utility in the food industry, influencing attributes like color, flavor, and preservation. In tandem, flavonoids and phenolic acids, sharing a chemical kinship as plant-derived substances with anti-inflammatory and antioxidant attributes, have been proven in clinical studies to potentially reduce the risk of chronic diseases such as specific cancers, type 2 diabetes, and cardiovascular ailments. Furthermore, phenolic acids foster gut health by promoting the growth of beneficial intestinal bacteria [14].

Oxazole is an organic compound characterized by a distinctive five-membered heterocyclic ring housing oxygen and nitrogen atoms [15]. It occupies a unique and pivotal role in the realm of organic chemistry. Its significance resonates widely across numerous scientific disciplines and industrial sectors. Furthermore, oxazole compounds exhibit a broad spectrum of applications, spanning from pharmaceuticals to antimicrobial agents and dyes. They frequently take center stage in drug development, serving as fundamental structural components in various medicinal compounds, thereby earning their place in the arsenals of researchers and chemists alike. In addition, oxazole derivatives, with their one-of-a-kind chemical properties, assume critical roles in the progression of our comprehension of chemical reactions, materials science, and agriculture. Their adaptability and versatility persistently yield substantial contributions to expanding scientific knowledge and the pragmatic utilization of these compounds across an extensive array of domains. Additionally, oxazoles have been investigated for their potential as cognitive enhancers, adrenergic antagonists, fungicides, anthelmintic, antihypertensive, and antiulcer agents [16]. Moreover, they have been explored for applications in pesticides, agrochemical fungicides, and insecticides.

In addition, the presence of C-H v, C=C aromatic at 2923.04 cm^{-1}, and 1609.12 cm^{-1}, respectively, from FTIR data has been validated by GCMS result as mass spectrum in **Figure 2** displays the presence of 1-ethyl-3-methyl-benzene with molecular ion $[M^+]$ at *m/z* 120. It corresponds with the MS properties of authentic standard 1-ethyl- 3-methylbenzene.

Figure 1. FTIR spectral of *Baeckea frutescens.*

Table 1. FTIR data of *Baeckea frutescens.*

Wavelength (cm^{-1})	Functional group
3308.23	O-H *v*
2923.04	C-H *v*
1720.39	C=O aldehyde
1690.35	C=N *v*
1609.12	C=C aromatic
1448.62	C=C aromatic
1313.80	C-O *v*

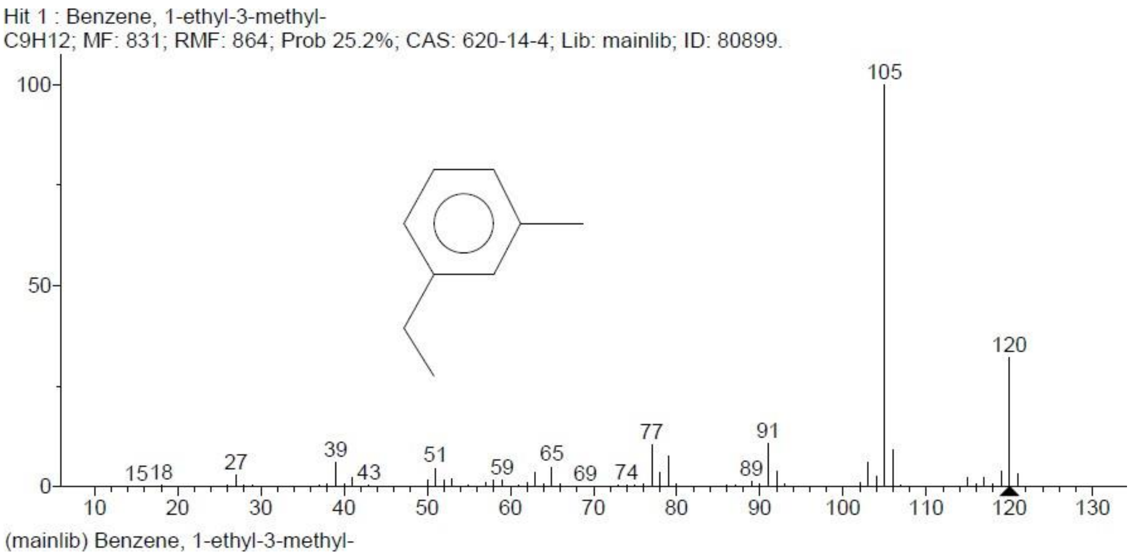

Figure 2. Gas chromatography-mass spectra of *Baeckea frutescens.*

CONCLUSION

The comprehensive exploration of *Baeckea frutescent's* molecular composition through ATR-FTIR spectrum analysis has unveiled the presence of noteworthy antioxidant compounds, specifically phenolic acids and flavonoids. These compounds, renowned for their robust antioxidative and anti-inflammatory attributes, hold immense promise in the realm of soap production. Their inclusion in soap formulations extends the utility of hygiene products beyond conventional cleansing, aligning with the preferences of health-conscious consumers and potentially offering benefits for skin health such as moisturizing, anti-aging, and protection. Furthermore, this innovation enriches our scientific understanding and addresses the evolving societal needs for holistic well-being and skincare solutions. Moving forward, we will perform GCMS analyses on flavonoids and oxazoles to advance our research. Note that these compounds provide an added layer of protection against harmful microorganisms, reinforcing the hygiene and health benefits of these products. Integrating phenolic acids, flavonoids, and antimicrobial oxazole compounds into soap products represents a tangible step toward enhancing healthcare and personal hygiene practices in an era where health and wellness are paramount. In addition, it bridges the gap between tradition and a more health-focused approach to personal care.

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