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UHPLC-MS profiles and antidiarrheal activity of *Quercus coccinea* münchh. and *Quercus robur* L. employing *in vivo* technique

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Introduction: *Quercus* L. genus (Oak) belongs to the family Fagaceae and their galls are used commercially in leather tanning, dyeing, and ink preparation. Several *Quercus* species were traditionally used to manage wound healing, acute diarrhea, hemorrhoid, and inflammatory diseases. The present study aims to investigate the phenolic content of the 80% aqueous methanol extract (AME) of *Q. coccinea* and *Q. robur* leaves as well as to assess their anti-diarrheal activity.

Methods: Polyphenolic content of *Q. coccinea* and *Q. robur* AME were investigated using UHPLC/MS. The antidiarrheal potential of the obtained extracts was evaluated by conducting a castor oil-induced diarrhea *in-vivo* model.

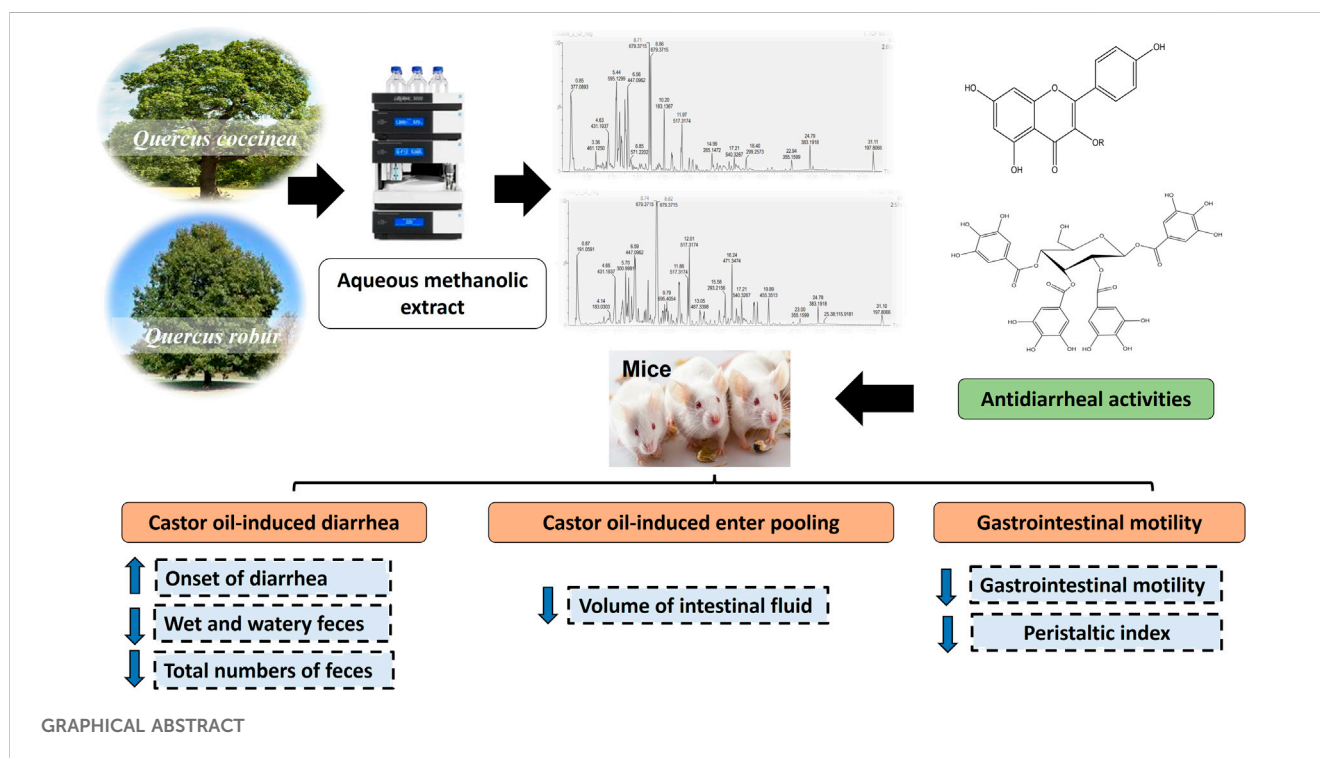
Result and Discussion: Twenty-five and twenty-six polyphenolic compounds were tentatively identified in *Q. coccinea* and *Q. robur* AME, respectively. The identified compounds are related to quercetin, kaempferol, isorhamnetin, and apigenin glycosides and their aglycones. In addition, hydrolyzable tannins, phenolic acid, phenyl propanoides derivatives, and cucurbitacin F were also identified in both species AME of *Q. coccinea* (250, 500, and 1000 mg/kg) exhibited a significant prolongation in the onset of diarrhea by 17.7 %, 42.6%,

Abbreviations: Polyphenols, They are a large classis of the plant secondary metabolites which possess number of hydroxyl groups on aromatic ring and they exerts a wide variety of biological activity; UHPLC-MS analysis, High-performance liquid chromatography coupled with mass spectroscopy technique is used to separate, identify and quantify different compounds present in a mixture. The median lethal dose: (LD₅₀), The dose of compounds or extract lethal for 50% of the experiential animals; *In vivo* study, It means that the research work is done with or within an entire, living organism; Gavage, Introduction of the drug into the stomach using oral tube; Peristaltic index, Percentage of the distance travelled by the charcoal meal relative to the total length of the small intestine; Diarrhea, Is the frequent passage of a watery movable stool; Anti-diarrheal drug, Are the drugs which control diarrhea and decrease the passage of stool through the intestine; Hydrolyzable tannins, Are a class of compounds containing glucose or polyol central core esterified with gallic acid (gallotannins) or with hexahydroxydiphenic acid (ellagitannins); Proanthocyanidins, They are oligomeric and polymeric products of the flavonoid biosynthetic pathway and their building units are catechin and epicatechin; Galls, Is a large, round, indefinitely apple-like, it is commonly found on many *Quercus* species. Galls are result due to chemicals injected by the larva of certain kinds of gall wasp in the family Cynipidae.

and 79.7% respectively while AME of *Q. robur* at the same doses significantly prolonged the onset of diarrhea by 38.6%, 77.3%, and 2.4 folds respectively as compared to the control. Moreover, the percentage of diarrheal inhibition of *Q. coccinea* was 23.8%, 28.57%, and 42.86% respectively, and for *Q. robur* 33.34%, 47.3%, and 57.14% respectively as compared to the control group. Both extracts significantly decreased the volume of intestinal fluid by 27%, 39.78%, and 50.1% for *Q. coccinea* respectively; and by 38.71%, 51.19%, and 60% for *Q. robur* respectively as compared to the control group. In addition, AME of *Q. coccinea* exhibited a peristaltic index of 53.48, 47.18, and 42.28 with significant inhibition of gastrointestinal transit by 18.98%, 28.53%, and 35.95 % respectively; while AME of *Q. robur* exhibited a peristaltic index of 47.71, 37, and 26.41 with significant inhibition of gastrointestinal transit by 27.72%, 43.89%, and 59.99% respectively as compared with the control group. Notably, *Q. robur* showed a better antidiarrheal effect in comparison with *Q. coccinea* and, the highest effect was observed for *Q. robur* at 1000 mg/kg as it was nonsignificant from the loperamide standard group in all measured parameters.

KEYWORDS

antidiarrheal, polyphenolic compounds, *Quercus coccinea*, *Quercus robur*, UHPLC-MS



1 Introduction

Diarrhea is commonly well-defined as an unusual passage of the unformed stools or liquid accompanied by increasing defecation rate, and abdominal discomfort (Guerrant et al., 2001). Diarrhea can be categorized into acute, persistent, and chronic based on the duration of the symptoms. Acute diarrhea lasts less than 2 weeks and is caused by a bacterial and viral infection or by some medications. It can be resolved quickly while the persistent one starts suddenly and lasts from two to four weeks. On the other hand, chronic diarrhea lasts more than four

weeks. Chronic and persistent diarrhea is commonly due to a functional disorder (Wingate et al., 2001; Thapar and Sanderson, 2004). Although diarrhea can be prevented and treated, it still represents one of the most serious health problems in the world, particularly in children under five years of age. It is a more challenging condition in developing countries. It is considered the second leading cause of mortality of death among children globally (Jaradat et al., 2016). The treatment of diarrhea usually focuses on reducing the pain associated with bowel movements. However, some of the available treatments are ineffective, unsafe, antagonize the effect of each other (Sinan et al., 2020), and/or

promote resistance (Sinan et al., 2021). Most people in developing countries depend on folk medicine for the treatment of different diseases including diarrhea (Park, 2021). Medicinal plants are culturally accepted, available, and inexpensive in comparison to modern medicine. Several reports reported the use of medicinal plants as anti-diarrheal agents since they can stimulate water absorption and decrease electrolyte secretion and intestinal motility (Agbor et al., 2004; Behera et al., 2006; Oben et al., 2006). They can also stimulate antispasmodic properties (Lozoya et al., 2002). The potency of medicinal plants as anti-diarrheal agents depends on their content of secondary metabolites. It was reported that medicinal plants containing tannins, flavonoids, saponins, alkaloids, steroids, and terpenoids exhibited anti-diarrheal activity (Chitme et al., 2004; Yadav and Tangpu, 2007; Brijesh et al., 2009). Flavonoids can inhibit intestinal motility and hydro electrolytic secretions (Venkatesan et al., 2005). Tannins form protein tannates through denaturation of the intestinal mucosa proteins. This effect can decrease the secretion and intracellular Ca^{2+} inner current or may activate the calcium pumping system encouraging muscle relaxation (Belemtougri et al., 2006). *Quercus* L. genus (family Fagaceae) is commonly known as oak. It comprises about 450 species of deciduous or evergreen trees widely spread in North Africa, Asia, Europe, and North, Central, and South America (Burlacu et al., 2020). The barks, woods, and galls of *Quercus* species have been commercially used since ancient times due to their tannins content. Oak galls were used in leather tanning, dyeing, and ink preparation (Şöhretoğlu and Renda, 2020). *Quercus* species were traditionally used for several disorders such as antimicrobial, wound healing, acute diarrhea, hemorrhoids, and inflammatory diseases (Şöhretoğlu and Renda, 2020). The anti-inflammatory, antibacterial, hepatoprotective, antidiabetic, gastroprotective, and antioxidant activity were assessed in previous reports (Moharram et al., 2015; Taib et al., 2020). It was reported that the biological effects of *Quercus* species were attributed to their high content of polyphenolic compounds which possess anti-inflammatory, antioxidant, and anti-diarrheal activities (Salem et al., 2016; Drózdź and Pyrzyńska, 2018; Vong et al., 2018). The identified polyphenolic compounds in *Quercus* species were related to flavonoid, tannins and proanthocyanidins classes (Şöhretoğlu and Renda, 2020).

Q. robur L., commonly known as the English or pedunculate oak, is a large, lobed, deciduous, and broadleaved tree, which is native to Europe (Eaton et al., 2016). It was traditionally used for sore throats, anal fissures, hemorrhoids, and skin problems. Its decoction is used for diarrhea treatment, rectal bleeding, and dysentery (Frag et al., 1998). The amount of hydrolysable and condensed tannins in *Q. robur* and other *Quercus* species is seasonally dependent. It was reported that the highest insect attack on oak leaves occurs in early spring, which links to the time when the amount of hydrolysable tannins is minimum and condensed tannins are almost free (Scalbert and Haslam, 1987; Moharram et al., 2015; Formato et al., 2022). Previous reports revealed that hydrolyzable tannins belonging to hexahydroxydiphenylesters nucleus were identified from *Q. robur* leaves, bark, and wood (Scalbert et al., 1988; Herve du Penhoat et al., 1991; Vivas et al., 1995). Moreover, catechin derivatives were detected from its bark (Vovk et al., 2003). The UHPLC-QTOF-MS profiling for *Q. robur* leaves (Moharram et al., 2015; Unuofin and Lebelo, 2021; Formato et al., 2022) and stem bark (Unuofin and Lebelo, 2021) indicated the presence of phenolic acid, flavonoids, and triterpenoids. Moreover, isolation of hydrolysable tannins and

flavonoids from *Q. robur* leaves were reported by Moharram et al. (2015), Unuofin and Lebelo (2021), Formato et al. (2022).

Q. coccinea Münchh (Scarlet oak, red oak, or Spanish oak) is a deciduous tree with broadleaf. Its common name comes from the color of its leaves in autumn. It is native to the eastern and central parts of the United States, but it is also widely distributed around the world (Burns and Honkala, 1990; Yang et al., 2019). In general, there is little information about the chemistry of *Q. coccinea* but like other oak species, it produces galls that are rich in phenolic compounds (Grieve, 1984). The present study aimed to tentatively identify the polyphenolic compounds in *Q. coccinea* and *Q. robur* leaves using UHPLC-MS. We also investigated the anti-diarrheal activity of both species against experimentally induced diarrhea.

2 Material and methods

2.1 General experimental material

Solvents used for polyphenols extraction were supplied from El Nasr Pharmaceutical Chemicals Co., Egypt) while that for HPLC (Merck, Darmstadt, Germany), loperamide (Sigma-Aldrich, MO, United States), castor oil and charcoal were purchased from a general market.

2.2 Plant material

Q. coccinea Münchh. and *Q. robur* L. leaves were collected from the International Garden, Cairo, Egypt in November 2021. They were kindly identified by Dr. Therese Labib, a plant taxonomist at Mazhar Botanical Garden, Giza, Egypt. A voucher samples are 34 Qro/2022 and 34 Qro/222 for *Q. coccinea* and *Q. robur* respectively, were kept at the Herbarium of the Pharmacognosy Department Faculty of Pharmacy, Helwan University, Cairo, Egypt.

2.3 Sample preparation and extraction of phenolic compounds

Q. coccinea and *Q. robur* air-dried leaves (600 g) were extracted with 80% aqueous methanol under reflux (4×3 L) followed by filtration and evaporation under vacuum to yield 80 g and 100 g extracts of *Q. coccinea* and *Q. robur*, respectively. The residue was defatted with petroleum ether followed by evaporation of the solvent to yield 60 g and 85 g methanol-soluble portions of *Q. coccinea* and *Q. robur*, respectively. The samples were stored at -15°C until UHPLC/MS analysis.

2.4 UHPLC-MS separation and identification

UHPLC-MS/MS studies were achieved using the Waters SYNAPT G2 LC/Q-TOF (Waters Corporation, Milford, MA, United States) system. The separation of the target compounds was implemented on the column of RP C₁₈ Waters Acquity UPLC BEH (Waters, 1.7 μm , 2.1 mm \times 100 mm). The mobile phase used was a mixture of solvent (A) acetonitrile and solvent B) 0.1% formic acid/water with a flow rate

TABLE 1 UHPLC profile tentative secondary metabolites identification for *Q. coccinea* and *Q. robur* leaves 80% aqueous methanol extract.

No	Rt (min)	Exact mass	[M-H] ⁻	MF	Name of compound	Species		References
						<i>Q. c</i>	<i>Q. r</i>	
1	0.87	192.06	191.0591	C ₇ H ₁₂ O ₆	Quinic acid	+		Nishimura et al. (1984), Sardans et al. (2014)
2	1.76	170.02	169.0149	C ₇ H ₆ O ₅	Gallic acid	+	+	Rahman et al. (2015)
3	1.18	342.10	341.1069	C ₁₅ H ₁₈ O ₉	Caffeic acid hexoside		+	Pacifico et al. (2019)
4	2.27	462.08	461.1343	C ₂₁ H ₁₈ O ₁₂	Kaempferol hexoside		+	Mahgoub et al. (2021)
5	3.11	462.08	461.125	C ₂₁ H ₁₈ O ₁₂	Di methyl ellagic acid <i>O</i> pentoside		+	Gao et al. (2021)
6	4.29	194.06	193.0522	C ₁₀ H ₁₀ O ₄	Ferulic acid	+	+	Aung et al. (2020)
7	4.55	432.11	431.1937	C ₂₁ H ₂₀ O ₁₀	Apigenin- <i>O</i> -hexoside	+	+	Deveoglu et al. (2012)
8	4.63	432.11	431.1849	C ₂₁ H ₂₀ O ₁₀	Methoxy apigenin- <i>C</i> -pentoside	+	+	
9	4.66	432.11	431.1483	C ₂₁ H ₂₀ O ₁₀	Afzelin	+	+	Xu et al. (2018)
10	4.89	432.11	431.1574	C ₂₁ H ₂₀ O ₁₀	Vitexin	+	+	Elsayed et al. (2022)
11	4.89	432.11	431.1574	C ₂₁ H ₂₀ O ₁₀	Isovitexin	+	+	
12	5.22	434.08	433.0383	C ₂₀ H ₁₈ O ₁₁	Quercetin- <i>O</i> -pentoside	+		Sakar et al. (2005)
13	5.44	596.14	595.1299	C ₂₆ H ₂₈ O ₁₆	Quercetin- <i>O</i> -pentose hexoside	+	+	Unuofin and Lebelo (2021)
14	5.73	610.15	609.1497	C ₂₇ H ₃₀ O ₁₆	Rutin		+	Aung et al. (2020)
15	5.91	302.04	301.0370	C ₁₅ H ₁₀ O ₇	Quercetin	+	+	
16	5.99	464.10	463.0890	C ₂₁ H ₂₀ O ₁₂	Isoquercitrin	+		
17	6.08	302.01	300.9991	C ₁₄ H ₆ O ₈	Ellagic acid	+	+	Unuofin and Lebelo (2021)
18	5.44	594.16	593.1479	C ₃₀ H ₂₆ O ₁₃	Tribuloside	+		Unuofin and Lebelo (2021)
19	6.30	198.05	197.0818	C ₉ H ₁₀ O ₅	Ethyl gallate	+	+	Ou et al. (2015)
20	6.35	600.11	599.1036	C ₂₈ H ₂₄ O ₁₅	Kaempferol hexose gallate		+	Mahgoub et al. (2021)
21	6.59	448.10	447.0962	C ₂₁ H ₂₀ O ₁₁	Kaempferol glucouronopyranoside	+	+	Meng et al. (2001)
22	6.67	478.11	477.1068	C ₂₂ H ₂₂ O ₁₂	Isorhamnetin- <i>O</i> -hexoside	+		Romussi et al. (1988), Jiang et al. (2017), Formato et al. (2022), Stefi et al. (2022)
23	7.59	418.09	417.1580	C ₂₀ H ₁₈ O ₁₀	Kaempferol pentoside		+	Saunier et al. (2022)
24	8.55	462.12	461.1156	C ₂₂ H ₂₂ O ₁₁	Isorhamnetin- <i>O</i> -rhamnoside		+	Arámbula-Salazar et al. (2015)
25	8.72	680.22	679.3714	C ₂₈ H ₄₀ O ₁₉	Ferulic acid triglucoside	+	+	Usui et al. (2017)
26	9.55	286.05	285.0393	C ₁₅ H ₁₀ O ₆	Kaempferol	+	+	Bursal and Boğa (2018)
27	10.21	184.04	183.1367	C ₈ H ₈ O ₅	Methyl gallate	+	+	Ou et al. (2015)
28	11.97	518.32	517.3173	C ₃₀ H ₄₆ O ₇	Cucurbitacin F	+	+	Unuofin and Lebelo (2021)
29	13.12	824.20	823.1816	C ₃₄ H ₃₀ O ₁₄	Kaempferol diacetyl- <i>p</i> -coumaroylrhamnoside	+		Unuofin and Lebelo (2021), Formato et al. (2022)
30	13.48	198.05	197.1186	C ₉ H ₁₀ O ₅	Syringic acid	+	+	Dar Ms Fau - Ikram and Ikram (1979)
31	15.76	634.08	633.3814	C ₂₇ H ₂₂ O ₁₈	Strictinin or its isomer	+	+	Nishimura et al. (1986), Moharram et al. (2015)
32	21.056	788.11	787.3824	C ₃₄ H ₂₈ O ₂₂	Tetra galloyl glucose	+	+	Amr et al. (2021)

of 0.4 mL/min. The gradient sequence was 0–1 min, 5% A; 1–16 min, 5%–99.5% A; 16–26 min, 99.5% A; 26–26.1 min, 99.5%–5% A; 26.1–28 min, 5% A. The column temperature was sustained at 40°C. All solvents used were filtered by a 0.45 µm membrane filter as well as the extract (5 mg) was dissolved in methanol (1 mL) at 5,000 ppm. The

non-targeted MS1 and MS2 data were composed at the range of *m/z* 100–2,000. The automated data-dependent acquisition (DDA) attitude was applied in the MS2 scans, and the non-targeted selections of 5 precursor ions were fragmented with ramping of the collision energy from 10 to 50 eV. The attained MS records were confirmed

by Waters Mass Fragment software (MassLynx4.1, Waters, MA, United States).

2.5 Animals

Swiss albino male mice at age of 5–7 weeks (20–25 g) were provided by the Biological Products and Vaccines breeding unit, Helwan, Egypt. Mice were kept in the standard condition and were free to access standard pelleted diet and water. All *in vivo* experiments were permitted by the scientific research ethics committee (Faculty of Pharmacy, Helwan University, no: 13A2022). The experiments were performed according to the European Community Directive (86/609/EEC), and the NIH Guidelines for the Care and Use of Laboratory Animals (8th edition).

2.6 Determination of median lethal dose (LD₅₀)

For the determination of the LD₅₀, mice (25 g) received orally 80% aqueous methanol extract (AME) dissolved in Tween 80 and

distilled water with the aid of tween 80 with increasing doses till 5 g/kg. At the same time, the control group mice were administrated Tween 80 and distilled water. Mice's general behavior and mortality percentage were noticed over 24 h (Mady et al., 2022).

2.7 *In vivo* anti-diarrheal activity

2.7.1 Castor oil-induced diarrhea in mice

Castor oil-induced diarrhea in mice was performed according to Zhao et al. (2018). Forty-eight mice were fasted for 18 h and then classified into eight groups (n = 6). Group I received orally the vehicle and was considered an untreated control, group II taking loperamide orally (5 mg/kg) and served as the standard group. The mice in groups III–VIII received the AME of *Q. coccinea* and *Q. robur* orally using gavage and the concentrations were 250, 500, and 1,000 mg/kg b. wt. After one hour from oral administration of castor oil (0.2 mL) to each mouse and they were kept individually in a plastic cage, where its floor was covered with white blotting paper. For each

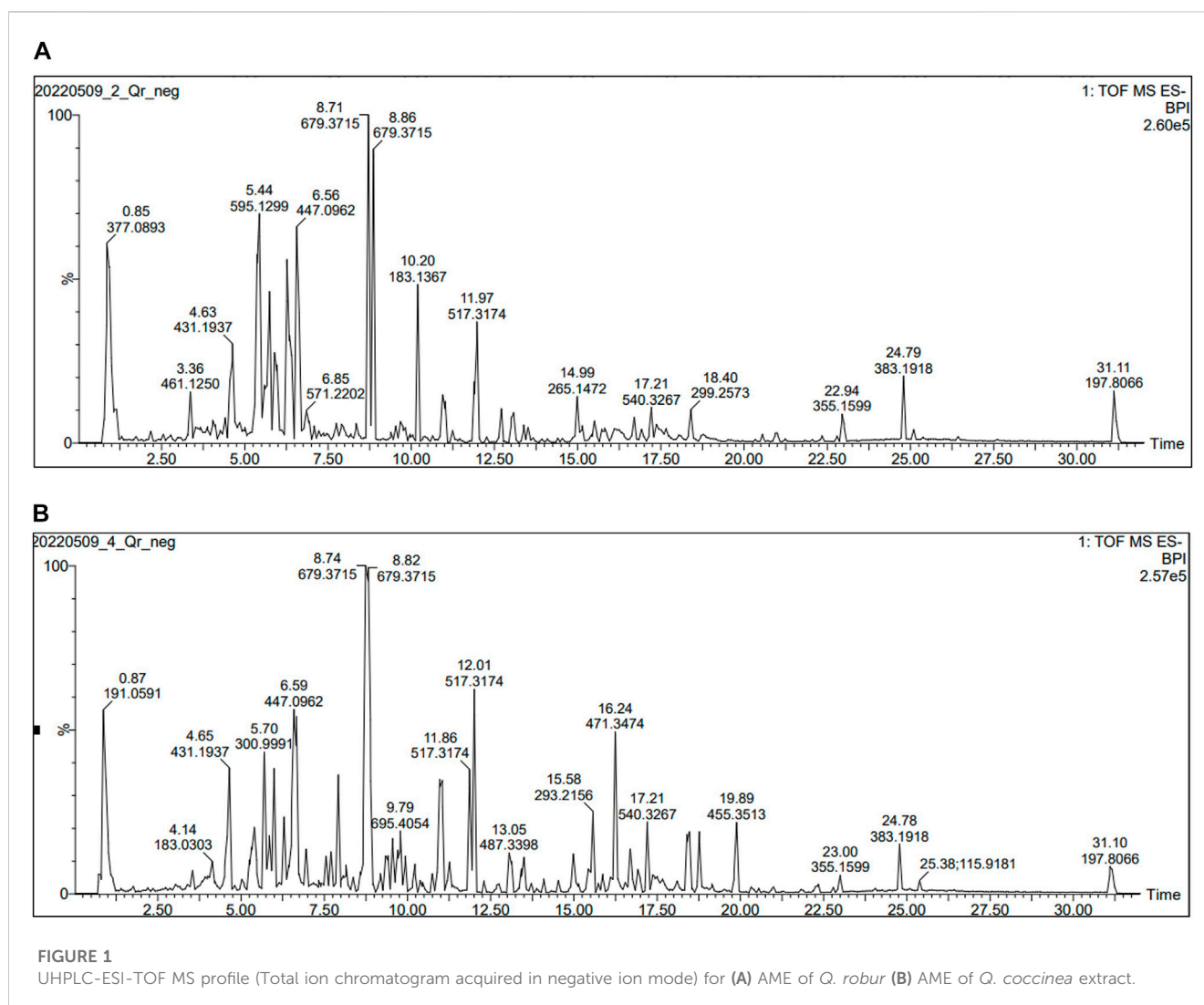


TABLE 2 MS/MS fragmentation for the major compounds present in *Q. coccinea* and *Q. robur* leaves 80% aqueous methanol extract.

Rt (min)	Exact mass	[M- H] ⁻	Daughter fragment	Name of compound	Species	
					<i>Q. c</i>	<i>Q. r</i>
1.18	342.10	341.1069	179.05	Caffeic acid hexoside		+
4.63	432.11	431.1849	299.99	Methoxy apigenin-O-hexoside	+	+
5.44	596.14	595.1299	301.02	Quercetin O-pentose hexoside	+	+
5.99	464.10	463.0890	301.01	Isoquercitrin	+	
6.08	302.01	300.9991	229.01	Ferulic acid	+	+
6.27	594.16	593.1479	285.05	Tribuloside		+
6.59	448.10	447.0962	286.03	Kaempferol hexoside	+	+
6.67	478.11	477.1068	315.04	Isorhamnetin-O-hexoside	+	
8.72	680.22	679.3714	517.31	Ferulic acid triglucoside	+	+

mouse, the onset of diarrhea was recorded, and the numbers of normal, wet, and watery feces were calculated for 4 h.

$$\text{Percentage of inhibition} = \frac{\text{Mean number of WFC} - \text{Mean number of WFT}}{\text{Mean number of WFC}} \times 100$$

Where WFC and WFT were the wet Feces for the control and test groups, respectively.

2.7.2 Castor oil-induced enter pooling in mice

The mice were divided as in the previous method. After one hour of the administration of AME and standard, each mouse received castor oil (0.5 mL) orally then 1 h later, mice were sacrificed using cervical dislocation and their abdomens were opened to isolate small intestines after ligation from the pylorus to the caecum. The intraluminal fluid was squeezed and placed in a graduated tube to determine the volume (Abdela, 2019).

2.7.3 Castor oil triggered charcoal meal transit test

Fasted forty-eight mice were categorized into eight groups as previously described (Mekonnen et al., 2018). After one hour of the administration of AME and loperamide, each mouse in all groups was given castor oil (0.5 mL). One hour later, each mouse received 5% charcoal 0.5 mL orally suspended in distilled water. Thirty minutes later, all mice were sacrificed to isolate their small intestines (Mekonnen et al., 2018). The intestinal length traveled by the charcoal was recorded and represented as a percentage of the total intestine length (peristaltic index) (Mady et al., 2022).

$$\text{Peristalsis index} = \frac{\text{Distance traveled by charcoal meal}}{\text{Length of small intestine}} \times 100$$

$$\% \text{ inhibition} = \frac{\text{Mean Peristalsis index of control} - \text{Mean Peristalsis index of test}}{\text{Mean Peristalsis index of control}} \times 100$$

2.7.4 Statistical analysis

The results were stated as mean value \pm SEM. Statistical analysis was done using Graph Pad Prism, version 8 (GraphPad Software Inc., United States), by one-way analysis of variance, followed by a Tukey's test to measure the statistical

significance between various groups. The value $p < 0.05$ was considered as significant.

3 Results

3.1 Identification of *Q. robur* and *Q. coccinea* polyphenols

UHPLC-MS analysis for the AME of both *Q. coccinea* and *Q. robur* leaves led to the tentative identification of twenty-five and twenty-six compounds in *Q. coccinea* and *Q. robur*, respectively belonging to phenolic acids, flavone, and flavonol aglycone and glycosides in addition to few tannins' compounds (Table 1 and Figure 1). They were identified based on their molecular ion peak, fragmentation pattern, and comparison with the previously published data. The major compounds present in both *Quercus* species were further subjected to MS/MS to establish their structure (Table 2). Quercetin-O pentoside, quercetin pentose hexoside, rutin, isoquercitrin, and quercetin represented the quercetin derivatives. Kaempferol glucouronopyranoside, afzelin, kaempferol glucogallate, kaempferol hexoside, kaempferol pentoside, kaempferol diacetyl-*p*-coumaroylrhamnoside, tribuloside, and kaempferol represented the main kaempferol derivatives. Isorhamnetin-O-hexoside and isorhamnetin-O-rhamnoside were examples of isorhamnetin compounds. Apigenin-O-hexoside, methoxy apigenin-O-pentoside, vitexin, and isovitexin were examples of apigenin derivatives. Quinic, syringic, gallic, and ellagic acids represented the phenolic acids while ethyl and methyl gallate, and dimethyl ellagic acid-O-pentoside were examples of phenolic acid derivatives, caffeic acid hexoside, ferulic acid, and ferulic acid tri glucosides were examples of phenylpropanoids. Strictinin or its isomer and tetra galloyl glucose are related to hydrolysable tannins. Cucurbitacin F, an example of triterpene, was observed in both species. Isoquercitrin and isorhamnetin hexoside represented the major compounds in *Q. coccinea* while tribuloside and caffeic acid hexoside were the major

TABLE 3 Effects of *Q. coccinea* and *Q. robur* AME on fecal characteristics in castor oil-induced diarrhea in mice.

Groups	Doses (mg/kg)	Onset of diarrhea	Number of normal feces	Number of wet feces	Number of watery feces	Total feces number	% of diarrheal inhibition
Control		63.00 ± 5.63	0.67 ± 0.21	3.50 ± 0.22	7.17 ± 0.31	11.33 ± 0.33	—
Loperamide	5	170.03 ± 1.28 ^a	3.50 ± 0.22 ^a	1.17 ± 0.31 ^a	1.33 ± 0.21 ^a	6.17 ± 0.31 ^a	66.66
<i>Q. coccinea</i>	250	74.17 ± 2.95 ^b	1.00 ± 0.00 ^b	2.67 ± 0.21 ^b	3.83 ± 0.40 ^{ab}	7.17 ± 0.48 ^a	23.8
	500	89.83 ± 2.98 ^{ab}	1.67 ± 0.21 ^b	2.50 ± 0.26 ^b	3.00 ± 0.37 ^{ab}	7.00 ± 0.26 ^a	28.57
	1,000	112.2 ± 2.86 ^{ab}	2.67 ± 0.21 ^a	2.00 ± 0.22 ^a	2.50 ± 0.22 ^a	6.83 ± 0.60 ^a	42.86
<i>Q. robur</i>	250	87.33 ± 0.79 ^{ab}	2.00 ± 0.37 ^{ab}	2.33 ± 0.21 ^b	2.67 ± 0.21 ^{ab}	7.00 ± 0.52 ^a	33.34
	500	111.7 ± 9.71 ^{ab}	2.50 ± 0.22 ^a	1.83 ± 0.17 ^a	1.67 ± 0.21 ^a	5.50 ± 0.43 ^a	47.3
	1,000	152.5 ± 0.93 ^a	3.00 ± 0.37 ^a	1.50 ± 0.22 ^a	1.50 ± 0.22 ^a	5.83 ± 0.48 ^a	57.14

Data presented as: Mean ± SEM ($n = 6$);

^asignificant from control

^bsignificant from loperamide.

components in *Q. robur*. Methoxy apigenin-*O*-pentoside, quercetin pentose hexoside, ellagic acid, kaempferol hexoside, and ferulic acid triglucoside were the major compounds detected in *Q. coccinea* and *Q. robur* leaves AME. Our study revealed that UHPLC MS/MS analysis of *Q. robur* leaves AME was consistent with the previously reported data (Moharram et al., 2015; Unuofin and Lebelo, 2021; Formato et al., 2022) which revealed that the identified flavonoids based on kaempferol and quercetin nucleus while our result demonstrated in addition to this compounds the presence of some tannins and flavonoids compounds related to apigenin and luteolin moiety. Moreover, Moharram et al. (2015) revealed the isolation of compounds **2**, **27**, **31** and **17** which identified also in our result. This difference in the results could be attributed to alterations in cultivation conditions, climate changes, and geographical origin of *Quercus* species (Holopainen et al., 2018; Stefi et al., 2022), while the components of *Q. coccinea* were identified for the first time.

3.1.1 Compound 3 (caffeic acid hexoside)

The TOF-MS/MS spectrum of compound **3** displayed a molecular ion peak at m/z 341.1070 [M-H]⁻ with deprotonated caffeic acid ion fragment at m/z 179.0542 [M-162]⁻ after losing 162 Da (hexose moiety) (Supplementary Figure S1) (Pacífico et al., 2019).

3.1.2 Compound 8 (methoxy apigenin-C-pentoside)

The MS/MS fragmentation spectrum of compound **8** showed a molecular ion peak at m/z 431.1846. The peaks at m/z 299.9988 represented [M-H]⁻ and [M-132]⁻, respectively. A fragment ion peak was detected resulting from the loss of 132 Da corresponding to pentose moiety. It was attributed to methoxy apigenin, supporting the suggestion of the *O*-linkage of the sugar moiety (Supplementary Figure S3) (Zengin et al., 2021).

3.1.3 Compound 13 (Quercetin pentose hexoside) and compound 16 (Isoquercitrin)

The MS/MS spectrum of compounds **13** and **16** displayed a deprotonated molecular ion at m/z 300.0291 corresponding to

quercetin as the aglycone moiety in both compounds. It was created, by the loss of 132 Da (dehydrated pentose moiety) and 162 Da (dehydrated hexose moiety) from the main peak of **13** at m/z 595.1299 [M-H]⁻ (Supplementary Figure S4) (Unuofin and Lebelo, 2021) while in case of **16**, it resulted from the neutral loss of 162 Da (dehydrated hexose moiety) from the parent ion m/z 463.0890 [M-H]⁻ (Supplementary Figure S5) (Zengin et al., 2021).

3.1.4 Compound 4 (Kaempferol hexoside) and compound 18 (Tribuloside)

The presence of kaempferol aglycone was suggested in both compounds since their MS/MS revealed a deprotonated molecular ion peak at m/z 285.0393 for kaempferol moiety which resulted from the loss of neutral 308 Da (dehydrated coumaroyl hexose moiety) from the main peak at m/z 593.1479 [M-H]⁻ (Unuofin and Lebelo, 2021) in compound **18** (Supplementary Figure S7), as well as, due to the loss of neutral 162 Da (dehydrated hexose moiety) from the parent peak at m/z 447.0962 [M-H]⁻ for compound (**4**) (Supplementary Figure S2) (Formato et al., 2021).

3.1.5 Compound 17, ellagic acid

The MS/MS (Supplementary Figure S6) spectrum of compound **17** displays the molecular ion peak at m/z 300.9991 [M-H]⁻ with a daughter peak at m/z 229.0138 [M-H]⁻ consistent with the loss of CO₂ and CO groups (77 Da) (Yan et al., 2014) (Supplementary Figure S6).

3.1.6 Compound 22 (Isprhamnetin)

Compound **22** was identified based on its MS/MS data which displays a molecular ion peak at m/z 477.1068 [M-H]⁻ with a fragment peak at m/z 314.0459 [M-162]⁻ due to the loss of hexose moiety (162 Da) (Romussi et al., 1988; Jiang et al., 2017) (Supplementary Figure S8).

3.1.7 Compound 25 (Ferulic acid triglucoside)

In compound **25**, the loss of 162 Da (hexose moiety) from the molecular ion peak 679.3714 [M-H]⁻ produced a fragment ion at m/z 517.3174 [M-162]⁻ (Kulshreshtha and Rastogi, 1971; Usui et al., 2017) (Supplementary Figure S9).

TABLE 4 Effects of *Q. coccinea* and *Q. robur* AME on castor oil-induced enter pooling.

Group	Dose (mg/kg)	Volume of intestinal fluid (mL)	% of inhibition
Control		0.78 ± 0.031	—
Loperamide	5	0.28 ± 0.012 ^{a,c,d}	64.52
AME of <i>Q. coccinea</i>	250	0.57 ± 0.019 ^{a,b,c,d,e}	27.09
	500	0.47 ± 0.013 ^{a,b,d,e}	39.78
	1,000	0.39 ± 0.010 ^{a,b,c,e}	50.10
AME of <i>Q. robur</i>	250	0.48 ± 0.014 ^{a,b,d,e}	38.71
	500	0.38 ± 0.011 ^{a,b,c}	51.19
	1,000	0.31 ± 0.009 ^{a,c}	60

Data presented as: Mean ± SEM ($n = 6$).

^aSignificant from control

^bSignificant from loperamide

^cSignificant from *Q. robur* 250 mg

^dSignificant from *Q. robur* 500 mg

^eSignificant from *Q. robur* 1,000 mg.

TABLE 5 Effects of *Q. coccinea* and *Q. robur* on gastrointestinal motility in mice.

Group	Dose (mg/kg)	Length of small intestine (cm)	Distance traveled by charcoal (cm)	Peristaltic index (%)	% of inhibition
Control		52.83 ± 0.75	34.83 ± 0.60	66.01 ± 1.65	—
Loperamide	5	53.83 ± 0.70	12.33 ± 0.33 ^{a,c,d}	22.95 ± 0.81 ^{a,c,d}	65.23
AME of <i>Q. coccinea</i>	250	52.50 ± 1.06	28.00 ± 0.63 ^{a,b,c,d,e}	53.48 ± 1.88 ^{a,b,d,e}	18.98
	500	53.33 ± 0.56	25.17 ± 0.70 ^{a,b,d,e}	47.18 ± 1.17 ^{a,b,d,e}	28.53
	1,000	54.00 ± 0.58	22.83 ± 0.83 ^{a,b,d,e}	42.28 ± 1.46 ^{a,b,d,e}	35.95
AME of <i>Q. robur</i>	250	52.67 ± 1.26	25.00 ± 0.58 ^{a,b,d,e}	47.71 ± 2.16 ^{a,b,d,e}	27.72
	500	53.17 ± 0.60	19.67 ± 0.56 ^{a,b,c,e}	37.04 ± 1.33 ^{a,b,c,e}	43.89
	1,000	53.00 ± 0.89	14.00 ± 0.68 ^{a,c,d}	26.41 ± 1.22 ^{a,c,d}	59.99

Data presented as: Mean ± SEM ($n = 6$).

^aSignificant from control

^bSignificant from loperamide

^cSignificant from *Q. robur* 250 mg

^dSignificant from *Q. robur* 500 mg

^eSignificant from *Q. robur* 1,000 mg.

3.2 In vivo anti-diarrheal activity

3.2.1 Acute toxicity study

The dose of 5 g/kg of both extracts, which is the maximum tested dose in the acute study, did not reveal any mortality or change in the behavior of the animals. Therefore, the selection of the AME at a dose of 250, 500, and 1,000 mg/kg was considered to be safe and this result was agreeing with previously reported LD₅₀ for *Q. robur* (Moharram et al., 2015).

3.2.2 Castor oil-induced diarrheal model

The obtained data showed that *Q. robur* and *Q. coccinea* AME significantly delay in a dose-dependent manner the onset of diarrhea (Table 3), in addition they decrease ($p < 0.05$) the total number of wet feces following castor oil administration comparing to the control and treated groups, especially in the case of *Q. robur*

AME at 500 (1.83 ± 0.17) ($p < 0.001$), and 1,000 (1.50 ± 0.22) ($p < 0.0001$). In addition, *Q. robur* AME significantly decreased the average total feces number in comparison with the control at 250, 500, and 1,000 mg/kg b. wt ($p < 0.0001$). The highest defecation inhibition percentage was also detected with *Q. robur* AME at 1,000 mg/kg (57.14) which was non-significant from loperamide as the antidiarrheal drug (66.66).

3.2.3 Castor oil-induced enter-pooling activity

After treatment with AME of *Q. robur* and *Q. coccinea*, it was found that they exerted in a dose dependent manner a significant suppression of castor oil-induced enter pooling ($p < 0.001$) compared with the untreated group (Table 4). Accordingly, after measuring the intestinal fluid volume, they were 0.48, 0.38, and 0.31 mL for 250, 500, and 1,000 mg/kg b. wt respectively with *Q. robur* AME and 0.57, 0.47, and 0.39 mL for 250, 500, and 1,000 mg/kg b. wt respectively with *Q.*

coccinea AME. The results were compared with 0.78 and 0.28 mL for the untreated control and loperamide groups, respectively.

3.2.4 Castor oil triggered charcoal meal transit test

The results showed that *Q. robur* and *Q. coccinea* AME suppressed gastrointestinal transit movement and affected peristaltic index ($p < 0.0001$) in a dose-dependent manner, which was confirmed by decreasing the mean of the distance moved by charcoal ($p < 0.0001$) compared with the control groups (Table 5). Furthermore, *Q. robur* showed a non-significant change from the standard group at 1,000 mg/kg.

4 Discussion

Hydro-methanol solution (80% aqueous methanol) can extract non-polar and polar phytochemicals (Zayede et al., 2020) so we used it to extract the polyphenolic constituents from both *Q. coccinea* and *Q. robur* leaves as well as to tentatively identify the polyphenolic constituents by UHPLC/MS. The antidiarrheal activity of both AME was assessed *in vivo* in three diarrhea models using castor oil as a stimulant laxative. Ricinoleic acid, the pharmacologically active component present in castor oil is liberated by the effect of the lipase enzyme present in the small intestine's upper part (Kulkarni and Pandit, 2005). Ricinoleic acid binds and activates the prostanoid EP3 receptor and motivates the endogenous prostaglandins synthesis from the intestinal arachidonic acid. Prostaglandins can advance gastrointestinal motility, exert a laxative effect, and modify the electrolytes and water movement in and out the lumen of the intestine (Tunaru et al., 2012; Adeniyi et al., 2017). Consequently, the use of castor oil to encourage diarrhea is similar to diarrhea pathophysiology, which rationalizes its use in the current research. Previous reports revealed that the anti-diarrheal effect of traditional medicinal plants is usually associated to their secondary metabolites as flavonoids, and tannins (Meite et al., 2009; Emudainohwo et al., 2015). Plants used to tackle diarrhea were found to exert their effect through different mechanisms including antiparasitic effect, suppression of gastric motility, and peristaltic index (Özbilgin et al., 2013). Our results revealed that *Q. robur* and *Q. coccinea* AME displayed a significant result on all measured parameters including the diarrhea onset, and the number of wet, watery, and total feces which indicated that they exert their antidiarrheal through an antisecretory mechanism. It is worth mentioning that the flavonoids, phenolic acids, and tannins were detected in the HPLC/MS of the AME which are well-known for their antidiarrheal effect *via* prostaglandin production inhibition (Hämäläinen et al., 2011). Furthermore, previous reports revealed that tannins have the ability to form complex with protein that help in the precipitation of the intestinal mucosa proteins making it more resistance to alteration by chemicals and consequently reduce intestinal secretion and peristaltic movements (Kumar and Upadhyaya, 2012). Another antidiarrheal mechanism for the drug is manifested by reducing the propulsive movement of the gastrointestinal smooth muscles. Consequently, enter pooling and motility tests were assessed to give more evidence for the antidiarrheal mechanism of the extract. In the castor oil-induced enter pooling method, *Q. robur* and *Q. coccinea* AME significantly decreased the intraluminal fluid accumulation compared with the control group. Meanwhile, castor oil improves prostaglandins production which motivates fluid secretion by avoiding the reabsorption of water and electrolytes (Pierce et al., 1971).

The AME of both species inhibited the gastrointestinal hypersecretion and enter pooling *via* increasing reabsorption of water and electrolytes otherwise through the inhibition of the induced intestinal fluid accumulation which again could be attributed to their polyphenolic content. Additionally, the method of the charcoal meal was measured to explain another antidiarrheal mechanism by monitoring gastrointestinal content transposition because of gastrointestinal motility reduction (Qnais et al., 2005; Ezekwesili-Ofili et al., 2016). The current results suggested that the AME of both species significantly inhibited the intestinal transit by reducing the gastrointestinal motility of charcoal meal, which led to an increase in the intestinal content passage time therefore it was suggested that the AME act on all intestinal parts as well as its anti-motility property (Islam et al., 2013).

Our results revealed that the two *Quercus* species are rich in flavonoids which are supposed to inhabit intestinal motility, water, and electrolyte secretions by interfering with the auto-acid and prostaglandins activity (Tiwari et al., 2011). Our results revealed also the presence of quercetin and its glycosides which yield quercetin in the intestine and it was reported that the plants containing quercetin, can produce antidiarrheic effects mainly due to its antihistamine and anti-inflammatory activities (Carlo et al., 1994) also other reports revealed that quercetin exerts its antidiarrheic action through inhibition of gastrointestinal release of acetylcholine (Lutterodt, 1989; Perrucci et al., 2006). Moreover, our result revealed the presence of hydrolyzable tannins which are recognized for their antidiarrheal activity through denaturing intestinal mucosa proteins by forming protein tannate, which makes them stronger to chemical changes and so reduces secretion (Sardans et al., 2014; Sanda et al., 2020). In addition, tannins possess an anti-motility activity due to their anti-spasmodic effect, which inhibits secretion and intestinal motility *via* decreasing Ca^{2+} influx or improving calcium outflow (Bamisaye et al., 2013). Moreover, the presence of gallic acid or its derivatives (strictinin and tetra galloyl glucose) which produce gallic acid on hydrolysis in the extract may enhance the antidiarrheal activity since gallic acid has anti-secretory activity through inhibition of $H^+ K^+ -ATPase$ activity (Chen et al., 2006), moreover it was reported that ellagic acid possesses antidiarrheal activity (Xiao et al., 2013). Therefore, it is speculated that the effectiveness of crude extract may be owing to the interaction between the different constituents in the extract leading to good activity and/or a decrease in possible toxicity of some distinct compounds. Otherwise, the individual action of different constituents found in the extract may collectively contribute to the efficacy of the extract. This finding justifies the ethnomedicinal use of the *Q. coccinea* and *Q. robur* extract in the treatment of diarrhea.

5 Conclusion

The current results revealed that the *Q. robur* and *Q. coccinea* leaves extracts showed antidiarrheal activity, which could be responsible for their inhibitory effect on gastrointestinal motility, and secretion. The secondary metabolites identified in the extracts mainly flavonoids and tannins could be owing to the *Quercus* species' antidiarrheal effect through different mechanisms. Conversely, more research is required to establish the mechanism of action for antidiarrheal activity and to purify the active compounds responsible for the activity. Moreover, the research results gave evidence for the traditional uses of *Quercus* species and direct that they can be used for the treatment of diarrhea.

Data availability statement

The datasets presented in this study can be found in online repositories. The data presented in the study are deposited in the <https://doi.org/10.6084/m9.figshare.21829956.v2> repository Supplementary Material.

Ethics statement

The animal study was reviewed and approved by Faculty of Pharmacy Helwan University No. 13A2022.

Author contributions

MM, RI, FE, FM, and EE-S developed the research design, ME, KL, and L-YC carried out the UHPLC data, MM, RI, FE, and FM, identified the compounds, EE evaluated the biological activity, MM, RI, EE-S, FE, and FM wrote the text. All authors revised the text before publication.

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Conflict of interest

The authors stated that there are not any commercial or financial relations that could be interpreted as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2023.1120146/full#supplementary-material>

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