



Utilizing spent coffee waste as a sustainable source for producing bioactive Gemini benzo-imidazolium cationic surfactant for cleaning water surface pollution

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ABSTRACT

Water contamination is a severe environmental problem that has detrimental effects on the health of living organisms. One of the most significant contaminants of water is petroleum films resulting from oil slicks. Using bio-based surfactants generated from renewable resources or waste can be a sustainable strategy for addressing such a problem. In such direction, novel groups of bioactive gemini cationic surfactants (**5a-d**) with benzo-imidazole moiety were designed as petro-dispersing and antimicrobial agents using an efficient method. Spectroscopic techniques, including FT-IR, ¹H, and ¹³C NMR, were utilized to analyze their chemical structures. Initially, the mixture of free fatty acids was extracted from the dried spent coffee waste using the Soxhlet method and elucidated by gas chromatography flame ionized detector (GC-FID). It was then esterified with methanol and followed by the reduction process in abundance of NaBH₄ to afford the desired mixed fatty alcohols. Finally, the resulting mixed fatty alcohols were esterified with monochloroacetic acid to produce mixed fatty acids chloroacetate. In parallel, 1H-benzo[d]imidazole, was synthesized by fusing o-phenylenediamine with formic acid in the presence of KOH. The resulting compound was then subjected to further treatment with KOH and separately refluxed with various alkylene glycol bis(2-chloroacetate) (**3a-d**) to produce **4a-d**. Finally, the quaternization process of the tertiary nitrogen in **4a-d** with mixed fatty acids chloroacetate (**1**) was carried out to obtain the bioactive gemini cationic surfactants (**5a-d**) bearing benzo-imidazole moiety. The physicochemical properties of the extracted oil and the surface-active indices of the synthesized gemini cationic surfactants (**GCS**) were elucidated. Also, the antimicrobial indices were investigated using the in vitro disc diffusion method on different species of bacteria and fungi. The results revealed that **GCS** (**5a-d**) compounds showed the highest inhibition zone diameter (IZD) ranged from 40-36 mm against gram-negative bacteria compared to gram-positive, due to them to the chemical composition of the quaternary benzo-imidazole moiety. Furthermore, the efficiency of **GCS** (**5a-d**) was determined in dispersing and collecting thin films of petroleum in different types of water. It was interesting to note that the majority of designed **GCSs** have shown the highest capabilities in dispersing and collecting thin films of petroleum from water with a thickness ranging from 0.15 to 0.90 mm.

1. Introduction

Many sources, such as oil tanker accidents and pipelines, cause the release of large quantities of petroleum oils into the hydrosphere. This leads to significant issues due to the pollution of surfaces of water with petroleum, which has negative impact on marine life. Different approaches can help solve this concern, including mechanical techniques that can be used to remove the thick petroleum layer. However, these techniques should not be used for removal of thin layers, which is very

environmentally hazardous. Colloid-chemical approaches were applied to overcome this problem by developing agents that have petro-collecting and petro-dispersing properties [1,2].

Conventional and gemini-cationic surfactants are the most effective agents for dispersing and collecting petroleum thin films formed on the water surface during oil processing stages. These surfactants have proven to be highly efficient in cleaning up petroleum films during oil processing [3,4].

Recently, there has been a notable shift towards development

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renewable and eco-friendly materials [5,6]. This shift has been driven by the growing demand for surfactants that can biodegrade after use [7,8]. A variety of natural sources can be utilized to obtain raw materials such as oleochemicals [9,10], carbohydrates [11,12], and amino acids [13,14] to manufacture eco-friendly surfactants. Among commercial surfactants that attract attention in the cosmetic industry are the surfactants with pyridinium and imidazolium moieties [15]. Today, there is a growing interest in the wide variety of applications for biodegradable and renewable surfactants. These surfactants are considered more environmentally friendly when compared to traditional commercial and petrochemical surfactants. The challenges of manufacturing, high costs, and environmental concerns remain issues that need to be resolved [16,17]. Recently, many reports have discussed a new cationic surfactant derived from fatty acids (oleic and stearic acid). It has been found that the biodegradation of surfactants is a result of the presence of ester bonds in the surfactants [17,18]. One of the issues that affect global health is the diseases caused by microbial infections. To overcome these problems, available marketed antibiotics can be used, and, on the other hand, new antimicrobial material has been developed. Therefore, the discovery of novel chemotherapeutic materials becomes necessary to decrease the resistance and reduce the duration of treatment [19]. Numerous research reports on antimicrobials have demonstrated that the compounds of benzo-imidazole's showed significant effectiveness against the microbes [20].

Benzo-imidazole is widely recognized for its diverse biological and pharmacological activities, making it a valuable bioactive agent in therapeutic medicine. Drug resistance is one of the serious problems that responsible for the spreading of diseases around the world [21]. In the present studies, authors have designed classes of antimicrobial drugs with novel heterocyclic moieties that developed with low cost and are eco-friendly. Benzo-imidazoles display a range of interesting biological properties such as antiviral [22], anticancer [23], antibacterial [24], anti-inflammatory [25], antifungal [26], anti-ulcer [27] and antihypertensive [28] activities. An effective option for reducing microbial activity is the use of cationic surfactants, which block the membrane protein of the cell at the interface between the membrane and water [29–32]. The activity of these compounds was utilized due to the presence of cationic groups.

Coffee and its derivatives have become culturally established. Over 10 million metric tons of coffee beans were produced annually in the 2018–2019 crop year, nearly double the amount produced 30 years prior [33]. The increase in demand due to population growth has resulted in various environmental issues such as the wasteful use of organic materials and the non-sustainable use of scarce resources [34]. Once the beverages are prepared, the spent coffee grounds (SCGs) generated are almost equal to the initial mass of coffee beans used, not including any additional waste materials generated during the production process [35]. The composition of SCG varies depending on cultivation area, extraction way, types of coffee beans, and roasting conditions. The oil fraction in the SCG contains percentages ranging from 7.9 % to 26.4 %, while the crude fiber content falls between 19.7 % and 22.1 %. Additionally, the SCG also contains alkaloids, proteins, and other compounds [36]. The content of oil content in the coffee source depends on their types which range from 11 to 20 wt% [37,38]. The oil that yields from defective coffee beans ranged from 10 % to 12 % [39], while in SCG contain 10–15 wt% [40,41]. The recycling of environmentally polluting materials to obtain eco-friendly materials become is very necessary. Accordingly, waste frying oil has been used to produce surfactants as eco-friendly material that having the ability to clean the water surfaces [42,43].

The present study focuses on the recycling of SCG for producing eco-friendly surface-active materials with petro-collecting and petro-dispersing properties to clean water surfaces from petroleum pollutants. The synthesized compounds were confirmed using various spectroscopic techniques, including FT-IR and NMR analysis. The surface properties of the synthesized materials were thoroughly examined. Both

petro-collecting and petro-dispersing activities were reported. Additionally, antimicrobial efficiency was tested against different microorganisms, including bacteria and fungi.

2. Synthesis and instrumental

2.1. Chemicals and Instrumental

Brazilian spent coffee grounds sample is collected from El-sultan café, as waste product from coffee. Benzene-1,2-diamine, acetonitrile, ethylene glycol and monochloroacetic acid were purchased from sigma-Aldrich chemical Co. Sodium borohydride (NaBH_4), *p*-toluene sulphonic acid, propylene glycol, pentane-1,5-diol, and hexane-1,6-diol were purchased from Fisher Scientific company. Formic acid, *n*-hexane, dichloromethane (DCM), tetrahydrofuran (THF), diethyl ether, ethyl acetate, ethanol absolute, methanol, and petroleum ether were purchased from El-Nasr Pharmaceutical Chemical Co. A Thermo-Scientific Nicolet iS10 FT-IR spectrometer was used to give the FT-IR spectra for all synthesized compounds. NMR spectra including ^1H NMR and ^{13}C NMR were obtained by Bruker Avance 400 MHz instrument in $\text{DMSO}-d_6$ solutions using tetramethylsilane (TMS) as the internal standard.

2.2. Synthesis

2.2.1. Extracting of mixed fatty acids from spent coffee grounds (SCGs)

According to Scheme 1, one kilogram of spent coffee grounds (SCGs) was dried overnight at 65 °C, resulting in a moisture content of 43.63 %. The oil was extracted using an automatically extraction system SOX-THERM [44]. 50.0 g of the dried SCG into a thimble and then placed into a unit of Soxhlet extractor, which put into a conical flask containing 200 ml *n*-hexane (with a solid to solvent ratio 1:4) under reflux condition. The reaction was left for extraction for 7 hrs. By using a rotatory evaporator under reduced pressure, *n*-hexane evaporated [44], and the obtained coffee oil yield was 152.1 g. Fatty acids content in the obtained oil were characterized by gas chromatography.

2.2.2. Fractionation of mixed fatty acids

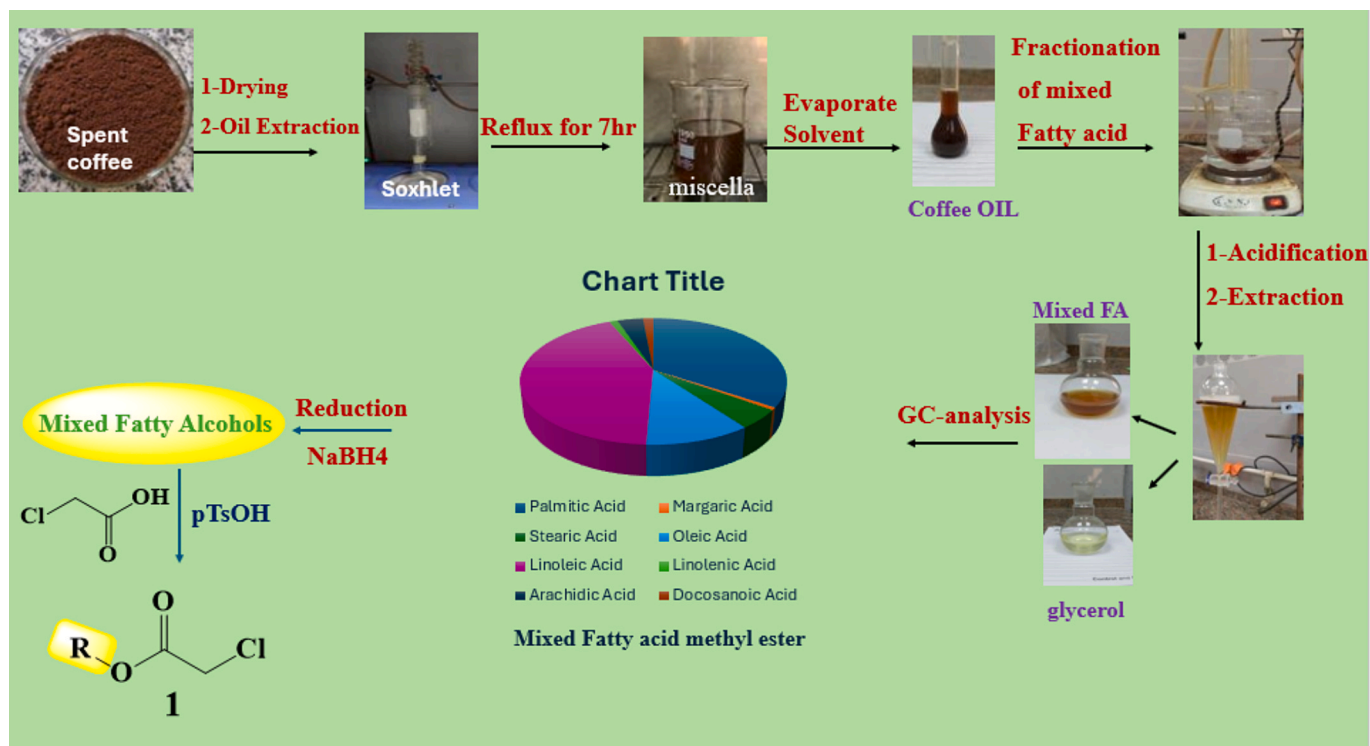
About 10.0 g coffee oil was weighed in 150 ml round flask and treated with 6 % alcoholic potassium hydroxide solution under reflux with condenser for two hours in water-bath till homogenous solution was obtained. Then, the reaction mixture was acidified by 30 % sulfuric acid to obtain mixed fatty acids. The resulting mixed fatty acids were extracted using *n*-hexane and then evaporated using a rotary evaporator under reduced pressure to obtain the desired product.

2.2.3. Detection of mixed fatty acids composition extracted from of SCGs

The methyl esters of mixed fatty acids (FAMES) can be prepared from the extracted lipids obtained from SCGs using BF_3 in methanol and the FT-IR spectrum confirms its structure (Fig. 1a) [45]. FT-IR (ν/cm^{-1}): 2928, 2854 cm^{-1} (C–H aliphatic), 1738 cm^{-1} (C=O of ester), 1165 cm^{-1} (C–O of methyl ester) (Fig. 1a). FAMES were separated and can be detected using a flame ionization detector (FID) in a HP 6890 plus gas chromatography system. The temperature of the injector and detector was maintained constant (250 °C) and at rate of 4 °C/min, the temperature of column started to increase from 140 °C up to 240 °C and maintained through 10 mins and then 1 μL of FAMES was injected at flow rate of 1.2 mL/min of carrier gas (helium). As shown in Table 1 [46], the percentage chart of the whole fatty acids in sample were illustrated Fig. S1.

2.2.4. Reduction process of mixed FAMES and synthesis of mixed fatty chloroacetate (1)

A solution of 2.0 mmol FAMES dissolved in 20 ml diethyl ether was stirred with 10.0 mmol of NaBH_4 for 20 min at room temperature. Then, 20 ml of methanol was slowly added drop by drop to the mixture mentioned above while refluxing for 20 h. Once the reaction was



Scheme 1. A stepwise chart for the extraction of FFA from spent coffee and Synthesis of mixed fatty acids chloroacetate.

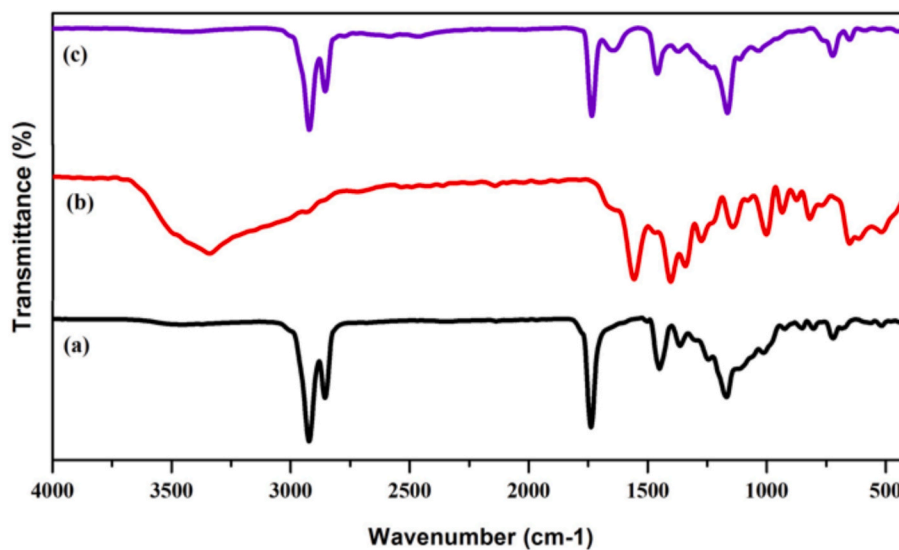


Fig. 1. FT-IR spectra of (a) Methyl ester of mixed fatty acids, (b) Mixed fatty alcohols and (c) mixed fatty acids chloroacetate.

completed and allowed to cool down, pale-yellow needles (mixed fatty alcohols) were obtained with 85–90 % yield by extracting with 2 N HCl and ethyl acetate [47]. FT-IR (ν/cm^{-1}): 3342 cm^{-1} (OH of alcohol), 2928 cm^{-1} (C–H aliphatic), 1145 cm^{-1} (C–O of alcohol), (Fig. 1b).

Then, 1.1 mol mixed fatty alcohols and 1.3 mol monochloroacetic acid were dissolved in dry benzene in the presence of 0.1 mol of *p*-TsOH as a catalyst. The reaction mixture was refluxed using Dean Stark apparatus in order to remove water molecules from the reaction [48]. The compound (1) was extracted using a saturated solution of Na₂CO₃, and then benzene was evaporated by a rotatory evaporator under reduced pressure. FT-IR for compound (1): (ν/cm^{-1}) 1734 cm^{-1} (C=O of ester), 2921, 2854 cm^{-1} (C–H aliphatic), 1165 cm^{-1} (C–O of ester), 722 cm^{-1} (C–Cl of chloroacetate), (Fig. 1c).

2.2.5. Synthesis of 1H-benzo[d]imidazole (2)

In sealed tube, a mixture of 9.25 mmol Benzene-1,2-diamine and 11.73 mmol formic acid was fused under solvent free condition for 2 h. While the reaction progress was monitored using TLC. After the reaction reached completion, the mixture was cooled and neutralized using sodium hydroxide. Finally, the obtained white crystals were filtrated, washed with water, recrystallized from water, and then dried to afford compound 2 with a 90–95 % yield.

1H-benzo[d]imidazole (2). FT-IR: (ν/cm^{-1}) at 3386 cm^{-1} (N–H of benzo-imidazole), 3061 cm^{-1} (C–H of aromatic ring), 1619 cm^{-1} (C=N of imine), 1590 cm^{-1} (C=C of aromatic ring), 1274 cm^{-1} (C–N bond) and 1458, 1501 cm^{-1} (C–C of aromatic ring), (Fig. 2).

Table 1
Fatty acid composition of extracted coffee oil.

Fatty acids	RT* (min)	Area %
Palmitic acid (C _{16:0})	4.49	34.38 ± 1.12
Margaric acid (C _{17:0})	5.68	0.64 ± 0.04
Stearic acid (C _{18:0})	6.68	4.86 ± 0.23
Oleic acid (C _{18:1})	6.80	10.80 ± 0.57
Linoleic acid (C _{18:2})	7.30	42.87 ± 1.15
Linolenic acid (C _{18:3})	8.14	1.23 ± 0.17
Arachidic acid (C _{20:0})	9.66	3.72 ± 0.22
Behenic acid (C _{22:0})	13.37	1.50 ± 0.08
∑ SFA	--	45.10
∑ UFA	--	54.90

*RT, Retention time by min

2.2.6. Synthesis of alkylene glycol bis(2-chloroacetate) (3a-d)

A mixture of 2.5 mol of monochloroacetic acid and 1.0 mol of ethylene glycol was dissolved in dry benzene in presence of 0.1 mol of *p*-TSOH as a catalyst. The reaction mixture was refluxed using Dean Stark apparatus in order to remove water molecules from the reaction [48]. Ethane-1,2-diyl bis(2-chloroacetate) **3a** was extracted by using saturated solution of Na₂CO₃. Compound **3a** was obtained via the evaporation of benzene using a rotary evaporator under reduced pressure as colorless viscous liquid, yield: 85–90 %. Propane-1,3-diyl bis(2-

chloroacetate) (**3b**) is a colorless viscous liquid, yield 82–90 %, pentane-1,5-diyl bis(2-chloroacetate) (**3c**) is pale yellow powder with yield 70–80 % and hexane-1,6-diyl bis(2-chloroacetate) (**3d**) is colorless crystals, yield 85–90 % were synthesized with the same procedure with using compounds propane-1,3-diol pentane-1,5-diol and hexane-1,6-diol, respectively. The obtained products were characterized by using FT-IR spectroscopy.

FT-IR (ν/cm^{-1}) for compounds (**3a-d**) at 2858–2860, 2928–2960 cm^{-1} (C–H aliphatic), 1746–1750 cm^{-1} (C=O of ester), 1158–1197 cm^{-1} (C–O), 781–788 cm^{-1} (C–Cl), (Fig. 3).

2.2.7. Synthesis of alkane-diyl bis(2-(1H-benzo[d]imidazol-1-yl) acetate) (4a-d)

A mixture of 5.63 mmol benzo-imidazole and 7.13 mmol KOH in acetonitrile was stirred under reflux until the base was consumed. Then, 2.82 mmol of compound **3a-d** was added to the reaction mixture with continuous stirring under reflux for 24 hr. The unwanted precipitate was removed from hot solution. After allowing for the evaporation of volatile components under reduced pressure, the final product was obtained as pale brown semi-solid with 70–80 % yield. Propane-1,3-diyl bis(2-(1H-benzo[d]imidazol-1-yl) acetate) (**4b**) is pale yellow semi-solid with yield of 75–80 %, pentane-1,5-diyl bis(2-(1H-benzo[d]imidazol-1-yl) acetate) (**4c**) is white solid with yield of 75–80 % and hexane-1,6-diyl

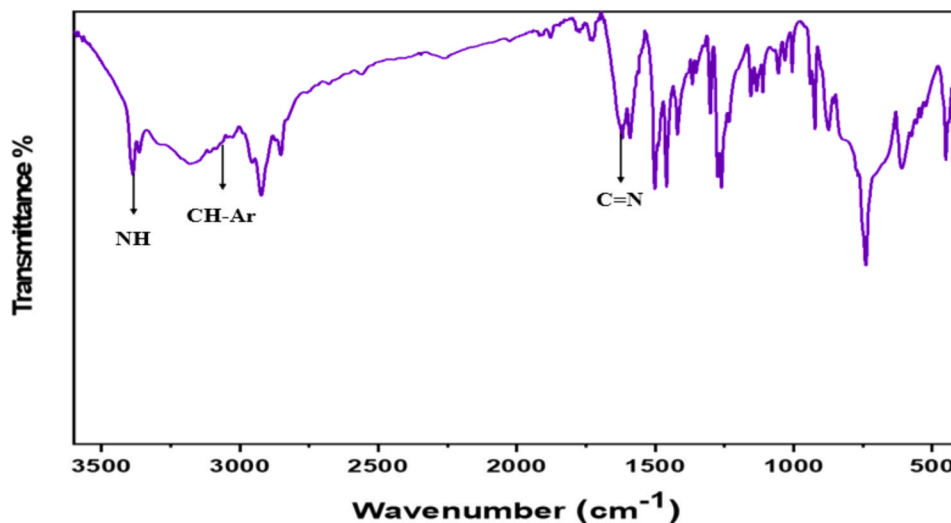


Fig. 2. FT-IR of 1H-benzo[d]imidazole.

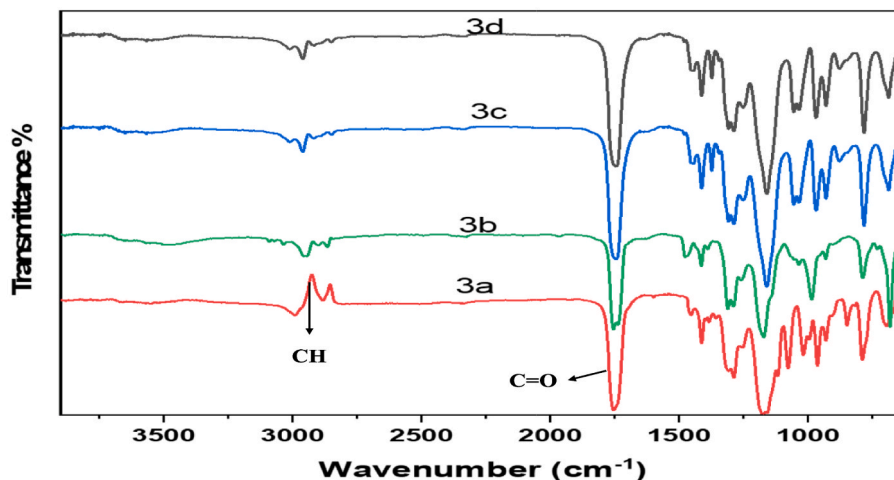


Fig. 3. FT-IR of alkylene glycol bis(2-chloroacetate) (3a-d).