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Phytochemical analysis of *Eucalyptus camaldulensis* leaves extracts and testing its antimicrobial and schistosomicidal activities

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Abstract

Background: The emerging evolution of antibiotic and anthelmintic resistance and inefficiency of some synthetic drugs elicit the need to investigate new drug sources. In this context, *Eucalyptus camaldulensis* is an evergreen tree that has been widely used in traditional medicine for the treatment of various health disorders.

Results: Organic solvent extracts from *Eucalyptus camaldulensis* leaves were assessed for their antimicrobial activity. Among these extracts, ethyl acetate (EtOAc) and water extracts showed the highest antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Candida albicans*, and *Aspergillus niger*. EtOAc extract was further subjected to vacuum liquid chromatography (VLC) technique for the isolation of its polyphenolic ingredients. VLC yielded 13 fractions (1–13) that were assayed for their antimicrobial activities. Six of these fractions, namely 4, 5, 6, 7, 8, and 9, showed antimicrobial activity against all the tested microbes except the fungus *Aspergillus niger*. Fractions 5 and 6 having considerably the highest antimicrobial activity with inhibition zones ranged from 5 to 14 mm. Moreover, fraction 5 was tested as a larvicidal agent against miracidia and cercariae of *Schistosoma mansoni*. At concentration of 200 mg/L, the mortality rates of miracidia and cercariae of *Schistosoma mansoni* were 30%, 20%, 50%, 40%, 80%, 20%, and 100%, 80% at 5, 10, 15, and 20 min, respectively. Chromatographic isolation of the EtOAc extract led to identification of six compounds: gallic acid (1), taxifolin (2), methyl gallate (3), quercetin (4), luteolin (5), and hesperidin (6).

Conclusions: Ethyl acetate extract from *Eucalyptus camaldulensis* leaves showed a potent antimicrobial and antischistosomal activity. This activity may be attributed to the six phenolic compounds identified through structure elucidation. Thus, these compounds can be good candidates for treatment of microbes and for the control of schistosomiasis.

Keywords: *Eucalyptus camaldulensis*, VLC, Polyphenolics, Antimicrobial, Antischistosomal, Miracidia, Cercariae

Background

Eucalyptus is the most important genera in the botanical family Myrtaceae; it is widely distributed in different regions around the world, with more than 800 species (Hassine et al. 2013). *Eucalyptus camaldulensis* Dehnh, commonly known as the river red gum, is endemic in Australia (Singab et al. 2011). Leaves of *E. camaldulensis* are known to possess several biological and pharmacological activities, including antioxidant (Singab et al. 2011), cytotoxic (Singab et al. 2011; Daniela et al. 2007; Meshkani et al. 2014), antimicrobial (Ghalem and Mohamed 2008), larvicidal (Medhi et al. 2010), pesticidal (Batish et al. 2008), and anti-dermatophytes (Falahati et al. 2005). Literature survey revealed the isolation and identification of some chemical ingredients from different parts of *E. camaldulensis* including eucalyptanoic acid (Begum et al. 2002), flavonoids (Abd-Alla et al. 1980), acylated pentacyclic triterpenoids (Siddiqui et al. 1997), and essential oils (Ghalem and Mohamed 2014; Gakuubi 2016).

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Medicinal plants have a long history in the treatment of various ailments and diseases especially infectious diseases due to their therapeutic benefits (Saad et al. 2017). The microbial resistance to the existence antibiotics is well known, so it is very important to search for alternative antimicrobial agents from natural source like plants or herbs to overcome this challenge (Ghareeb et al. 2015).

Various medicinal plants were proven efficient drug candidates to control tropical parasitic diseases such as schistosomiasis (El-Sayed et al. 2011a; de Moraes 2015; El-Sayed et al. 2011b). Although praziquantel, the currently used drug for human infections of schistosomiasis, is safe and effective, it has some problem such as the emerging resistance to some laboratory strains of *Schistosoma* (Fallon and Doenhoff 1994; Doenhoff et al. 2009). Similarly, the chemical molluscicide used to control the snail's intermediate hosts of schistosomiasis is cost-effective and has some environmental side effects (King and Bertsch 2015). In this context, some plants are widely tested as molluscicides against snails due to their toxic effects. Other plants are not toxic to snails, but due to their powerful antioxidant capacity, they were used as supplementary antioxidants to control schsitosome infection within snails (Mossalem et al. 2017). Moreover, *E. camaldulensis* itself was shown to reduce the outcome of *S. mansoni* infection in *Biomphalaria alexandrina* (Mossalem et al. 2018).

Numerous studies have showed that ordinary antioxidants in plants are intimately linked to their bio functionalities such as the reduction of chronic diseases and inhibition of pathogenic bacteria growth, which are often associated with the termination of free radical proliferation in biological systems (Covacci et al. 2001; Ghareeb et al. 2018a; Sobeh et al. 2018). Therefore, the aim of the current study was to evaluate the in vitro antimicrobial and antischistosomal activity of *E. camaldulensis* leaves' extracts as well as the chromatographic isolation of the ethyl acetate extract followed by the structural elucidation of pure isolated compounds.

Methods

Collection of plant material, extraction, and fractionation

The fresh leaves of *Eucalyptus camaldulensis* Dehnh were collected from Giza governorate during June, 2016. The identification and authentication of the plant was carried out by Dr. Therese Labib, Department of Flora and Taxonomy, El-Orman Botanical Garden, Giza, Egypt. A voucher specimen (No. E25/4/3) is kept in the herbarium of the garden. Details of extraction of *E. camaldulensis* leaves using organic solvents and testing the free radical scavenging activity of the obtained extracts are described in Mossalem et al. (Mossalem et al. 2018).

Instruments, chemicals, and reagents

The ^{1}H and ^{13}C-NMR analyses were recorded on BRUKER 400 (400 and 100 MHz for ^{1}H and ^{13}C-NMR, respectively). For column chromatography, we used the following chemicals and materials: Sephadex LH-20 (Pharmacia Fine Chemicals), Silica gel (60–200 mesh) (S.D. fine-CHEM Ltd.) and Whatmann No. 1 sheet filter papers (Whatmann Ltd., Maidstone, Kent, England). Thin layer chromatography with aluminum sheet (20 × 20) percolated with silica gel 60 F_{254} was obtained from E. Mark (Darmstadt, Germany).

Vacuum liquid chromatography (VLC) of the ethyl acetate extract

The ethyl acetate extract (10 g) was subjected to vacuum liquid chromatography (VLC) using silica gel GF254 (300 g) as stationary phase and eluted via gradient mix elution system with increasing polarity including *n*-hexane, *n*-hexane/EtOAc (80:20, 60:40, 40:60, and 20:80; *v*/*v*), EtOAc, CH_2Cl_2, CH_2Cl_2/methanol (MeOH) (80:20, 60:40, 40:60, and 20:80; *v*/*v*), MeOH and acetone to afford 13 fractions (Table 1). The major fraction 4 (0.3 g) was eluted with *n*-hexane/EtOAc (40:60) and then rechromatographed for further purification using Sephadex LH-20 sub-column initially eluted with 5% MeOH, and the polarity was increased with different ratios of MeOH until reaching 100% MeOH. The resulting fractions were combined on the basis of paper chromatography (PC) eluted by two common eluents, 15% acetic acid (15%AcOH) and BAW (*n*-butanol to acetic acid to water; 4:1:5; *v*/*v*/*v*/upper layer) to afford compound 1 (10 mg). Similarly, another major fraction 5 (0.5 g) was eluted with *n*-hexane/EtOAc (20:80) and then rechromatographed using Sephadex LH-20 sub-column to afford compound 2 (15 mg). Fraction 6 (0.5 g) was eluted with EtOAc then rechromatographed using Sephadex LH-20 sub-column to afford compound 3 (10 mg). Fraction 7

Table 1 Vacuum liquid chromatography (VLC) of the ethyl acetate extract

Fraction	Elution system	% or *v*/*v*	Yield (g)
1	*n*-hexane	100%	0.0000
2	*n*-hexane/EtOAc	80:20	0.2037
3	*n*-hexane/EtOAc	60:40	0.2987
4	*n*-hexane/EtOAc	40:60	0.3505
5	*n*-hexane/EtOAc	20:80	0.5470
6	EtOAc	100%	0.5210
7	CH_2Cl_2	100%	0.20
8	CH_2Cl_2/MeOH	80:20	2.7269
9	CH_2Cl_2/MeOH	60:40	2.2427
10	CH_2Cl_2/MeOH	40:60	0.2636
11	CH_2Cl_2/MeOH	20:80	0.0888
12	MeOH	100%	0.0461
13	Acetone	100%	0.0239

(0.15 g) was eluted with CH_2Cl_2 then rechromatographed using Sephadex LH-20 sub-column to afford compound 4 (10 mg). Fraction 8 (2.5 g) was eluted with CH_2Cl_2/MeOH (80:20) then rechromatographed using Sephadex LH-20 sub-column to afford compound 5 (10 mg). Finally, fraction 9 (2.20 g) was eluted with CH_2Cl_2/MeOH (60:40) then rechromatographed using Sephadex LH-20 sub-column to afford compound 6 (10 mg).

Antimicrobial activity

Disc agar plate method has been recognized to assess the antimicrobial activities of organic solvent extracts from *E. camaldulensis* as well as the selected ethyl acetate VLC fractions (El-Neekety et al. 2016). Four different test microbes, *Staphylococcus aureus* (G+ve bacterium), *Pseudomonas aeruginosa* (G-ve bacterium), *Candida albicans* (yeast), and *Aspergillus niger* (fungus), were selected to evaluate the antimicrobial activities. The bacterial and yeast test microbes were grown on a nutrient agar medium. On the other hand, the fungal test microbe was cultivated on Czapek-Dox medium. The culture of each test microbe was diluted by distilled water (sterilized) to 10^7 to 10^8 colony-forming units (CFU)/mL then 1 mL of each was used to inoculate 1-L Erlenmeyer flask containing 250 mL of solidified agar media. These media were put on the previously sterilized petri dishes (10 cm diameter having 25 mL of solidified media). Filter paper discs (5 mm Ø, Whatmann No. 1 filter paper) loaded with 0.2 mg of each extract. The discs were dried at room temperature under sterilized conditions. The paper discs were placed on agar plates seeded with test microbes and incubated for 24 h, at the appropriate temperature of each test organism. Antimicrobial activities were recorded as the diameter of the clear zones (including the disc itself) that appeared around the discs (Hathout et al. 2016; Madkour et al. 2017).

Effect of *E. camaldulensis* extracts against *Schistosoma mansoni* larvae

Miracidia and cercariae of *S. mansoni* were obtained from Schsitosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute. The selected concentration was 200 mg/L (Mossalem et al. 2018). A double concentration of fraction 5 *n*-hexane/EtOAc (20:80 *v*/*v*) was prepared in 5 ml volume of water. Then, it was added to a petri dish containing 50 fresh hatched miracidia in 5 mL of water (final volume is 10 mL). Another 10 mL dechlorinated tap water containing 50 fresh hatched miracidia served as control (Mohamed et al. 2012). Following 5, 10, 15, 20, 25, and 30 min, the petri dishes were examined under dissecting microscope and mortalities in miracidia were recorded (the larvae were considered dead when they stopped movement completely for 1 min) (Mahmoud and Ibrahim 2011). The same experiment was repeated with cercariae. Percentages of dead miracidia and cercariae were compared by Chi-square test (Mohamed et al. 2012).

Results

In vitro antimicrobial activity

The in vitro antimicrobial activity of organic solvent leaf extracts of *E. camaldulensis* was evaluated via disc agar method against four pathogenic strains of microbes, *Staphylococcus aureus* (G+ve bacteria), *Pseudomonas aeruginosa* (G-ve bacteria), *Candida albicans* (yeast), and *Aspergillus niger* (fungus), and compared to Neomycin and Cyclohexamide as standard antibiotics. The antimicrobial activities of the tested extracts were expressed in clear zone of inhibition (mm). The results in Table 2 and Fig. 1 revealed that water extract showed the highest inhibition zones (13, 11, 11, and 9 mm, for *S. aureus*, *P. aeruginosa*, *C. albicans*, and *A. niger*, respectively. Ethyl acetate extract exhibited antimicrobial activity very close to the water extract with inhibition zones of 11, 10, 10, and 8 mm for *S. aureus*, *P. aeruginosa*, *C. albicans*, and *A. niger*, respectively. The other solvent extracts showed antimicrobial activities according to the following order: *n*-butanol > dichloromethane > petroleum ether > 90% methanol. Further fractionation of ethyl acetate extract using VLC yielded 13 fractions, six of them (fractions 4, 5, 6, 7, 8, and 9) exhibited antimicrobial activities against all test microbes except *A. niger*. While the fractions (1, 2, 3, 10, 11, 12, and 13) are inferior in the antimicrobial activity, fraction 5 showed the highest antimicrobial activity among the fractions tested. Inhibition zones were 15, 13, and 14 mm against *S. aureus*, *P. aeruginosa*, and *C. albicans*, respectively (Table 3 and Fig. 2).

Table 2 In vitro antimicrobial activity of organic solvent extracts of *E. camaldulensis* leaves

Solvent extracts	Clear zone (mm)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
1. 90% MeOH	–	8	–	–
2. EtOAc	11	10	10	8
3. Water	13	11	11	9
4. Dichloromethane	6	6	–	–
5. *n*-Butanol	8	8	7	6
6. Petroleum ether	–	–	6	9
Antibiotic				
Neomycin[1]	17	20	21	–
Cyclohexamide[2]	–	–	–	25

[1]Neomycin was used at 200 microgram per disc
[2]Cyclohexamidewas used at 200 microgram per disc

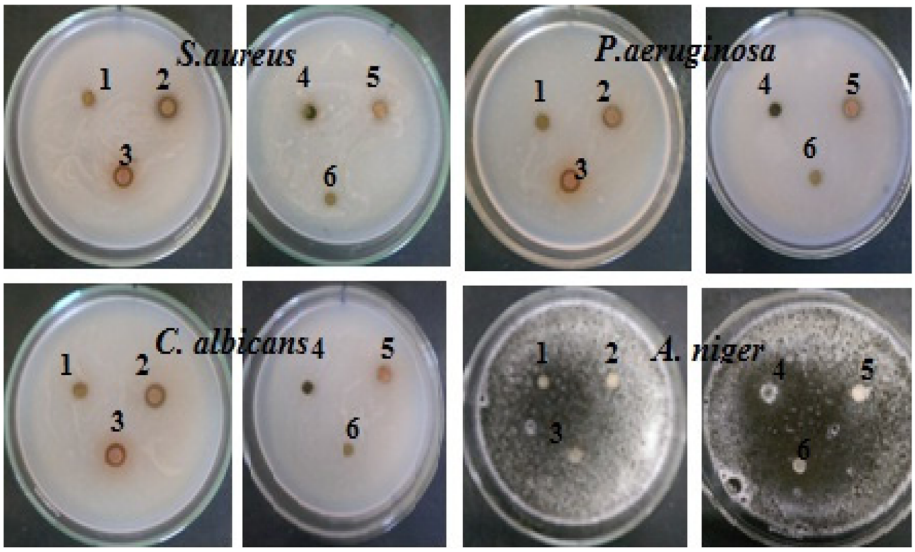

Fig. 1 The antimicrobial activity of organic solvent fractions from methanolic extract of *E. camaldulensis* leaves

Miracidicidal and cercaricidal effect

Observation of *S. mansoni* miracidial and cercarial mortalities following exposure to 200 mg/L of fraction 5 *n*-hexane/EtOAc (20: 80 *v*/*v*), isolated from ethyl acetate extract of *E. camaldulensis* leaves by VLC technique, showed a gradual increase in mortalities rate with increasing exposure time. After a few minutes from exposure, both miracidia and cercariae exhibited morphological changes started by inactivation of their internal parts and ended via complete death with extended exposure time. Mortalities rates of miracidia were 30%, 50%, 80%, and 100% after 5, 10, 15, and 20 min of continuous exposure to 200 mg/L of the fraction, respectively. As indicated in Table 4 by 20 min of exposure, all the miracidia were found dead. Moreover, cercariae exhibited a similar pattern of gradual increase in mortality rate with prolonged exposure time. Complete death (100%) of cercariae was recorded after 30 min of continuous exposure.

Table 3 In vitro antimicrobial activity of VLC fractions of ethyl acetate extract of *E. camaldulensis* leaves against different test microbes

Ethyl acetate VLC fractions	Clear zone (mm)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
1				
2	–	–	–	–
3	–	–	6	–
4	8	8	12	–
5	15	13	14	–
6	11	12	11	–
7	7	7	6	–
8	9	10	8	–
9	9	10	10	–
10	–	–	–	–
11	–	–	–	–
12	–	–	–	–
13	–	–	–	–
Antibiotic				
Neomycin	18	19	21	–

Chromatographic isolation of the ethyl acetate extract

Six phenolic compounds were isolated from the EtOAc extract of *E. camaldulensis*. Based on ^{1}H-NMR spectra, chromatographic data, and available data in the literature; their chemical structures were elucidated as gallic acid (1), taxifolin (2), methyl gallate (3), quercetin (4), luteolin (5), and hesperidin (6) (Fig. 3).

Discussion

In vitro antimicrobial activity

Microbial infections still have a great public issue, and there is a dramatic increase in the microbial resistance to the existence antimicrobial agents (Collins and Lyne 1985). Most medicinal plant extracts showed a synergistic antimicrobial activity (Co-activity), which may be attributed to the collaborative action between their mixed constituents like flavonoids, anthraquinones, coumarins, and phenolic acids (Murugan et al. 2013).

Reviewing the literature revealed that the antibacterial activity of three solvent extracts (methanol, dichloromethane, and petroleum ether) of *E. camaldulensis*

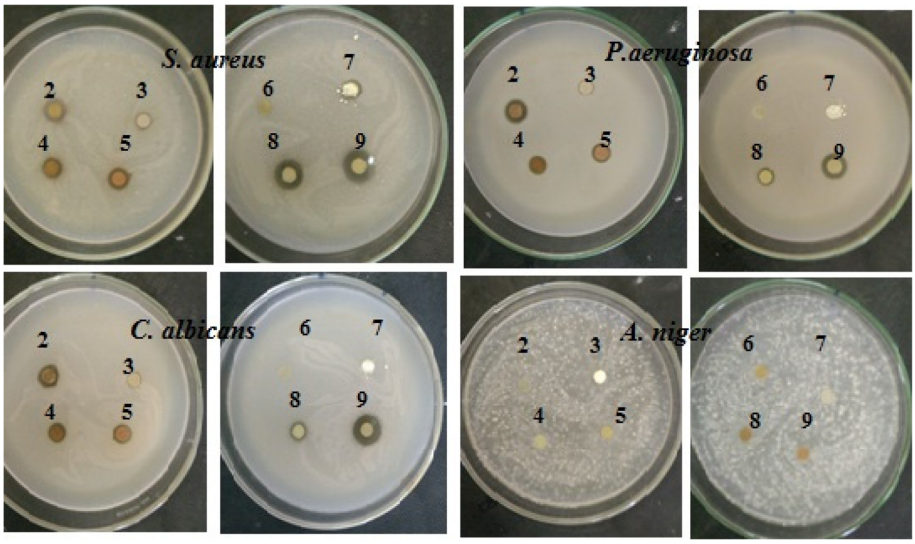

Fig. 2 The antimicrobial activity of VLC fractions from ethyl acetate extract of *E. camaldulensis* leaves

leaves growing in Nigeria was evaluated against six microbial species, namely *Klebsiella* spp., *Salmonella typhi*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*; for the methanolic extract, it showed strong activity with inhibition zones of 14, 16, 14, 15, 15, and 16 mm while for dichloromethane were 15, 15, 13, 13, 14, and 14 mm, respectively, for the abovementioned species, and there is no any activity was recorded with petroleum ether (Saad et al. 2017). Moreover, the in vitro antimicrobial activity of the Nigerian *E. camaldulensis* was evaluated against six strains of *Helicobacter pylori* (Adeniyi et al. 2009). Previous studies stated that polyphenolic compounds are responsible for the antimicrobial activity of the plant extracts (Funatogawa et al. 2004; Buzzini et al. 2008; Min et al. 2008). To sum up, *E. camaldulensis* exhibited noticeable antimicrobial potentials which may be return to their unique chemical profile.

Table 4 *Schistosoma* miracidial and cercarial mortality (%) post exposure to 200 mg/l from VLC fraction (5)

Exposure time (min)	Percentage of dead miracidia		Percentage of dead cercariae	
	Control	Exposed	Control	Exposed
5	0	30***	0	20***
10	0	50***	0	40***
15	0	80***	0	50***
20	0	100***	0	80***
25	0	100***	0	90***
30	0	100***	0	100***

***High significant at $p < 0.001$ compared to control

Toxicity to *Schistosoma mansoni* larvae

Recently, there has been an interest to control schistosomes infection within snails rather than killing these intermediate hosts. This method has many advantages including maintenance of natural biodiversity in aquatic habitats and minimizing the environmental impacts related to application of chemical molluscicides (Mossalem et al. 2018). Plants rich with antioxidant molecules such as flavonoids and phenolic acids compounds are great targets for exogenous source of antioxidants that can maintain the balance between oxidative stress result from infection or other stressors and antioxidant system within organisms (Ghareeb et al. 2018b). Indeed, exposure of *B. alexandrina* to 90% defatted methanol extract from *Punica granatum* peels reduced the infection rates of snails with *S. mansoni* to 20% compared to 95% in infected unexposed snails (Mossalem et al. 2017). The same reduction in infection rate was obtained when snails exposed to ethyl acetate extract from *E. camaldulensis* leaves (Mossalem et al. 2018).

The results obtained from testing the larvicidal properties of fraction 5 *n*-hexane/EtOAc isolated from ethyl acetate extract of *E. camaldulensis* leaves indicate that this fraction possesses a potent toxic effect to miracidia and cercariae of *S. mansoni*. At concentration of 200 mg/L from fraction 5 *n*-hexane/EtOAc (20: 80 *v*/*v*), 100% mortality was recorded in miracidia and cercariae after 20 and 30 min of continuous exposure, respectively. Mossalem et al. (Mossalem et al. 2018) showed that ethyl acetate extract of *E. camaldulensis* possesses a powerful antioxidant activity and its administration to *B. alexandrina* snails prior and during pre-patent infection with *S. mansoni* resulted in a significant decrease in the

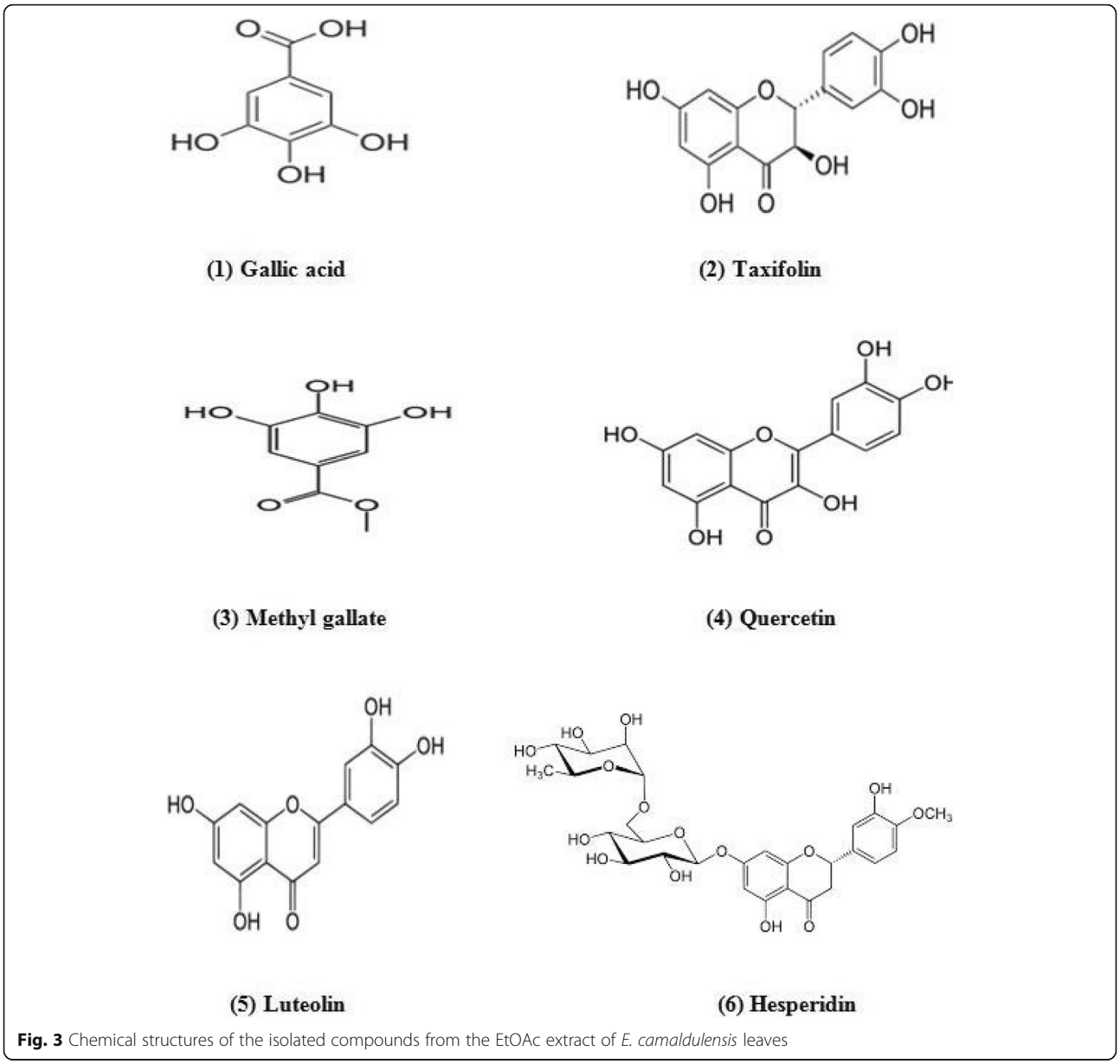

Fig. 3 Chemical structures of the isolated compounds from the EtOAc extract of *E. camaldulensis* leaves

infection rate of snails and increased the survival rate of snails. In the same context, Al-Sayed et al. (Al-Sayed et al. 2014) reported a potent molluscicidal activity of 80% MeOH extract of *E. globulus* against *B. alexandrina* snails and also showed strong miracidicidal and cercaricidal activity (80% and 100% mortality, respectively) after a 2 h exposure. A wide range of plants were proven to have potent schistosomicidal prosperities. The seeds of *Nigella sativa* either in the form of powder or as extracts had larvicidal potency against *S. mansoni* miracidia and cercariae. LC_{50} values of this drug were 1 and 2 mg/L for miracidia and cercariae, respectively (Mansour et al. 2002; Mohamed et al. 2005). Studies of the effect of this plant on adult female and male worms suggested that the possible mechanism of its action against *S. mansoni* parasite is mediated by inhibiting important antioxidant enzymes in the worms (Mohamed et al. 2005). The plants *Allium sativum* and *Allium cepa* (powder) at concentrations of 50 and 100 mg/L caused 50% mortalities to *S. mansoni* miracidia, respectively (Mantawy et al. 2012). Also, tannins extracted from *P. granatum* at a concentration as low as 0.39 mg/L killed 100% of miracidia after 50–150 min and 50% of miracidia within 25.1–48.3 min (Abozeid et al. 2012).

Reports from other countries on testing different plant species for their larvicidal effect showed their potential as miracicidal and cercaricidal agents. Extracts of the leaves and fruits of *Piper marginatum*, *Protium heptaphyllum*,

and *Capsicum annuum* from Brazil show a remarkable effect on the cercariae of *S. mansoni*. In the case of the oils of *Piper marginatum* and *Capsicum annuum*, 90–96% of the cercariae of *S. mansoni* were killed within 15 min (Frischkorn et al. 1978). Moreover, extracts from *Phytolacca dodecandra*, *Tamarindus indica*, *Acacia nilotica*, *Hibiscus sabdariffa* and *Tacca leontopetaloides* from Sudan were toxic to miracidia and cercariae at concentration from 50 to 100 mg/L (Elsheikh et al. 1990).

The toxic effect of *n*-hexane/EtOAc fraction isolated from ethyl acetate extract of *E. camaldulensis* leaves may be exerted in a different mode of action that differ from the abovementioned plants. Since *E. camaldulensis* has a powerful antioxidant activity and is rich with bioactive molecules such as gallic acid, taxifolin, quercetin, luteolin, and hesperidin. One possible explanation is that exposure of the larvae to 200 mg/L of the fraction led to prod-oxidative acts or their interference with critical reactive oxygen species required for maintenance of cellular functions and physiological processes (Bouayed and Bohn 2010).

Structural elucidation of the isolated compounds from the ethyl acetate extract

Compound 1 was isolated as off white powder, m.p. 250–251 °C. On paper chromatography (PC), it showed violet and deep violet spots under long and short UV light respectively as well as positive result with $FeCl_3$. R_f values are 0.82 (BAW) and 0.52 (15% AcOH). ^{1}H-NMR spectra (400 MHZ, DMSO-d_6) showed a characteristic signal in the aromatic region for two identical aromatic protons at δ_H 7.12 ppm (2H, *s*, H-2,6). The ^{1}H-NMR and chemical data were in agreement with the reported data of 3,4,5-trihydroxybenzoic acid (gallic acid) (Chanwitheesuk et al. 2007).

Compound 2 was isolated as a yellow powder, m.p. 230–232 °C. On PC, it showed a yellow fluorescence colored spot under long UV light turned to bright yellow with ammonia vapors. R_f values are PC 0.76 (BAW) and 0.07 (15% AcOH), an indication of its nature as aglycone.^{1}H-NMR spectra (400 MHZ, DMSO-d_6) revealed the presence of three aromatic protons at B-ring was resonated at δ_H 6.93 (1H, d, J = 1.25 Hz, H-2′), 6.87 (1H, dd, J = 8.0, 1.5 Hz, H-6′), and 6.80 ppm (1H, d, J = 8.0 Hz, H-5′). In addition, the presence of two meta-coupled protons at A-ring appeared at δ_H 5.89 (1H, d, J = 1.25 Hz, H-6) and 5.95 ppm (1H, d, J = 1.25 Hz, H-8), while the C-ring protons were resonated at δ_H 4.89 (1H, d, J = 11.5 Hz, H-2) and 4.52 ppm (1H, d, J = 11.5 Hz, H-3). Based on the abovementioned spectral and chromatographic data, this compound could be identified as 3,5,7,3′,4′-pentahydroxy-flavanone (dihydroquercetin or taxifolin) (Kuspradini et al. 2009; Usman et al. 2016).

Compound 3 was isolated as off white powder, m.p. 200–202 °C. On PC, it showed violet and deep violet spots under long and short UV light respectively. R_f values are 0.65 (BAW) and 0.70 (15% AcOH) and positive results with $FeCl_3$. ^{1}H-NMR spectra (400 MHZ, DMSO-d_6) showed two sets of protons, the first one for the methoxy protons at δ_H 3.78 ppm (3H, *s*, –OCH_3) and the second one for the two aromatic protons at δ_H7.14 ppm (2H, *s*, H-2, 6). The ^{1}H-NMR and chemical data were in agreement with that of methyl gallate (Choi et al. 2014).

Compound 4 was isolated as yellow powder, m.p. 311–313 °C. On PC, it showed a yellow fluorescence colored spot under long UV light turned to bright yellow with ammonia vapors and green color with $FeCl_3$. R_f values are 0.67 (BAW) and 0.07 (15% AcOH) which were in the range of aglycones. ^{1}H-NMR spectra (400 MHZ, DMSO-d_6) showed different sets of resonances; two meta-coupled aromatic protons at A-ring were appeared at δ_H 6.19 (1H, d, J = 2.1 Hz, H-6) and 6.41 ppm (1H, d, J = 2.1 Hz, H-8). Three aromatic protons located at B-ring appeared at δ_H 7.53 (1H, d, J = 2.1 Hz, H-2′), 6.88 (1H, d, J = 8.0 Hz, H-5′), and 7.68 ppm (1H, dd, J = 8.0, 2.1 Hz, H-6′). The most downfield protonappeared at δ_H 12.5 ppm (1H, s, 5-OH). On the basis of ^{1}H-NMR spectra and chromatic data, the compound could be identified as 5,7,3′,4′-flavon-3-ol (quercetin) (Huang et al. 2013).

Compound 5 was isolated as pale yellow powder, m.p. 320–322 °C. On PC, it showed a dark purple florescence spot under long UV light. R_f values are 0.74 (BAW) and 0.07 (15% AcOH) which were in the range of aglycones. ^{1}H-NMR spectra (400 MHZ, DMSO-d_6) showed different sets of aromatic protons; two meta-coupled protons at A-ring were appeared at δ_H 6.25 (1H, d, J = 2.1 Hz, H-6) and 6.54 ppm (1H, d, J = 2.1 Hz, H-8). Another characteristic signal for proton in position 3 at C-ring was appeared at δ_H 6.73 ppm (1H, *s*, H-3). Moreover, a characteristic pattern for three protons at B-ring appeared at δ_H 7.0 (1H, d, J = 8.1 Hz, H-5′), 7.49 (1H, dd, J = 8.0, 2.1 Hz, H-6′), and 7.98 ppm (1H, d, J = 2.1 Hz, H-2′). A most down field proton appeared at δ_H 13.04 ppm (1H, *s*, 5-OH) due to the effect of intermolecular hydrogen bond with adjacent carbonyl group at C-ring. Based on ^{1}H-NMR and chromatographic data, the compound could be identified as 5,7,3′,4′-tetrahydroxy-flavone (luteolin) (Tshikalange et al. 2005; Sato et al. 2000).

Compound 6 was isolated as pale yellow powder, m.p. 250–252 °C. On PC, it showed a yellowish green florescence spot under long UV light. R_f values are 0.56 (BAW) and 0.78 (15% AcOH) indicating to its glycosidic nature. ^{1}H-NMR spectra (400 MHZ, DMSO-d_6) revealed the presence of set of resonances were appeared at δ_H 11.97 ppm (1H, br s, 5-OH) for the most downfield proton at position 5 at A-ring. Three aromatic protons of 1,3,4-trisubstituted B-ring were resonated at δ_H 6.95 (1H, d, J = 2.1 Hz, H-2′), 6.85 (1H, J = 8.2 Hz, H-5′), and 6.81 ppm (1H, dd, J = 8.2, 2.1 Hz, H-6′). Also, two

meta-coupled aromatic protons were resonated at δ_H 6.13 (1H, d, $J = 2.1$ Hz, H-8) and 6.10 ppm (1H, d, $J = 2.1$ Hz, H-6). C-ring protons appeared at δ_H 5.35 (1H, dd, $J = 11.2$, 5.1 Hz, H-2), 3.15 (1H, dd, $J = 16.0$, 11.0 Hz, H-3a), and 2.51 ppm (1H, dd, $J = 16.0$, 5.0 Hz, H-3b). Moreover, two characteristic anomeric protons of sugar moieties were located at δ_H 5.07 (1H, d, $J = 7.5$ Hz, H-1″) and 4.57 ppm (1H, br s, H-1‴). Methoxy and methyl protons were resonated at δ_H 3.78 (3H, s, 4-OCH_3) and 1.09 ppm (3H, d, $J = 6.0$ Hz, H-6), respectively. Accordingly, on the basis of ^{1}H-NMR spectral data and chromatographic features, the compound could be identified as 3′,5,7-trihydroxy-4′-methoxyflavanone-7-*O*-rutinoside (hesperidin or hesperetin 7-*O*-rutinoside) (Areias et al. 2001; Cuyckens et al. 2001).

Conclusions

Eucalyptus camaldulensis was considered as a prolific source for several biologically active metabolites. The ethyl acetate leaves' extract of this plant showed considerable antimicrobial and antischistosomal activity. Further fractionation by vacuum liquid chromatography (VLC) yielded 13 fractions from which 6 fractions exhibited antimicrobial activities. The most powerful antimicrobial fraction (5) also showed potent larvicidal activity against *S. mansoni* larvae. Chromatographic isolation of ethyl acetate extract led to identification of six phenolic compounds that could be responsible for the observed activity.

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Availability of data and materials
All the data obtained during the study are presented in this manuscript. Any further enquiries for additional information are available upon request from the corresponding author.

Authors' contributions
MAG, MRH, and HSM conceived and designed the study. MAG, MRH, HSM, and MSA performed the experiments. MAG, MRH, and MSA analyzed the data. MAG and MRH wrote the first draft, revised, and edited it. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Ethical approval had been granted approval by the Ethics Committee of Theodor Bilharz Research Institute (TBRI).

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Not applicable.

Competing interests
The authors declare that they have no competing interest.

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