PHYTOCHEMICAL SCREENING, AND EVALUATION OF ANTIMICROBIAL POTENTIAL OF *COFFEA ARABICA* L. BEANS AND WHOLE CHERRIES EXTRACTS OF KHOLANI CULTIVAR FROM SOUTHWEST OF SAUDI ARABIA

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(Received 13th Sep 2023; accepted 16th Nov 2023)

Abstract. Kholani Coffee is a high-quality *Coffea arabica* cultivar in southwest Saudi Arabia. The objective of our research was to determine the phytochemical profile of the Kholani cultivar using LC-MS/MS analysis of whole coffee cherries (WCC) and coffee green beans (CGB) aqueous extract and GC–MS analysis of (CGB) n-hexane extract. Additionally, well diffusion method was used to assess the antimicrobial efficacy of the extracts against six pathogenic microorganisms. LC-MS/MS analysis of water extracts produced 34 phytochemical compounds, of which 16 were identified based on previous studies; 18 were identified from *Coffea arabica* extracts for the first time; these compounds may be responsible for the distinctive characteristics of the Kholani cultivar. Including four compounds with high-quality separation (100%) and the following molecular formulas: $C_{21}H_9N_3O_2S$, $C_{22}H_{45}NS_4$, $C_{23}H_{28}N_{10}O_6$, and $C_{27}H_{22}N_{10}O_{10}$. Ten phytochemicals were produced by GC-MS analysis of n-hexane extract, with varied intensities and retention times. The most significant compounds were 2-ethyl nitrobenzene and prenyl vinyl acetylene with the highest intensity. Our findings showed that all examined bacterial strains were sensitive to coffee extracts. Where (WCC) water extract was shown to have the largest inhibition zone against *Proteus mirabilis.* Neither *Penicillium digitatum* nor *Aspergillus niger* was sensitive to the (CGB) water extract or n-hexane. Our investigations showed that the Kholani cultivar of coffee cherries and green beans is a good source of several phytonutrients.
Keywords: *coffee green beans, n-hexane extract, water extract, LC-MS/MS, GC-MS*

Introduction

More than 50 tropical and subtropical nations worldwide produce coffee cherries, which are one of the most important agricultural products in the world, Brazil is the biggest producer (Legesse, 2020). Coffee cherries have historically been valued as rich source of nutrients, stimulants, and health-improving characteristics for people (Little et al., 1966; La Vecchia and Tavani, 2007; Nemzer et al., 2021). Due to cultural and traditional variations, coffee is consumed both roasted and unroasted as brewed coffee, drinks, and supplements. It is generally traded as green coffee beans, made either by sun-drying techniques or wet processing (Tounekti et al., 2018).

Arabian coffee originates from Ethiopia (Africa) and grows widely in the southern part of the Arabian Peninsula (Yemen and southwestern Saudi Arabia). It is primarily farmed in the KSA's Asser (Hada Mountain region) at an altitude of about 1200-1800 meters and in Jazan provinces (Al-Dayer Bani Malek) (Pohlan and Janssens, 2010). The high-quality coffee from these regions, known as Kholani coffee, is well-known around the world.

Despite coming from a genetically limited population source, the allotetraploid nature of the Arabica coffee genome is thought to be a trait that enables it to adapt impressively through a large variety of environmental variations of intertropical zones (Chen, 2010).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2201_811831

This adaptation directly affects the phytochemical composition of the plant (El-Shaboury et al., 2017). Saudi Arabia produces some of the best coffee in the world (Lewin, 2004), as coffee is grown under mostly organic traditions without pesticides, herbicides, and artificial fertilizers (Al-Turki, 2002).

Nemzer et al. (2021) The principal component of commercial compounds sold as Coffeeberry® and "Neurofactor™" by VDF FutureCeuticals, Inc. is whole coffee cherries powder. They are made as Food Supplements utilizing a patented multi-step 70% ethanol/water extraction and purification procedure, followed by spray-drying. Previous studies have suggested that coffee cherries extract high in chlorogenic acid has significant antioxidant potential. Natural coffee cherry extracts may be able to replace developed antidiabetic and anti-Alzheimer medication molecules due to the high concentration of chlorogenic acid and caffeine they contain (Reyes et al., 2013; Robinson et al., 2021).

According to the diameter of the inhibitory zone, the diffusion method, a qualitative test, allows the classification of bacteria as either susceptible to or resistant to the tested plant extract (Palombo and Semple, 2001; Uzun et al., 2004; Cos et al., 2006; Ncube et al., 2008). Using phytochemicals from plant extracts, which are recognized as having antibacterial characteristics, can be extremely important in therapeutic treatment. However, coffee also possesses antimicrobial properties that work against a number of pathogens, including fungi and both gram-positive and gram-negative bacteria, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella choleraesius*, and *Escherichia coli* (Martínez-Tomé et al., 2011). Arabica coffee extracts were shown to have the highest antibacterial activity, compared to the other two species, in a recent study by Nassar et al. (2019) that looked at the antibacterial characteristics of Arabica, Turkish, and Brazilian coffee.

We now have a far better understanding of the physiological advantages of coffee and its many products thanks to an extensive global study. However, the total number of phytonutrients observed in coffee that have been published in earlier studies does not include all classes and types of compounds and their metabolites. According to Mullen et al. (2011, 2013), and Lang et al. (2013) contrary to earlier studies, coffee's chemical composition is significantly more complex and diverse. It needs the use of high throughput technologies and analytical capabilities to unravel this complexity.

So, the purpose of our study is to use LC-MS-MS and GC-MS analysis to determine the phytochemical ingredients of the *Coffea arabica* Kholani cultivar water extract of whole cherries and green beans as well as for the n-hexane *Coffea arabica* green beans extract. We also want to determine the antimicrobial effect of these extracts against six different microorganisms., three bacteria (*Proteus mirabilis*, *Staphylococcus aureus,* and *Shigella flexneri*) and three fungi (*Rhizopus stolonifer*, *Aspergillus niger*, and *Penicillium digitatum*) using the diffusion method. Chem Spider, PubChem, mz-Cloud spectral library, and previous literature were among the databases searched in this investigation to find compounds that could be identified by LC-MS-MS analysis (Nemzer et al., 2021). Also, to identify chemicals produced by GC-MS, the NIST library was used. (Joulain and Koenig, 1998).

Materials and methods

Plant material

Coffee cherries from the *Coffea arabica* Kholani cultivar were gathered and collected in December 2022 from El-faraa, Aseer province Abha city trusted coffee garden in a

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2201_811831

commercial harvesting stage (80% red of the overall skin color). After harvest, the coffee cherries were transferred to the processing facility where they were washed by floating in tap water before being wet processed. The samples were collected, dried in the dark, and powdered. Green coffee beans were obtained by wet processing and then the seed coat was removed. *Figure 1* shows a photograph of the studied *Coffea arabica* Kholani cultivar. The collected plant samples were identified according to the herbarium of the King Khalid University KSA College of Science.

Figure 1. *Photograph of the studied Coffea arabica Kholani cultivar*

Sample preparation

Water and n-hexane extract of the dried samples of *Coffea arabica* Kholani cultivar (whole cherries and raw beans) were prepared as follows: 25.0 g of the powdered samples were extracted with 250 mL of the solvent (water- n-hexane) using the Soxhlet method where the process was performed at temperature 80^{O}C for 180 minutes. After the extraction, the resulting solution was filtered through filter paper (Whatman no.1) Rotary evaporator was used to evaporate the solvent (Marlina et al., 2017). Then the resulting precipitate was weighed for each coffee extract and standardized according to Heinrich et al. (2022).

LC-ESI-TOF–MS analysis

Phytochemical chemical components in the crude water extract of the *Coffea arabica* Kholani cultivar (whole cherries and green beans) were identified using liquid chromatography-mass spectrometry (LC-MS-MS). Chromatographic analysis was carried out by reverse phase elution (Waters Symmetry LC18 column 250 × 4.6 mm,

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5 μm) on Agilent 6500 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF; Agilent Santa Clara, CA, USA) LC/MS system with Agilent 1200 Series Diode Array Detector (module G1315B; detection type: 1024-element photodiode array; light source: deuterium and tungsten lamps; wavelength range 190-950 nm). The solvents used in the mobile phase were (A) formic acid (0.1%, v/v); (B) acetonitrile + 0.1% formic acid; and (C) a gradient of (i) 20% over the first 20 minutes, (ii) 95% over the next 20 minutes, and (iii) 35% over the final 27 to 30 minutes of the run; Agilent Technologies' Mass-Hunter software was used to analyze the mass using the following parameters: flow rate: 0.2 ml/min; injection volume: 3 L; ESI parameters: both negative and positive ion mode; mass range: 100-1200 m/z; spray voltage: 4 kV; gas temperature: 325 °C; gas flow: 10 L/min; nebulizer pressure: 40 psi (Shaker et al., 2022).

GC–MS analysis

GC–MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). An electron ionization system with ionization energy of 70 eV and helium gas as the carrier gas were utilized in GC-MS detection. The flow rate of the helium gas was maintained at 1 mL/min. A temperature of 280 °C was set for the MS transfer line and injector. The oven temperature was programmed at an initial temperature of 50 °C (hold 2 min) to 150 °C at an increasing rate of 7 °C/min then to 270 at an increasing rate of 5 °C/ min (hold 2 min) then to 310 as a final temperature at an increasing rate of 3.5 °C/min (hold 10 min). The quantification of all the identified components was investigated using a percent relative peak area. Tentative identification of the compounds was performed based on the comparison. of their relative retention time and mass spectra with those of the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA), WILEY library data of the GC–MS system (Joulain and Koenig, 1998; El-Shaboury et al., 2021).

Antimicrobial activity

The antimicrobial activity of *Coffea arabica* extracts against six pathogenic microorganisms was evaluated using the agar well diffusion method. The bacterial strains used for the study included both Gram-negative (*Proteus mirabilis*, and *Shigella flexneri*) and Gram-positive (*Staphylococcus aureus*) pathogenic strains of bacteria and three fungi (*Rhizopus stolonifer*, *Aspergillus niger*, and *Penicillium digitatum*). Bacteria and fungi strains were obtained from the American Type Culture Collection the matured culture of bacteria was uniformly seeded over the surface of the medium (20 mL) in the Petri plates for 48 h for further use. For the bacterial culture, media of nutrient agar was used, and potato dextrose agar (PDA) media for the fungal culture (Dudeja et al., 2022). Gel puncture was used to create three 6 mm wells in each plate, and 100 uL of each extract solution was added to each well. Dimethyl sulfoxide (DMS) 10% was used as a negative control and 100 uL of Cefoxitin as a positive control (Heinrich et al., 2022).

The plates containing bacteria were incubated at (37°C) for 48 h and 72 h at 28 °C for fungal strains. The antimicrobial agents present in the extract were diffused out into the medium and interacted with the microorganism. The diameter of the inhibition zone that formed around each well (measured in mm) was used to determine the antibacterial activity (Ncube et al., 2008).

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Statistical analysis

The biological experiments were independently performed in triplicates and are represented as the mean ± standard deviation (SD). The data were subjected to aone-way analysis of variance (ANOVA) Statistica 7.1 was applied to process all the statistical analyses (Statsoft, 2007).

Results

Qualitative determination of compounds in the water extract of Coffea arabica green beans and whole cherries using LC–MS–MS analysis

The water extracts of *Coffea arabica* whole cherries and Coffee green beans were analyzed using LC-MS-MS technology, which generated 34 phytochemical compounds, of which 16 were identified based on previous literature and mentioned in *Table 1*, the other 18 phytochemical compounds were recorded in our study from *Coffea arabica* extracts of which five common compounds in (WCC and CGB) water extracts, four compounds related to (CGB) extract, and nine compounds related to (WCC) extract at different retention times as mentioned in *Table 2* and illustrated in *Figure 2*. *Table 1* shows that the water extract from *Coffea arabica* (WCC and CGB) contains many compounds including amino acids, phenolic acids, terpenes, and alkaloids. The 1,3,7,9-theacrine and methyl libertine alkaloid compounds were found to be related to (WCC) water extract; they both had the same chemical formula ($C_9H_{12}N_4O_3$) and were detected at a retention time of 5.91 minutes and a molecular ion peak at m/z 225.098. The phenolic acid component with the chemical formula $C_{16}H_{18}O_9$, which was found in both (CCW and CGB) water extract, may be chlorogenic acid (1-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, or 5-O-caffeoylquinic acid). The molecular formula C17H18O8 was identified as 4-Caffeoyl-1,5-quinide which was detected at a retention time of 6.16 min and a molecular ion peak at 351.1057 and was detected only in (CGB) water extract. Tricalysiolide A, Tricalysiolide B, Tricalysione A, and 4-carboxy atractyligenin are terpenes that were recorded in (WCC) water extract at different retention times, where Carnosol has the same molecular formula of Tricalysiolide A which it was $C_{20}H_{28}O_4$ and detected at 11.8 min as a retention time. De hydro cafestol and atractiloside II are terpenes, but they were recorded in (CGB) water extract only with molecular formula $C_{20}H_{26}O_2$ and $C_{25}H_{38}O_9$, respectively. The amino acid compound (phenylalanine) was recorded in (CGB) water extract with a molecular formula of $C_9H_{11}NO_2$ at a retention time of 4.30 min (*Table 1*).

Table 2 provides a list of 18 phytochemical compounds that have been identified in water extracts from (WCC and CGB) in our study and without any prior biological activities being noted in the previous literature along with their molecular weight, molecular formula, analyte peak name, quality, retention time, and suggested names. *Figure 2* shows the molecular structure of these compounds. The molecular formula for the compound with the largest molecular weight, 2,4-bis[4-(1,4-dioxa-7,13-dithia-10-azacyclopentadec-10-yl) phenyl] cyclobuta-1,3-diene-1,3-dio, is $C_{36}H_{50}N_2O_6S_4$ at retention time 7.72 min with the lowest quality 56%.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Table 1. *List of phytochemicals identified from WCC and GCB water extract using LC-MS-MS analysis based on previous literature. Where Rt (Retention time) – M/Z (Mass-to-charge ratio)*

Se	Molecular formula	Rt	M/Z	Suggested name	Type	Whole Cherries	Green Beans	Ref.
1	$C_9H_{12}N_4O_3$	5.91	225.0981	1,3,7,9-Theacrine	Alkaloids	+	-	Xue et al. (2019)
2				Methyl libertine	Alkaloids	+	-	Xue et al. (2019)
3	$C_{16}H_{18}O_9$	4.02	731.1795	Chlorogenic acid	Phenolic acid	+	+	Martins et al. (2014) Cheek et al. (2018)
4				3-O-Caffeoylquinic Acid	Phenolic acid	+	+	Martins et al. (2014)
5				4-O-Caffeoylquinic Acid	Phenolic acid	+	+	Martins et al. (2014)
6				5-O-Caffeoylquinic Acid	Phenolic acid	+	+	Martins et al. (2014)
7				1-O-Caffeoylquinic Acid	Phenolic acid	+	+	Martins et al. (2014)
8	$C_{20}H_{28}O_4$	11.80	355.1880	Tricalysiolide A	Terpenes	+	-	Wang et al. (2018) Cheek et al. (2018)
9				Carnosol	Diterpenes	+	-	Cheek et al. (2018)
10	$C_{20}H_{28}O_5$	10.68	371.1830	Tricalysiolide B	Terpenes	+	-	Wang et al. (2018) Cheek et al. (2018)
11	$C_{20}H_{26}O_2$	12.81	299.2008	Dehydrocafestol	Diterpenes	-	+	Janeiro (2017) Li et al. (2022)
12	$C_{18}H_{26}O_3$	12.04	313.1774	Tricalysione A	Terpenes	+	-	Wang et al. (2018)
13	$C_9H_{11}NO_2$	4.30	166.0861	Phenylalanine	Amino acid	-	+	Cheek et al. (2018)
14	$C_{20}H_{28}O_6$	10.00	497.1640	4-Carboxyatractyligenin	Diterpenes	+		Janeiro (2017) Li et al. (2022)
15	$C_{25}H_{38}O_9$	9.85	500.2855	Atractiloside II	Diterpenes	-	+	Janeiro (2017)
16	$C_{17}H_{18}O_8$	6.16	351.1057	4-Caffeoyl-1,5-quinide	Phenolic acid	-	+	de Paulis et al. (2004)

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Table 2. *List of the newly identified phytochemicals in Kholani coffee in our study from WCC and GCB water extract using LC-MS-MS analysis*

Se.	Mo. weight	Molecular formula	Analyte Peak Name	Quality	Rt	Suggested name	WCC	CGB
1	367.4	$C_{21}H_9N_3O_2S$	406.0051 / 1.56 M+K+	1.00	1.56	14,15-dioxa-8-thiapentacyclo [10.7.2.$0^{2,7}$.$0^{9,20}$.$0^{17,21}$] henicosa 1(19),2(7),3,5,9,11,17,20-octaene-4,10,19-tricarbonitrile	+	+
2	220.29	$C_{15}H_8S$	221.0418 / 2.17	0.96	2.17	16-thiapentacyclo [7.6.1.$0^{2,8}$.$0^{3,5}$.$0^{10,15}$] hexadeca-1,3(5),6,8,10,12,14-heptaene	+	-
3	451.9	$C_{22}H_{45}NS_4$	452.2508 / 5.78	1.00	5.78	4-dodecyl-1,7,10,13-tetrathia-4-azacyclopentadecane	-	+
4	561.8	$C_{34}H_{31}N_3OS_2$	562.1978 / 7.68	0.99	7.69	9-[5-[(4-phenyl-1,3-thiazol-2-yl) sulfanyl] pentyl]-N-(pyridin-2-ylmethyl) fluorene-9-carboxamide	-	+
5	400.41	$C_{11}H_{24}N_6O_8S$	423.1264 / 3.70 M+Na+	0.98	3.71	acetamide;2-[[[[3-(1-amino-2-hydroxyethyl)-1,2,4-oxadiazol-5-yl] methylamino]-dihydroxy-λ^4-sulfanyl] amino]-3-hydroxybutanoic acid	+	+
6	340.6	$C_{13}H_{28}N_2S$	341.1212 / 4.01	0.93	4	1,4,7,10-tetrathia-13,17-diaz cyclo nonadecane	+	-
7	576.9	$C_{35}H_{44}OS_3$	577.2616 / 5.18	0.98	5.17	3-(2-naphthalen-1-ylsulfanyl-5-octylthiophen-3-yl)-5-octyl thiophene-2-carbaldehyde	+	-
8	654.9	$C_{33}H_{50}O_9S_2$	655.2966 / 9.41 M+H+	0.97	9.40	[2-(2-dodecoxyphenyl) sulfonylperoxysulfonylphenyl] nonanoate	-	+
9	735.1	$C_{36}H_{50}N_2O_6S_4$	735.2625 / 7.34	0.52	7.36	2,4-bis[4-(1,4-dioxa-7,13-dithia-10-azacyclopentadec-10-yl) phenyl] cyclobuta-1,3-diene-1,3-dio	+	-
10	252.30	$C_6H_{16}N_6O_3S$	253.1074 / 7.63	0.79	7.49	diaminomethylideneazanium;4-oxo-4-sulfidobutanoate	+	-
11	540.5	$C_{23}H_{28}N_{10}O_6$	541.2263 / 7.71	1.00	7.72	(2S)-2-[[4-[(2,4-diaminopteridin-6-yl) methylamino] benzoyl] amino]-5-oxo-5-(2-propoxycarbonylhydrazinyl) pentanoic acid	+	+
12	683.1	$C_{40}H_{50}N_4S_3$	683.3276 / 11.59	0.74	11.58	7,8-didecyl-4,11-dithiophen-2-yl-[1,2,5] thiadiazolo[3,4-b]phenazine	-	+
13	698.7	$C_{31}H_{42}N_{10}O_9$	699.3210 / 9.28	0.74	9.28	4-amino-1-[(2R,4R,5R)-4-hydroxy-3,5-dimethyloxolan-2-yl] pyrimidin-2-one;4-amino-1-[(2R,4R,5R)-4-hydroxy-5-methyl-3-methylideneoxolan-2-yl] pyrimidin-2-one;2-(4-amino-2-oxopyrimidin-1-yl)-5-ethyl-4-hydroxyoxolane-3-carbonitrile	+	+

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DOI: http://dx.doi.org/10.15666/aeer/2201_811831

Se.	Mo. weight	Molecular formula	Analyte Peak Name	Quality	Rt	Suggested name	WCC	CGB
14	330.30	$C_6H_{22}N_{10}O_6$	331.1724 / 9.31	0.60	9.34	[(2R,3S,4S)-4,5-dihydrazinyloxy-2,5-bis(hydrazinyloxymethyl)oxolan-3-yl] oxyhydrazine	+	-
15	352.50	$C_{13}H_{32}N_6O_3S$	353.2330 / 10.06	0.77	10.09	N,N-dimethylmethanesulfonamide;methane;3-methylbutan-2-one;N,N,1-trimethyltetrazol-5-amine	+	-
16	595.8	$C_{38}H_{29}NO_2S_2$	596.1718 / 10.38 M+H+	0.99	10.26	2-[[5-[5-(4-tert-butyl-N-naphthalen-2-ylanilino) thiophen-2-yl] thiophen-2-yl] methylidene]indene-1,3-dione	+	+
17	646.5	$C_{27}H_{22}N_{10}O_{10}$	647.1587 / 10.30	1.00	10.26	1-(2,4-dinitroanilino)-3-[4-[[4-[(2,4-dinitroanilino) carbamoylamino]phenyl]methyl]phenyl]urea	+	-
18	386.6	$C_{15}H_{26}N_6S_3$	387.1445 / 11.06	0.86	11.03	1-[1,6-bis[methyl(methylidenecarbamothioyl)amino] hexan-3-yl]-1-methyl-3-methylidenethiourea	+	-

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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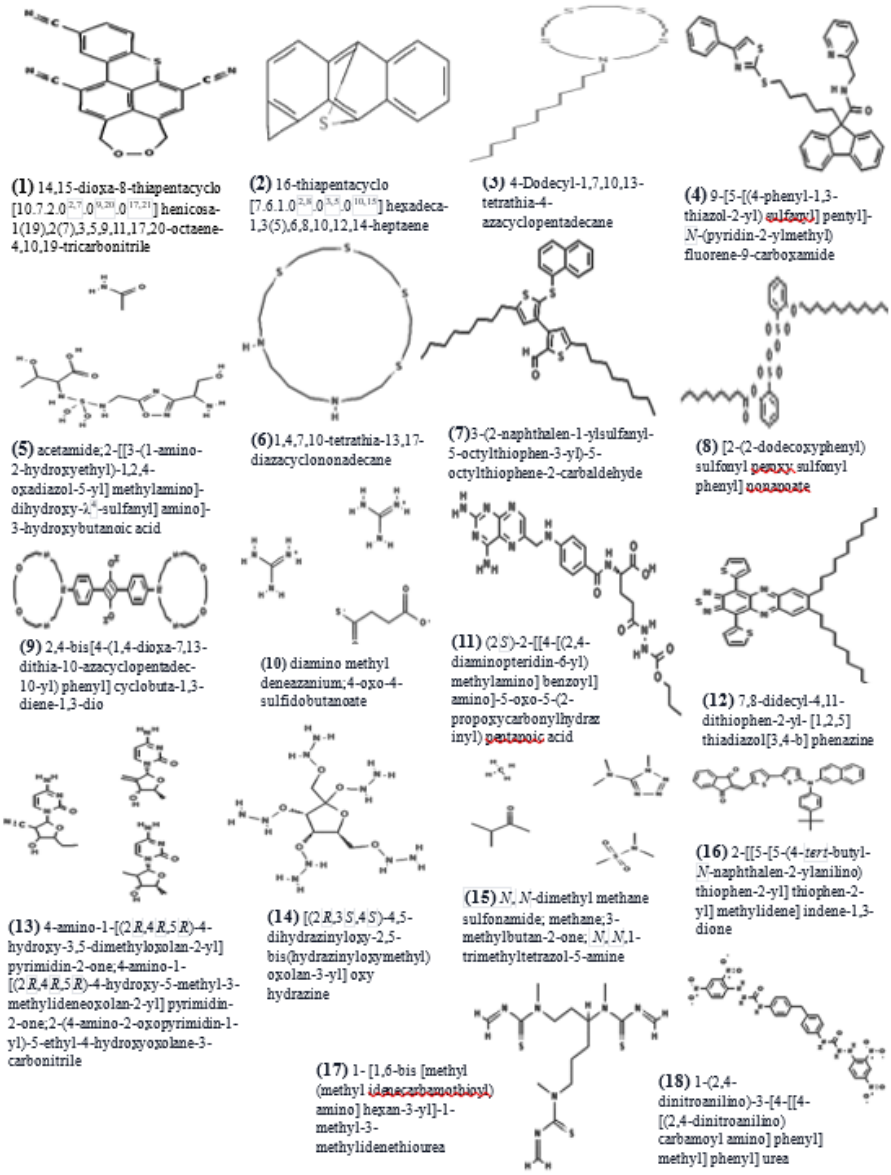

Figure 2. *The molecular structure of 18 phytochemical compounds new identified in Coffea arabica*

The compound with the lowest molecular weight, 220.29 g/mol, had a retention time of 2.17 min, a quality of 96%, and a recommended name of (16-thiapentacyclo [7.6.1.02,8.03,5.010,15] hexadeca-1,3(5),6,8,10,12,14-heptaene), its chemical formula is $C_{15}H_8S$. Four compounds were recorded with 100% quality of which two compounds were recorded in the two extracts with the molecular formula $C_{21}H_9N_3O_2S$- $C_{23}H_{28}N_{10}O_6$ at 1.56 and 7.72 retention time respectively, one compound recorded in (WCC) water extract with the molecular formula $C_{27}H_{22}N_{10}O_{10}$ at retention time 10.26 min, and one compound recorded in (CGB) water extract with the molecular formula $C_{22}H_{45}NS_4$ at retention time 5.78 min (*Table 2*). The mass spectrum of the four compounds that separated at high quality (100%) along their molecular structures is shown in *Figure 3*.

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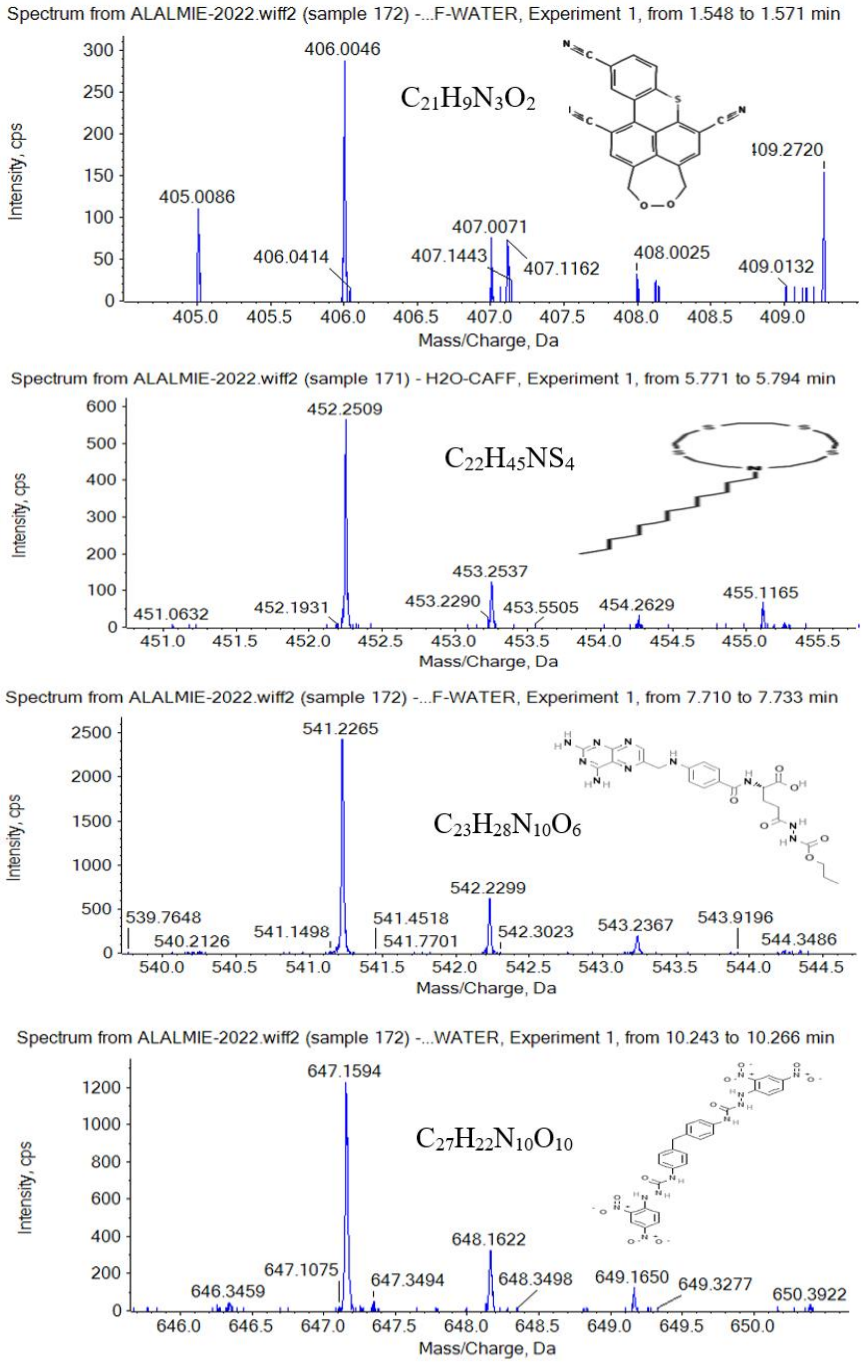

Figure 3. *The mass spectrum of the highest quality compounds (100%) among Kholani coffee green beans and whole cherries new reported 18 compounds*

GC-MS analysis of coffee green beans n-hexane extract

In our study, the phytochemicals of n-hexane extraction from *Coffea arabica* green beans were analyzed using the GC-MS analysis. The list of identified compounds along with molecular weight, compound formula, peak report area, and retention time are given in *Table 3*. *Figure 4* shows a representative GC-MS chromatogram of the n-hexane extract. GC-MS analysis showed the presence of 10 compounds in the n-hexane extract.

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DOI: http://dx.doi.org/10.15666/aeer/2201_811831

The highest molecular weight (268 g/mol) compound with the molecular formula $C_{19}H_{40}$ and decided name Nonadecane recorded at a retention time of 34.00 min with the lowest peak area. The lowest molecular weight (98 g/mol) compound with the molecular formula $C_6H_8D_2O$ and decided name 4,4-dideutero cyclohexa-2-en-1-ol was recorded at a retention time of 2.689 min. The highest peak area was (806998562) recorded with prenyl vinyl acetylene as decided name with a molecular weight of 120 g/mol and the molecular formula C_9H_{12} at a retention time of 5.137 min. 7-methyl-1,3,5-cyclo heptatriene was recorded at two different retention times 4.952 and 5.509 min with 106 g/mol molecular weight and C_8H_{10} molecular formula. Phytochemicals of the decided names 2,6,11-trimethyl dodecane and 2,6,10-trimethyl dodecane (Farnesan) were recorded at two different retention times 12.385 and 9.166 min with the same molecular weight of 212 g/mol and molecular formula $C_{15}H_{32}$. *Figure 5* shows MS fragmentation form of *Coffea arabica* green beans compounds with high intensity with their molecular structures.

Table 3. *the list of identified phytochemicals of (CGB) n-Hexane extraction using GC-MS analysis*

Peak no	Mol. Formula	Mol. weight	Decided Name	RT	Peak Report area
1	$C_6H_{14}O$	102	2-Ethyl-1- butanol	2.521	520640825
2	$C_6H_8D_2O$	98	4,4-Dideutero cyclo hexan -2-en-1-ol	2.689	229772517
3	C_8H_{16}	112	Ethyl cyclohexane	4.430	4564628
4	C_8H_{10}	106	7-Methyl-1,3,5-Cyclo heptatriene	4.952	599540031
5	$C_8H_9NO_2$	151	2-Ethyl nitrobenzene	5.001	297741985
6	C_9H_{12}	120	Prenyl vinyl acetylene	5.137	806998562
7	C_8H_{10}	106	7-Methyl-1,3,5-Cyclo heptatriene	5.509	219349150
8	C_9H_{12}	120	Benzene, propyl	6.562	8176154
9	$C_{15}H_{32}$	212	2,6,10-Trimethyl dodecane (Farnesan)	9.166	4994616
10	$C_{15}H_{32}$	212	2,6,11-Trimethyl dodecane	12.385	4242738
No	$C_{19}H_{40}$	268	Nonadecane	34.00	Low

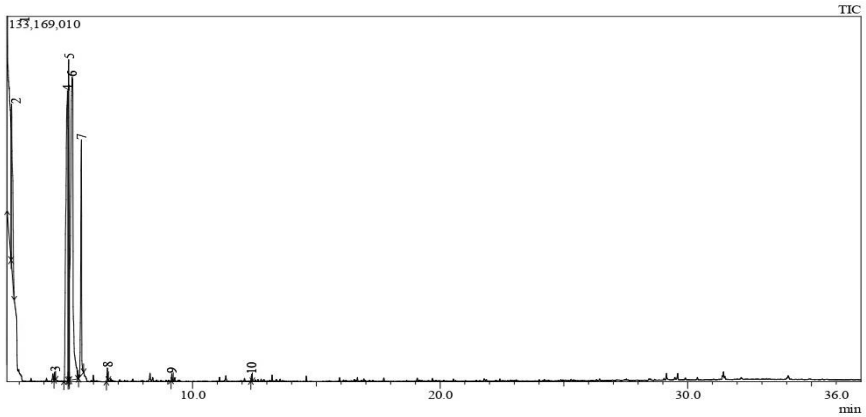

Figure 4. *Chromatogram of GC-MS analysis of n-hexane (CGB) extraction*

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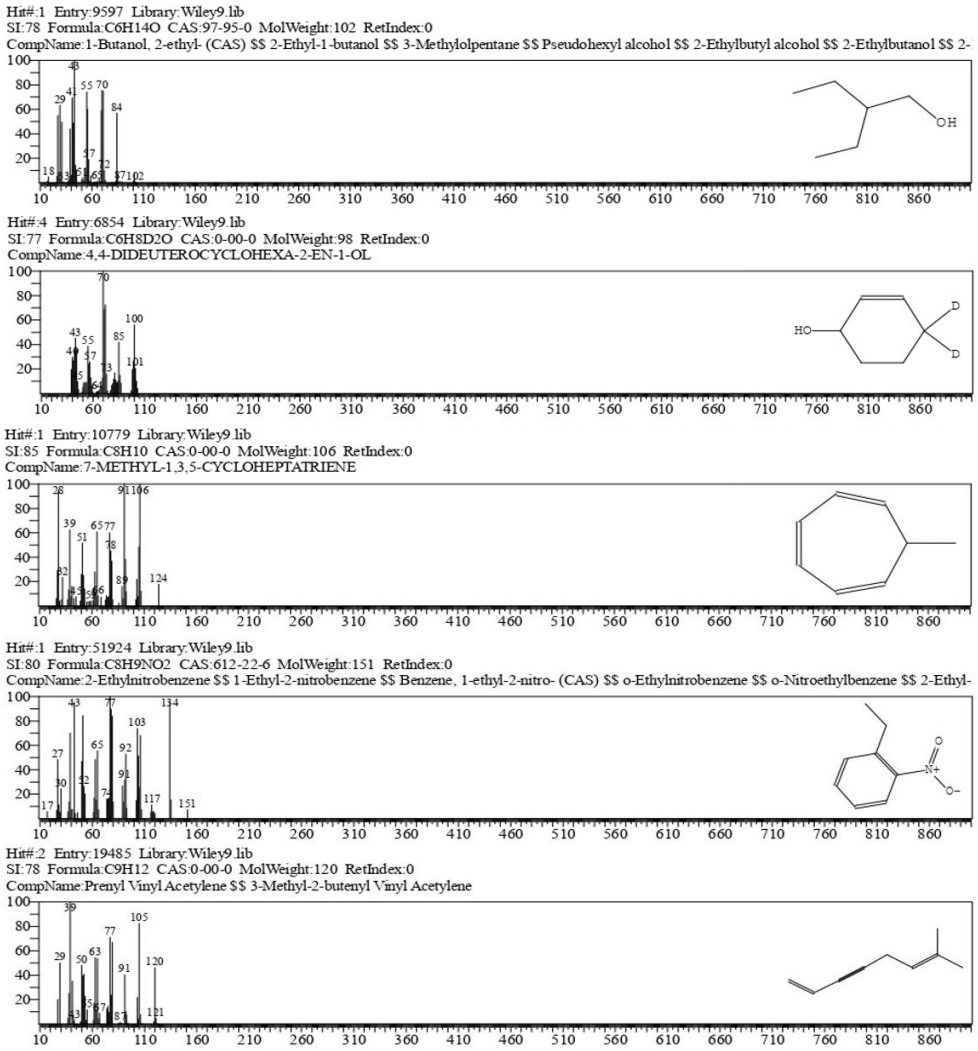

Figure 5. *Shows the MS fragmentation form of Coffea arabica green bean compounds with high intensity and their molecular structures*

Antimicrobial potential of (WCC and CGB) water extract and (CGB) n-hexane extract

Table 4 contains the results for the antimicrobial assessment of three extracts of *Coffea arabica* kholani cultivar (WCC and CGB) water extract and (CGB) n-hexane extract as inhibition zone mm. The negative control didn't show any antimicrobial activities, while the positive control showed a halo indicative zone in the range between (26.93±1.63 mm to 36.83±0.76 mm). In comparison to the antimicrobial activities of the positive control and the coffee extracts, the agar diffusion antibacterial test revealed that (WCC) water extract exhibited promising activity against *Proteus mirabilis* (Gram-negative) bacteria with the highest inhibition zone of 39.03±3.61 mm, compared with 36.83±0.76 mm inhibition zone generated by positive control, so (WCC) water extract considered strong antibacterial agent against *Proteus mirabilis*. In addition, the inhibition zone was 33.20±2.85 mm in the case of the other Gram-negative bacteria strain *Shigella flexneri,* it was 24.09±2.61 mm in the case of the Gram-positive bacteria *Staphylococcus aureus*.

Antibacterial test using (CGB) water extract is more effective on Gram-positive bacteria *Staphylococcus aureus* strain with a 36.34±1.52 mm inhibition zone diameter

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DOI: http://dx.doi.org/10.15666/aeer/2201_811831

and has a moderate effect on *Proteus mirabilis* with a 27.12±2.72 mm inhibition zone diameter. (CGB) n-hexane extract has a high antibacterial potential against all tested bacterial strains *Proteus mirabilis, Shigella flexneri,* and *Staphylococcus aureus* compared with the positive control with an inhibition zone of 36.00±2.64 mm, 36.23±1.66 mm, and 36.92±1.38 mm, respectively (*Table 4*).

Table 4. *Antimicrobial activities of (WCC), (GCB) water extract, and (CGB) n-hexane extract*

	Extract	Bacteria			Fungi		
		Proteus mirabilis	**Shigella flexneri**	**Staphylococcus aureus**	**Penicillium digitatum**	**Rhizopus stolonifer**	**Aspergillus niger**
Zone of inhibition (mm)	(WCC) water	39.03±3.61[a]	33.20±2.85[b]	24.09±2.61[c]	24.23±0.97[c]	33.34±2.48[b]	24.85±2.02[c]
	(CGB) water	27.12±2.72[a]	33.33±2.33[b]	36.34±1.52[b]	30.19±1.70[c]	36.00±2.37[b]	No
	(CGB) n-hexane	36.00±2.64[a]	36.23±1.66[a]	36.92±1.38[a]	No	42.01±2.64[b]	27.00±2.64[c]
	PC	36.83±0.76[a]	34.90±0.85[a]	35.52±0.50[a]	28.20±3.00[b]	35.90±0.78a	26.93±1.63[b]
	NC	NO	No	No	No	No	No
F.value		11.913	1.551	34.095	180.887	7.737	160.799
P < 0.05		0.003	0.275	0.000	0.000	0.009	0.000

NO, no inhibition activities; NC, Negative control for (DMSO) 10%, PC, Positive control (Cefoxitin). ± SD of the mean for n = 3. a-c Means in a row without a common superscript letter are significantly different (P < 0.05)

The antifungal trends obtained from (CGB) n-hexane extract had a maximum activity against *Rhizopus stolonifer* with a 41.01±3.64 mm inhibition zone diameter and has a moderate effect on *Aspergillus niger* with a 27.00±2.64 mm inhibition zone diameter. A high antifungal potential of (CGB) water extract on *Rhizopus stolonifer* generated a 36.00±2.37 mm inhibition zone compared with a 35.90±0.78 mm inhibition zone generated by positive control. *Penicillium digitatum* had a high susceptibility using (CGB) water extract with a 30.19±1.70 mm inhibition zone, while positive control generated a 28.20±3.00 mm inhibition zone. (CGB) water extract has no antifungal effect on *Aspergillus niger*. Antifungal test of (WCC) water extract showed a moderate effect against *Penicillium digitatum* and *Aspergillus niger* strains with 24.23±0.97 mm and 24.85±2.02 mm inhibition zone respectively, but in the case of *Rhizopus stolonifer* showed 33.34±2.48 mm inhibition zone diameter (*Table 4*).

Discussion

Phytochemical analysis of water extract of Coffea arabica green beans and whole cherries using LC–MS/MS

The analysis of (WCC and CGB) water extract using LC-MS-MS techniques generated 34 phytochemicals including 18 compounds identified for *Coffea arabica* whole cheery and green beans for the first time using Chem Spider, PubChem, mz-Cloud spectral library, from which 14 phytochemicals were generated from (WCC) water extract, 9 phytochemicals were generated from (CGB) water extract as they share 5 compounds (*Table 2*). The newly reported compounds' unique occurrence in the kholani cultivar compared to others may be the result of environmental adaptation. Our results agree with Mullen et al. (2011, 2013), and Lang et al. (2013) who revealed that the number of

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DOI: http://dx.doi.org/10.15666/aeer/2201_811831

phytonutrients reported in previous studies for coffee does not encompass all classes and types of chemicals and their metabolites. The chemical composition of coffee is far more complex and diverse than was previously reported. Unraveling this complexity requires the implementation of high throughput methods and analytical capabilities. Due to the highly rich biochemistry of plants, which includes many semi-polar molecules, including essential secondary metabolite groups, which can best be separated and detected by LC-MS techniques, LC-MS-based approaches are likely to be of special importance in plants (De Vos et al., 2007; Nemzer et al., 2021).

Our study revealed that the water extracts of (WCC and CGB) have a noticeable antimicrobial effect against examined bacterial and Fungai strains. (WCC) Water extract showed a broad spectrum of antimicrobial effects due to its recorded effect on all tested microbial strains which may be due to the diverse chemical contents of the extract and maybe because of the presence of the newly reported compounds which need future investigation for its biological activities (*Table 4*). We recorded in our study 4 phytochemicals out of the newly reported compounds with high abundance which have 100% quality according to the LC-MS/MS report. The mass spectrum of these compounds with their molecular structure and formula are shown in *Figure 3*.

Coffea arabica Kholani cultivar from South-west Saudi Arabia, characterized as A high-quality coffee, is well-known around the world (Tounekti et al., 2017) This may be due to the unique phytochemical composition which means the presence of many phytochemicals results in distinctive taste and aroma. Saud and Salamatullah (2021) revealed in their review of the analysis of *Coffea arabica* green beans using different methods and solvents that have 162 phytochemicals based on previous literature.

The diffusion method is a qualitative test that allows the classification of bacteria as susceptible or resistant to the tested plant extract according to the diameter of the inhibition zone (Palombo and Semple, 2001; Uzun et al., 2004; Cos et al., 2006; Ncube et al., 2008). In our study, the antibacterial sensitivity test using *Coffea arabica* (WCC and CGB) water extracts showed variable antibacterial activity according to the diffusion method. A highly antibacterial effect was recorded against *Proteus mirabilis* (Gram-negative bacteria) with (WCC) water extract although it has a moderate antibacterial effect against *Shigella flexneri* and a relatively low effect against *Staphylococcus aureus.* In contrast, (CGB) water extract exhibited a moderate antibacterial effect against Gram-negative bacteria (*Proteus mirabilis* and *Shigella flexneri*) and a relatively high effect against *Staphylococcus aureus* (*Table 4*).

These results may be due to the presence of antibacterial compounds which are more effective on Gram-negative bacteria (*Proteus mirabilis* and *Shigella flexneri*). While LC-MS analysis of (WCC) water extract revealed the presence of many oxygenated terpenes such as Tricalysiolide A, Carnosol, Tricalysiolide B, Tricalysione A, and 4-carboxyatractyligenin, but only two oxygenated terpenes recorded in (GCB) water extract, dehydrocafestol and Atractiloside II which they were identified based on the previous literature as mentioned in *Table 1*. These results agree with Guimarães et al. (2019) who evaluated the antibacterial potential of 33 terpenes. Their results revealed that the oxygenated terpenes showed strong antibacterial activity against all tested bacteria, especially Gram-negative bacteria. Also, our results are supported by Dessai et al. (2022). When they compared the antimicrobial potential of calcium hydroxide, triple antibiotic paste, and carnosic acid as intracanal medications against *Enterococcus faecalis*, their findings revealed that carnosic acid is an herbal derivative with potent antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Carnosic acid and carnosol are

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phenolic terpenes, they were previously mentioned by Wang et al. (2017) in their work. According to research in the pharmaceutical industry, phenolic terpenes have been employed as antioxidants to prevent illnesses (Zabot et al., 2014).

Our results clarify the presence of 1,3,7,9-theacrine as an alkaloid compound in (WCC) water extract, which is a family of methylxanthine, recently gained good attention due to its superior anti-inflammatory and analgesic activities (Zheng et al., 2002; Wang et al., 2010). Chlorogenic acid was recorded in both water extracts (WCC and CGB). These results agree with Nemzer et al. (2021) they clarify the high abundance of chlorogenic acid and caffeine in *Coffea arabica* whole cherries than in coffee bean extract, where chlorogenic acid had broad-spectrum antibacterial activities against many bacteria, such as *S. aureus* (Li et al., 2013), *Escherichia coli* (Li et al., 2006), *Pseudomonas aeruginosa* (Wang et al., 2019), *S. maltophilia* (Zhang et al., 2019), *Bacillus subtilis* (Wu et al., 2020), numerous pharmacological properties of chlorogenic acid include antibacterial, antioxidant, lipid-lowering, antiviral, anti-inflammatory, anti-cardiovascular, anticancer, and immunomodulatory activities (Miao and Xiang, 2020). So far, chlorogenic acid has been widely used in many fields such as medicine, food, health care, and the chemical industry.

The relatively high antibacterial effect of (CGB) water extract on *Staphylococcus aureus* (Gram-positive) bacteria may have resulted from the reported phenylalanine amino acid in the extract. This result agrees with Joondan et al. (2014) finding revealed that phenylalanine and L-Tyrosine were employed as antibacterial agents, and a variety of cationic surfactants generated from L-Phenylalanine and L-Tyrosine esters were produced and tested for antibacterial activity. The esters were more active against gram-positive than gram-negative bacteria. Our findings contradict those of Diaz-Hernández (2022), who found that aqueous and ethanol extracts of coffee green beans and roasted beans had no antibacterial action (MIC > 8 mgmL1) against the investigated bacterial strains in contrast to spent coffee grounds extracts.

Antifungal tests using C*offea arabica* (WCC and CGB) water extracts showed variable antifungal activity. Where (WCC) water extract has a broad-spectrum antifungal effect than (CGB) extract, where it has an effect against the three tested fungal strains by it has a relatively low effect on *Penicillium digitatum* and *Aspergillus niger* and a slightly high effect on *Rhizopus stolonifer*. But (CGB) extract has no effect on *Aspergillus niger.* These results may be due to the high terpenes and chlorogenic acid content of (WCC) water extract than (CGB) extract. Rao et al. (2010) showed that terpenoid phenols have strong antifungal properties against a variety of pathogens, such as *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

According to Sangta et al. (2021), antifungal activity against *Alternaria brassicicola*, *Pestalotiopsis* sp., and *Paramyrothecium breviseta* were tested using the extracted fraction from the coffee pulp. The outcomes demonstrated that the caffeic acid and epigallocatechin gallate in the polyphenol fraction actively inhibited pathogenic fungus. Because of the inclusion of coffee pulp, the high abundance of chlorogenic acid in (WCC) may boost its antifungal and antibacterial capabilities, whereas the high concentration of chlorogenic acid may influence *Aspergillus Niger* (Miao and Xiang, 2020). Ansari et al. (2013) and El-Khateeb et al. (2013) the capacity of plant phenolic compounds to inhibit pathogenic fungi is well established, among the potential routes of action are toxicity, cell apoptosis induction, hypha development inhibition, biofilm inhibition, disruption of cell membrane integrity, and others.

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GC-MS analysis of coffee green beans n-hexane extract

GC-MS analysis of *Coffea arabica* green beans n-hexane extract generates 10 phytochemical compounds at different retention times. The list of identified compounds along with other details is given in *Table 3*. *Figure 4* shows a representative GC-MS chromatogram of the n-hexane extract.

In our study the antimicrobial sensitivity test by agar diffusion method using (CGB) n-hexane extract showed variable antimicrobial activity. Where a highly antifungal effect was recorded against *Rhizopus stolonifer*, Although the extract has no effect on *Penicillium digitatum*. The extract has a moderate antifungal effect on *Aspergillus niger* Table 4. These results may be due to the presence of antifungal compounds which are more effective on *Rhizopus stolonifer Aspergillus niger* than *Penicillium digitatum*. Furthermore, these findings could be attributed to the outer layer of the polysaccharide (capsule) released by the organism, which functions as a barrier against any hazardous substances (Lamber, 2002).

The antibacterial sensitivity test of the extract showed the same high effect against the three tested bacterial strains, these results may be due to the presence of antibacterial phytochemicals in the n-hexane (CGB)extract. Our results revealed the presence of 2-ethyl-1- butanol which has a strong antifungal effect against some fungi [2,6,10-trimethyldodecane and, (Farnesan)] which has antifungal and antibacterial properties as previously recorded. Mao et al. (2015) studied the antifungal activity of volatile organic compounds such as 2-ethyl-1- butanol against some plant pathogens, the results showed strong inhibitory activity against the plant pathogens *Botrytis cinerea* and *Rhizoctonia solani* but showed weak inhibitory activity against *Fusarium oxysporum* and *Pythium ultimum*. Xu et al. (2009) evaluated the anti-fungal effects of Panax ginseng ether extracts using the GC-MS technique, they recorded 51 compounds of ginseng from them [2,6,10-trimethyldodecane (Farnesan)] showing that the extracts produced excellent antitumor and antifungal effects.

Also, GC-MS analysis revealed the presence of ethyl cyclohexane which acts as an antimicrobial agent as mentioned by Shoaib et al. (2019) who studied antimicrobial activities for cyclohexane derivatives against some bacterial and fungal strains. Test compound showed better antimicrobial properties against Gram-negative bacteria as compared to Gram-positive bacteria and fungi. Our results clearly the presence of 2-ethyl nitrobenzene, prenyl vinyl acetylene, and 2,6,11-trimethyl dodecane in n-hexane (CGB) extract which may have antibacterial properties (*Table 3*). Yadav et al. (2018) studied the antibacterial potential of nitrobenzene derivatives their results concluded that these compounds were more effective against *E. coli.* Benzene derivatives carrying nitro group substituents have a broad-spectrum antibiotic that is effective against a variety of susceptible and serious bacterial infections (Zhao and Hsu, 2014). Also, Araya-Cloutier et al. (2018) indicated that ring-prenylated compounds have more antibacterial activity against Gram-positive bacteria than chain-prenylated compounds and increase the hydrophobicity of the molecule potentially allowing easier diffusion across the bacterial membrane (Li et al., 2021). The endophytes' antibacterial ability was assessed against pathogenic bacteria that were multidrug resistant. Additionally, metabolite chemical diversity was calculated using LC-Q-TOF-MS/MS and GC-MS fingerprinting. The extract which contains multiple compounds from which 2,6,11-trimethyl dodecane exhibited significant antibacterial activity.

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DOI: http://dx.doi.org/10.15666/aeer/2201_811831

Conclusion

In the *Coffea arabica* whole cherries and green beans water extract, our research found the presence of 30 active phytochemicals. Using LC-MS-MS analysis, 18 of these phytochemicals were identified and demonstrated in our study from *Coffea arabica*, suggesting that they may be responsible for the biological activities and properties (such as odor, flavor, taste, and texture) of the kholani cultivar. The n-hexane extraction of *Coffea arabica* green beans also yielded 10 active phytochemicals, according to GC-MS analyses. The antimicrobial potential of water and n-hexane extracts were evaluated against 6 different pathogen which indicated that all examined bacterial strains were sensitive to the Coffea extracts. Where the highest inhibition zone 41.01±3.64mm was recorded when (CGB) n-hexane extraction used against *Rhizopus stolonifer*. Moreover, *Penicillium digitatum*, and *Aspergillus niger* were not sensitive to the n-hexane and water extract of (CGB) respectively. Whereas further studies are required, purification of newly identified active phytochemicals derived from *Coffea arabica* whole cherries and green beans, to assess their toxicity, antibacterial activity, and anticancer potential with a view to pharmaceutical usage.

Acknowledgments. The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University, Abha, Sudi Arabia, for funding this work through the General research Project under grant number R.G.P2/295/44.

Conflicts of Interest. The authors declare no competing financial interests.

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