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GC-MS and Bioactivity Analysis of the Acetone Methanol Extract of the Mesocarp of Cocoa Pod Husk and Potential Utilizations

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ABSTRACT

Cocoa pod husk (CPH), is a rich source of minerals (particularly potassium), fiber (including lignin, cellulose, hemicellulose, and pectin), and antioxidants (e.g. phenolic acids). It is composed of mesocarp, endocarp, and epicarp. The mesocarp consists of fiber (crude, NDF, and ADF-44-48%) and cellulose (53%). CPH's potential application to human health includes antioxidant, anti-inflammatory, anti-carcinogenic, immunomodulatory, vasodilatory analgesic, and antimicrobial activities. Fresh ripe cocoa pod husks were collected, and the mesocarp was separated and air dried followed by size reduction and extraction with acetone: methanol (7:3) (v/v) using the maceration process. GC-MS analysis was carried out to identify the phytochemical compounds present in the extract. The compounds identified in the extract highlight the great potential of the extract to improve life. When tested, the extract showed activity against nine different human pathogens at varying concentrations. Free radical scavenging activity was also determined using ABTS and DPPH assays, and the extract was found to be potent.

1.0 Introduction

Theobroma cacao is the only species of the *Theobroma* genus that is farmed for commercial purposes and is the most popular on the market [1]. Cocoa is thought to be a prehistoric tree that was already cultivated by the Olmecs and Mayans more than 3000 years ago [2]. Due to its unique chemical composition of more than 500 distinct compounds, cocoa has become a significant ethno-medicinal plant [3]. According to studies, cocoa pod husks have antioxidant, anti-inflammatory, anti-carcinogenic, immunomodulatory, vasodilatory, and analgesic activities and antimicrobial properties [3,4]. For the 2020–21 season, a record production volume of 5.024 million tones was anticipated [5]. The top three cocoa bean producers for the year mentioned above, in terms of thousand metric tons, were Côte d'Ivoire (2,150), Ghana (850), and Ecuador (340) [6]. CPH is the primary by-product of cocoa bean processing, accounting for 75 to 76 percent of the entire fruit [1]. Cocoa beans are the raw material for the production of chocolate and its derivatives, which are used in various forms worldwide [7]. CPHs are typically discarded on the farm after the cocoa beans are removed during the harvesting process. However, they can serve as organic fertilizer by increasing the amount of organic matter in the soil and facilitating the return and recycling of nutrients into plant-available forms after decomposition [8].

Meanwhile, untreated CPH left on the soil surface in cocoa farms may serve as a source of inoculum for plant diseases like black pod rot, caused by the presence of *Phytophthora* spp. This can result in an annual yield loss of 20 to 30 percent worldwide, with individual farms experiencing annual yield losses of 30 to 90 percent [9].

The cultivation of cocoa holds economic value for many countries, including Ivory Coast, Ghana, Ecuador, Nigeria, Indonesia, Malaysia, Brazil, and others [10]. However, commercial cocoa production poses a serious problem due to the large amounts of pod husks typically left behind in cocoa farms [11]. For every ton of dry cocoa bean produced, approximately 9-10 tons of fresh pod husks are generated [12].

Despite this, cocoa husks are a plentiful, affordable, and renewable source of bioactive substances such as dietary fiber, pectin, antioxidant compounds, minerals, and theobromine, which underscores the need to add value to them [1]. They are also rich in minerals (particularly potassium) and antioxidants like phenolic acids [1]. These potentials of CPH remain largely untapped. By using this lignocellulosic material and other cocoa pod husk components beneficially, it may be possible to reduce their negative environmental impact while generating economic benefits [13].

Cocoa pod husks have been reported to have activity against human pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [14]. The activity of CPH could be tested against other human pathogens. Additionally, analysis of the three major parts of the cocoa pod shows that the mesocarp contains significant amounts of fiber (crude, NDF, and ADF – 44-48%) and cellulose (53%), justifying further research focused on the mesocarp to determine its potential [1].

2.0 Materials and Method

Cocoa pod husks (CPH) were collected from a farm in Tsito, located in the Volta Region of Ghana. All solvents and

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reagents used in the study were purchased from commercial suppliers and were used without any further purification. The following solvents; ethyl acetate (EA), chloroform, diethyl ether, petroleum ether, acetic acid, anisaldehyde reagent (100 mL), and acetone, were obtained from MES Equipment, Fregeosco Limited, and other chemical shops in Circle-Accra, Ghana, each in 2.5L Winchester bottles. The remaining reagents; sulfuric acid, hexane, ethanol, and methanol, were sourced from the chemistry laboratory of the University of Education, Winneba (UEW), where the solvent extraction took place.

Several 19.5 cm by 6 cm glass plates were purchased from a local shop, prepared, and used as TLC plates. These plates were coated with silica gel (Silica Gel G6) produced by BDH. The silica gel contained 13% calcium sulfate and was specifically manufactured for TLC studies. A gas chromatography-mass spectrometer (Shimadzu GCMS QP2020) was used to analyze the crude extracts.

Eight strains of microbes were obtained from the School of Basic and Biomedical Sciences at the University of Health and Allied Sciences. These included Methicillin-resistant *Staphylococcus aureus* (NCTC 12493), *Staphylococcus aureus* (NCTC 12973), *Escherichia coli* (NCTC 12241), *Streptococcus mutans* (ATCC 700610), *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (ATCC 14028), *Klebsiella pneumoniae* (NCTC 13440), and *Candida albicans* (ATCC 90028).

The reagents used for the free radical scavenging activity tests on the crude extracts were methanol (Sigma-Aldrich, analytical grade), DPPH (Sigma-Aldrich, analytical grade), ABTS (Sigma-Aldrich, analytical grade), and DMSO (Sigma-Aldrich, analytical grade).

2.1 Sample Collection and Treatment

Ripe cocoa pods were harvested and cut in half using a cutlass to remove the pulp and seeds. The cocoa pod husks (CPHs) were weighed using an electronic balance, and with the help of a kitchen knife, the mesocarp was separated from the other layers. The mesocarp was then sliced into pieces about 5 cm in size to prepare them for air drying. Since the moisture content of fresh CPH is about 90%, quick drying is essential to prevent deterioration. This is achieved by slicing the fresh pod [1].

The sliced mesocarp was then cut into approximately 3 cm pieces and dried in the sun from around 7:30 am to 10:00 am each day for two days until surface moisture was removed, followed by air drying. After drying, the mass of each sample was weighed again.

2.2 Extraction by Maceration

The method used was as described by [4] with some modifications. The powder of CPH was weighed using an electronic balance model, Scout Pro with 400g capacity. A solvent system used in the extraction process was acetone: methanol (7:3). The ratio between the sample weighed in grams (g) and the solvent system in millilitres (mL) was to be 1:5. Maceration was then done for 72 hours to allow maximum digestion of compounds by solvents from the CPH at room temperature in an enclosed area protected from direct light. The solvent extract was separated from the residue with the aid of a piece of handkerchief and filter paper, then concentrated using a rotary evaporator at a temperature of 70^{o}C, to obtain crude concentrate.

2.3 Thin Layer Chromatography (TLC) Analysis

To determine the presence of different compounds and separate the crude mixture, TLC analysis was performed using different solvent systems as the mobile phase, and the stationary phase was made up of silica gel spread on a glass plate. The silica gel was freshly prepared and used. For the mobile phase, various solvent mixtures of different solvent polarities were employed. Among those which gave good separations were (V/V) mixtures, hexane: petroleum ether: acetic acid (23:23:3) (V/V) mixtures.

2.4 Analysis Using Gas Chromatography-Mass Spectrometry (GC-MS).

This analysis was done at the University of Cape Coast instrumental room. The method used was similar to that found in earlier published articles with some modifications to suit the purpose of this research [4]. The GC operation parameters used were Column oven Temp: 50.0 C, Injection Temp: 250.0 C, Split ratio: 50.0, Pressure: 84.0 KPa, Column flow: 1.62 mL/min and Linear velocity: 49.2 cm/sec. The column used was Rtx-5ms (30×0, 25µm×0.25mm) with an *Injection volume* of 1.0µL. the column oven temperature was 50^{o}C setting the initial temperature of 50^{o}C and a total programmed time of 32 minutes to reach a temperature of 300^{o}C. Flow control mode was linear velocity, using He carrier gas with a pressure of 84.0 kPa with a Column flow of 1.62 mL/min, linear velocity of 49.2 cm/sec. MS operation Parameters were set as follows; ion source temperature of 25^{o} C, interface temp: 270^{o} C, and mass scan range: 80 -550 m/z. The qualitative or quantitative parameters used were NIST 14, NIST14s, and Wiley 8' libraries for the identification of peaks. Quantifications were done using area normalization for total ion counts (TIC), and five reference ions were selected to improve sensitivity.

2.5 Antimicrobial Activity Determination

Antimicrobial activity tests were carried out using standard operation procedures as outlined below;

2.5.a *Micro-well broth dilution*

The broth microdilution technique was carried out following the methodology described in document M27-A3 published by the Clinical and Laboratory Standards Institute (CLSI) [15] with minor modifications.

100 uL of Mueller Hinton broth was dispensed into all the wells of each of the plates to be used. 100uL of the extract was then used to prepare well concentrations ranging from 100 – 0.1 mg/mL and wells 11 and 12 serving as the positive control (Broth + organism only) and negative control (Broth with no organism only) respectively for each microorganism on the columns A- H. This process was likewise done for voriconazole and chloramphenicol at 30 ug/mL in a separate plate as standard drug controls against all the test bacteria and fungi respectively.

Next, 100uL of each of the 0.5 McFarland standardized, test organisms on each column were added after which the plates were subjected to incubation at 37^{o}C for 24/48 h for bacterial and fungal strains respectively.

MIC values were then evaluated by visual analysis by adding Tetrazolium dye after 30 minutes. Each experiment was done thrice with the MICs recorded.

2.5.b *Determination of Minimum Bactericidal (MBC) and Fungicidal Concentration (MFC)*

To verify if the extracts were able to kill the microbial cells (bacteri-/fungi-cidal effect) the plates were also evaluated for MBC and MFC.

Briefly, aliquots from each well from susceptibility testing assays were transferred to plates containing Nutrient agar, which were incubated at 37^{o}C for 24/48 h. Results were then

evaluated by analysing the presence or absence of growth in the Nutrient agar or SDA.

2.6 Free radical scavenging activity (ABTS, DPPH) Determination

A 300 µL of freshly prepared stock, 0.6 mM of 1,1-diphenylpicrylhydrazyl (DPPH) was dissolved in 100 ml of methanol as a working solution. A 150 µl of this solution was added to 50 µl of the test compound (T) with concentrations of 1, 0.5, 0.25, 0.125,0.0625, 0.0315, 0.0156, and 0.0078 mg/mL and then incubated for 30 minutes at room temperature. The DPPH radical scavenging activity of the compound was then determined by measurement of the absorbance at 517nm for test compound (T) expressed as a percentage of the absorbance of a control sample (C) of DPPH without the compound. Subsequently, the antioxidant potential against ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was also determined by adding 10 mL of ABTS and 10 mL of aqueous 2.4 mM potassium persulphate to generate the ABTS free radical to form the stock solution. This stock was further diluted in 50 mL of methanol as a working solution. A 150 µL of the solution was then added to 50 µL of the prepared compound concentrations, vortexed, and incubated at 30ºC for 10 min. The absorbance was then recorded at 734 nm. The free radical scavenging activity for the compound with Ascorbic acid as a referenced positive control against DPPH and ABTS was therefore evaluated by inputting data into the relation: $\%Scavenging\ activity = \left[\frac{C-T}{C}\right] \times 100^{\%}$ [16].

A graph of concentration against the mean for the percentage inhibition scavenging activity was plotted using Microsoft Excel and the IC_{50} for each of the samples was determined by using the equation of the line.

3. RESULTS AND DISCUSSIONS

3.1 TLC Analysis.

Different spots were produced indicating that the extract contained a mixture of compounds.

3.2 GC-MS Analysis

The analysis revealed that many compounds were present in the extract. The names of the compounds are presented in table 1, with their retention time, molecular formulae as well as their molecular masses.

3.3 Structures and Utilization of Compounds

A total of 23 different compounds were identified in this sample. Many of these compounds interesting structure and bioactivities according to information on their identification from other biological sources. Below are the structures of some of the interesting compounds identified from the extract.

3.4 Minimum Inhibitory Concentrations and Bactericidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC) of CPH Extracts.

MBC/MFCs are denoted as MFCs and were defined as the *lowest* drug dilutions that yielded <3 colonies (approximately 99 to 99.5% killing activity) [34]. Also, lower MIC and MBC or MFC values indicate higher efficacy of drugs [35]. The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial ingredient or agent that is bacteriostatic (prevents the visible growth of bacteria) [36]. MICs are used to evaluate the antimicrobial efficacy of various compounds by measuring the effect of decreasing concentrations of antibiotic/antiseptic over a defined period in terms of inhibition of microbial population growth [37].

Table 3.Table1 Results of MIC and MBC/MFC of extracts (mg/mL).

Organism	MIC	MBC	MIC/MBC
EC	25.0	>50.0	N/A
KP	25.0	>50.0	N/A
MRSA	25.0	50.0	2.0 [bc]
PA	25.0	>50.0	NA
ST	25.0	>50.0	N/A
SA	25.0	>50.0	N/A
SM	25.0	>50.0	N/A
CA	50.0	>50.0	N/A

EC- *E. coli* KP- *Klebsillia* pneumonia, MRSA- Methicilin resistant *Staphyloccocus aureus*, PA- *Pseudomonas aeruginosa*, ST- *Samonella typhi*, SA- *Staphylococcus aureus*, SM- *Streptococcus mutans*, CA- *Candida albicans*. bs - bactericidal, bs – bacteriostatic NA- Not applicable

The tested organisms (EC, KP, MRSA, PA, ST, SA, SM, CA) have a MIC of 25.0 mg/mL, except *Candida albicans*, which have a higher MIC at 50.0 mg/mL. This indicates that the extract is equally effective at inhibiting the growth of most bacteria at 25 mg/mL, while it takes a higher concentration (50 mg/mL) to inhibit the fungal organism *C. albicans*. In most bacterial organisms tested, the MIC was found to be 25 mg/mL. This indicates that the extract has a consistent ability to inhibit the growth of both Gram-negative and Gram-positive bacteria at this concentration. The exception to this is *Candida albicans* (CA), where the MIC was observed to be 50 mg/mL. Gram-negative bacteria like *E. coli* and *P. aeruginosa* are known to have an outer membrane that provides an additional barrier to antimicrobial agents [38]. The MIC of 25 mg/mL for these organisms suggests that the extract can penetrate this barrier effectively enough to inhibit bacterial growth. The extract exhibited the same inhibitory effect against Gram-positive bacteria, *S. aureus* and MRSA. Given that Gram-positive bacteria have a simpler cell wall structure without an outer membrane, it is with no doubt that the MIC remains consistent across both Gram-negative and Gram-positive bacteria.

Fungal Activity with MIC of 50 mg/mL for *C. albicans* is consistent with reports that fungal cells often require higher concentrations of antimicrobial agents due to their complex cell wall structure [39]. The MBC values for most organisms were >50 mg/mL, indicating that the extract is bacteriostatic at the tested MIC values but may not be bactericidal until higher concentrations are reached. MRSA was the only organism for which the extract exhibited bactericidal activity at 50 mg/mL, with a MIC/MBC ratio of 2.0, which suggests that the extract is capable of both inhibiting and killing this organism at relatively close concentrations. This is significant, as MRSA is a resistant pathogen that poses challenges in clinical treatment [39]. The extract's potential to kill MRSA at moderate concentrations may make it a useful candidate for further investigation as an alternative treatment for resistant infections. For other bacteria and fungi, the extract's MBC exceeded 50 mg/mL, indicating that while the extract can inhibit growth at 25 mg/mL or 50 mg/mL, higher concentrations would be needed to achieve a killing effect. This aligns with findings from other studies that suggest some natural plant extracts possess inhibitory properties but require higher concentrations to be lethal [40].

In this study, for most organisms, the MIC/MBC ratio is not applicable (N/A) due to the MBC values being greater than 50 mg/mL. This suggests that the extract functions primarily as a bacteriostatic agent rather than bactericidal for

these organisms, except in the case of MRSA, where the ratio of 2.0 indicates bactericidal action.

Antioxidant activity can be determined using ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-ssulfonic acid) and or DPPH (1,1-diphenylpicrylhydrazyl) assay. DPPH is a stable free radical compound and has an absorbance in its oxidized form around 515-520 nm [41]. DPPH assay is a relatively quick and efficient method to evaluate free radical scavenging activity. DPPH can accept an electron or hydrogen radical to form a stable diamagnetic molecule and indicate a change in colour, from purple to yellow indicating a decrease in absorbance at 517nm. This demonstrates that the antioxidant found in a mixture solution interacts with the free radicals.

The ABTS assay is a colourimetric assay based on the ABTS cation radical formation. The ABTS cation radical exhibits a change of colour from slightly yellow to an intensely turquoise-coloured solution with an absorbance at 405 nm [41]. Employing this assay, we can determine the number of active signal-generating HRP labels present on the MB bioconjugates [41]. In this study, the percentage of inhibition was measured to determine the antioxidant activity of the CPH extracts which can inhibit free radicals at different concentrations.

From the results in Table 4, The IC_{50} value for the ABTS assay was 0.36475 mg/mL, which is of higher potency than that of the cocoa bean husks of 17.32–44.56 mg/mL [42]. This indicates the concentration at which 50% of the ABTS radicals were scavenged. The ABTS scavenging activity was highest at the highest concentration (1 mg/mL), with an activity of 96.26%. Meanwhile, as the concentration decreases, the scavenging activity also declines. The mean scavenging activity ranged from 96.26% at 1 mg/mL to 0.70% at 0.0078 mg/mL, showing a significant drop in the activity as the concentration decreased. The IC_{50} value for the DPPH assay was 0.038629 mg/mL, which is comparable to the potency of the IC_{50} of 27.2 μg/mL reported in the literature [43]. This suggests a much lower concentration is needed to achieve 50% inhibition than ABTS, indicating stronger scavenging activity in the DPPH assay. The DPPH scavenging activity is highest at 1 mg/mL, showing 100% inhibition. Like ABTS, activity decreases as the concentration lowers, with the lowest activity being 30.13% at 0.0078 mg/mL.

It is clear from the observations above that, there was a higher potency in the DPPH assay: The DPPH assay shows greater free radical scavenging activity across all concentrations, as indicated by the lower IC_{50} value (0.038629 mg/mL), compared to ABTS (0.36475 mg/mL). In both assays, the scavenging activity decreased with decreasing concentration, demonstrating a dose-dependent response [44]. The variability between replicates (EXP1 and EXP2) was generally low for most concentrations, especially in the DPPH assay, indicating consistent experimental results. However, in the ABTS assay, the standard deviation increased at lower concentrations, reflecting greater variability. The sample (SM) demonstrates strong free radical scavenging activity, particularly in the DPPH assay, which suggests that it may be more effective at neutralising DPPH radicals than ABTS radicals. The concentration-dependent decline in scavenging activity is typical of antioxidant assays [44], and the lower IC50 value in the DPPH assay implies stronger antioxidant potential.

4.0 Conclusion

The GC-MS analysis indicates that the mesocarp of cocoa pod husk is a rich source of many bioactive phytochemical compounds that can be extracted and utilised to improve life. The impressive free radical activity of the extract against ABTS and DPPH especially with an IC_{50} of 0.038629 against DPPH leaves much to be desired of cocoa pod husk as a potential source of active ingredients for cancer and other free radical complications combating humanity.

Table1. List of Compounds identified with masses and molecular formular of SM

S/N	Name of Compound	Retention time (t_R)	Molecular Formular	M/z
1	2-Pentanone, 4-hydroxy-4-methyl-	7.7	$C_{12}H_{20}O$	180.2
2	Bicyclo[4.1.0]heptane-7-methanol, 1,5,5-trimethyl-2-Methylene-, (1.alpha.,6.alpha.,7.alpha.)-	3.1	$C_6H_{12}O$	100.1
3	Phenol, 2-methoxy-4-(2-propenyl)-	8.1	$C_{10}H_{12}O_2$	164.1
4	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	8.9	$C_{15}H_{26}O$	222.2
5	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-	9.1	$C_{10}H_{10}O_3$	178.1
6	Tetradecanoic acid, methyl ester	10.3	$C_{15}H_{30}O_2$	242.2
7	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	10.6	$C_{15}H_{18}$	198.1
8	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	10.8	$C_9H_{10}O_3$	166.1
9	3-Eicosene, (e)-	11.0	$C_{20}H_{40}$	280.3
10	1-Methyl-4-(2-methyl-2-oxiranyl)-7-oxabicyclo[4.1.0]heptane	11.2		
11	9-Octadecenoic acid (z)-, methyl ester	12.3	$C_{19}H_{36}O_2$	296.3
12	Hexadecanoic acid, methyl ester	12.6	$C_{17}H_{34}O_2$	270.3
13	Palmitoleic acid	13.1	$C_{16}H_{30}O_2$	254.2
14	n-Hexadecanoic acid	13.3	$C_{16}H_{32}O_2$	256.2
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	14.8	$C_{19}H_{34}O_2$	293.3
16	Methyl stearate	15.1	$C_{19}H_{38}O_2$	298.3
17	9,12-Octadecadienoic acid (Z,Z)-	15.6	$C_{18}H_{32}O_2$	280.2
18	14-Pentadecynoic acid, methyl ester	17.8	$C_{16}H_{28}O_2$	252.3
19	Squalene	24.1	$C_{30}H_{50}$	410.4
20	Benzene, 1,2,3-trimethoxy-5-(1-propenyl)-, (e)-	24.9	$C_{11}H_{14}O_3$	194.1
21	Hexacosanoic acid, methyl ester	25.3	$C_{27}H_{54}O_2$	410.4
22	2-Tert-butyl-5-methylbenzoquinone	27.3	$C_{11}H_{14}O_2$	178.1
23	Vitamin E	27.8	$C_{29}H_{50}O_2$	430.4

Table 2. Compounds and their utilization

S/N	Name	Utilization	Reference
1	2-Pentanone, 4-hydroxy-4-methyl-	Antimicrobial	17
2	Bicyclo[4.1.0]heptane-7-methanol, 1,5,5-trimethyl-2-Methylene-, (1.alpha.,6.alpha.,7.alpha.)-	---	
3	Phenol, 2-methoxy-4-(2-propenyl)-	Antioxidant/ protection against DNA damage	18
4	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	Antimicrobial/ antioxidant	19
5	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-	…..	
6	1H-2-Benzopyran-1-one, 3,4-	Flavouring agent	20
7	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	Antioxidant/ autoinflammatory/phytotoxic	21
8	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	Antimicrobial/ antioxidant	22
9	3-Eicosene, (e)-	Pesticidal/insecticidal	23
10	1-Methyl-4-(2-methyl-2-oxiranyl)-7-oxabicyclo[4.1.0]heptane	…….	
11	9-Octadecenoic acid (z)-, methyl ester	Antimicrobial	24
12	Hexadecanoic acid, methyl ester	Antimicrodial/ active against *plasmodium berghei*	25
13	Palmitoleic acid	Ameliorates insulin resistance diabetes	26
14	n-Hexadecanoic acid	….	
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Antimicrobial/ anticancer/antioxidant	27
16	Methyl stearate	Nematodal	28
17	9,12-Octadecadienoic acid (Z,Z)-	……	
18	14-Pentadecynoic acid, methyl ester	Antioxidant/ antifungal/antioxidant/anticancer	29
19	Squalene	Antiaging/anti-inflammatory/ wound healing	30
20	Benzene, 1,2,3-trimethoxy-5-(1-propenyl)-, (e)-	Antimicrobial	31
21	Hexacosanoic acid, methyl ester	Antinflamatory/antibrotic	32
22	2-Tert-butyl-5-methylbenzoquinone	…..	
23.	Vitamin E	Antioxidant	33

Table 4.Free radical Scavenging Activity of SM

CONC.	ABTS activity [IC_{50} = 0.36475±0.00 mg/mL]			DPPH activity [IC_{50} = 0.038629±0.00 mg/mL]		
	EXP1	EXP2	MEAN±SD	EXP1	EXP2	MEAN±SD
1	96.26	96.26	96.26±0.00	100.00	100.00	100.00±0.00
0.5	92.06	90.65	91.36±0.99	98.07	98.51	98.29±0.32
0.25	71.03	61.21	66.12±6.94	82.44	83.93	83.18±1.05
0.125	25.70	34.58	30.14±6.28	64.14	67.11	65.63±2.10
0.0625	15.89	5.61	10.75±7.27	53.27	55.06	54.17±1.26
0.0313	10.28	2.34	6.31±5.62	44.05	47.17	45.61±2.21
0.0156	0.93	1.87	1.40±0.66	34.38	37.05	35.71±1.89
0.0078	0.47	0.93	0.70±0.66	30.06	30.21	30.13±0.11

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