Tropical Journal of Pharmaceutical Research November 2016; 15 (11): 2383-2390
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v15i11.12

Original Research Article

Larvicidal, nematicidal, antifeedant and antifungal, antioxidant activities of Mentha spicata (Lamiaceae) root extracts

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Received: 1 June 2016 *Revised accepted: 16 October 2016*

Abstract

Purpose: *To evaluate the larvicidal, nematicidal, antifeedant, and antifungal effects of 10 solvent extracts of Mentha spicata root.*
Methods: *Ten solvent extracts were investigated for their total flavonoid and phenolic content and screened for larvicidal, nematicidal, antifeedant, and antifungal activities. The total phenolic content of the extracts was determined using the Folin–Ciocalteu method, while total flavonoid content was determined by aluminium chloride ($AlCl_3$) colorimetric assay. Four solvents extracts were screened for antifungal activity against Aspergillus niger, Candida albicans, recultured Cryptococcus neoformans, and Microsporum audouinii using the agar diffusion method. The nematicidal activity of the compounds was evaluated against the juvenile Meloidogyne javanica organism, while larvicidal properties were evaluated against the urban mosquito Culex quinquefasciatus using a standard bioassay protocol. The antifeedant activity of marine acclimated Oreochromis mossambicus was used for evaluating ichthyotoxic potential.*
Results: *The total flavonoid content in the extracts ranged from 18.5 to 83.4 mg/g, and the amount of free phenolic compounds ranged from 14.7 to 91.9 mg/g of extract powder. The water extract of these plants exhibited significant antioxidant activity and significant levels of phenolics and flavonoids. The water extract exhibited higher larvicidal (LD_{50} = 11.77 µg/mL), nematicidal (LD_{50} = 11.78 µg/mL), antifeedant (LD_{50} > 40 µg/mL), and antifungal activities (minimum inhibitory concentration: 16 µg/mL) against M. audouinii compared with the other extracts.*
Conclusion: *These results show that the water extract of Mentha spicata may be used as a potential natural alternative source of nutritional and pharmaceutical ingredients.*

Keywords: *Mentha spicata, Larvicidal, Nematicidal, Antifeedant and Antifungal activities, Nutritional supplement, Pharmaceutical ingredients*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Mentha spicata Linn., commonly known as spearmint, belongs to the Lamiaceae family [1]. The genus *Mentha* consists of more than 25 species, among which the monoterpenes such as menthol, menthone, carvone, and pulegone are well known. Monoterpenes are widely used

by food and pharmaceutical industries as a flavour or in fragrance formulations. They are widely grown in temperate areas of the world, particularly in Europe, North America, North Africa, and Asia, although presently they are cultivated in regions all over the world.

The Lamiaceae family is a rich source of polyphenolic compounds and may therefore possess strong antioxidant properties [2]. Mints are herbaceous plants with high contents of essential oils. Due to their levels of secondary metabolites, they are commonly used as pharmaceuticals, fragrances, flavours, and foods [3]. The pharmacological activity of *Mentha* species, peppermint in particular, has been investigated extensively. Peppermint is used for gastrointestinal disorders (atony of the stomach, flatulence) and nervous system disorders (nervousness, insomnia, nervous sickness). Its antibacterial, antiviral, fungicidal, anti-inflammatory, choleretic, cholekinetic, and astringent activities have also been described [4]. Recent work examined the antioxidant activities of different *Mentha* species. Phenolic compounds play the most important role in the antioxidant properties of mint. The major phenolic constituents are caffeic acid derivatives and flavonoids (Figure 1). This study is the first to report the larvicidal, nematicidal, antifeedant, and antifungal activities of the root extract of *M. spicata*.

Research on herbs has recently become more pressing because of their potential antifungal activity. Here, we estimated the total phenolic and flavonoid contents of dried *M. spicata* root using various solvent extractions (hexane, chloroform, ethyl acetate, and water) and screened for larvicidal, nematicidal, antifeedant, and antifungal activities.

EXPERIMENTAL

All materials and reagents were purchased from Sigma Aldrich (Mississauga ON Canada) unless otherwise stated. (Chloroform, Ethyl acetate, Sodium carbonate, Aluminium chloride, Sodium nitrate, Catechin, Gallic acid)

Plant material

The fresh plant material used in this study, and fresh plant was collected (Juan 28, 2015) from Tiruchirappalli distric, Tamil Nadu, The plant was morphologically identified and authenticated by Anis Ahamed (One of the authors of this paper – Lecture in Botany department) the Botanist of Department of Botany, King Saud University (KSU), Riyadh, Saudi Arabia.

Extraction of solvent fractions

The shade-dried root powder of *M. spicata* (100 g) was immersed in 500 mL of double distilled (DW) water at 85 °C for 3 h (water extraction). The mixture was constantly stirred at every 24 hours interval of 72 h then the final filtrate volume found to be 1.2 L. Each extraction was performed three times. After filtration, the extracts was then concentrated under reduced pressure at 40 ^{o}C in a vacuum rotary evaporator. Afterwards, the concentrates were dried and weighed to determine total extractable compounds.

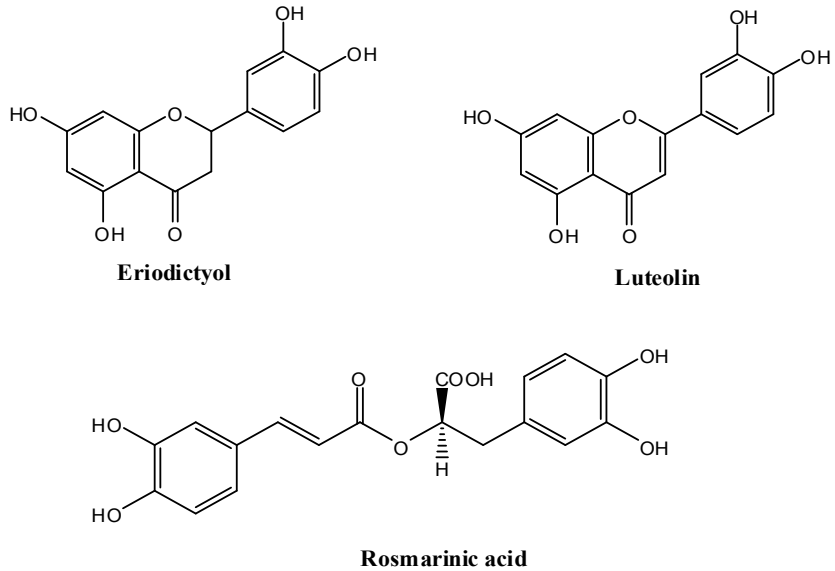

Figure 1: Phenolic compounds in *Mentha spicata*

Finally, the extracts were stored in a refrigerator at 4 °C until used. The aqueous layer further fractionated with chloroform ($CHCl_3$), ethyl acetate other solvent fractions were collected and concentrated with vacuum rotary evaporator.

Identification of total phenolic content

The total phenolic content of the extracts was determined by using the FC (Folin–Ciocalteu) method [5]. Briefly, 10 mg of each extract was dissolved in 1 mL of DW and different concentrations of gallic acid (0 - 1 mg/mL) were prepared in ethanol. 40 µL of each solution of *Mentha spicata* root extract or a standard reagent was mixed with 20 µL of 1 N FC reagent. Next, the mixture was incubated for 3 – 5 min at room temperature, followed by addition of 20 % sodium carbonate solution and incubated at RT for 30 min. Finally, the absorbance was measured at 700 nm wavelength, with a UV–visible spectrophotometer. Total phenolic content was determined from the standard calibration curve. Results were expressed as percentage of gallic acid equivalents (GAE) per 100 g dry mass.

Identification of total flavonoid content

Total flavonoid content was determined by aluminium chloride ($AlCl_3$) colorimetric assay [5]. The concentrations of the *Mentha spicata* root extract and standard (catechins) solutions were 10 mg/mL and 25, 20, 15, 10 and 5 µg/mL, respectively. The concentration (25 µL) of each sample or standard reagent was mixed with 125 µL of DW, followed by addition of 8 µL of 5 % sodium nitrate solution. The mixture was incubated at RT for 5 min. Aluminium chloride (10 %) solution was mixed with 15 µL of the above mixture. Finally, the absorbance was measured at 517 nm. Total flavonoid content was expressed a percentage of the catechins equivalent (CE) per 100 g dry mass obtained from the standard calibration curve.

Determination of larvicidal activity

The larvicidal activity was assessed by the procedure of the WHO (1996) with some modifications and as per the method [6]. *Mentha spicata* root extract was tested against the urban mosquito larvae (*Culex quinquefasiatus*) using standard bio assay protocol. Eggs of *C. quinquefasiatus* were obtained from drainage system. Eggs were placed in clean water and kept at room temperature for hatching. Larval development was monitored for seven days. The second stage larvae were collected at the tip of a pasture pipette and placed in cotton bud to remove excess water and transferred to the test vial. The larval mortality was observed using various concentrations of *Mentha spicata* root extract (10, 20, 30 and 40 mg/mL).

Assessment of nematicidal activity

Nematicidal activity was evaluated using juvenile nematodes of *Meloidogyne javanica* [7]. Assay system was prepared with 2 mL Milli Q water containing different concentrations of *Mentha spicata* root extract (10, 20, 30 and 40 mg / mL) in glass tubes. The different concentrations of solutions are prepared treated and control nematodes were held under the same conditions as used for colony maintenance. Total 25 juveniles of *M. javanica* were transferred in test tube, positive control (sample) and negative control (only solvent). Mortality was observed under a zoom stereomicroscope after 24 h of exposure.

Evaluation of antifeedant activity

Fingerlings (1.5 - 2.0 cm) of marine acclimated *Oreochromis mossambicus* were used for evaluating the ichthyotoxic potential [8]. Total 25 fingerlings fish were introduced in experimental and control glass bowls containing 1,000 mL seawater and chosen concentrations of *Mentha spicata* root extract. Immediate reflex changes and mortality were observed continuously for six hours at 1 h interval for the next 12 h. After 24 h of exposure, the number dead and live fish were counted.

***In vitro* antifungal screening**

Mentha spicata root extract were evaluated for their *in vitro* antifungal activity against *Aspergillus niger, Candida albicans, Cryptococcus neoformans* (recultured) and *Microsporum audouinii* using an disc diffusion method [9,10] with Sabouraud's dextrose agar (Hi-Media).

Determination of the minimal inhibitory concentration (MIC): Test sample was concentrated at two fold dilutions method (356, 128, 64, 32…., 0.5 µg/mL, etc). The microorganism suspensions at 10^6 cfu/mL (colony forming unit/mL) concentrations were incubated to the corresponding wells. The test sample solution (0.01 mL per disk) was added to sterile disks to prepare disks of potency with different concentration. The disks were stored at 4 °C until use.

The plates were incubated at 36 ºC at 48 h and observe for inhibition zones and then note the MIC. The minimum inhibitory concentration (MIC)

values were determined as the lowest concentration that completely inhibited visible growth of the microorganism.

Statistical analysis

All experiments were performed in triplicate and all data were expressed at least 3 independent evaluations and the standard deviations (SD) were also calculated using Microsoft Excel 2007 software (Microsoft, Redmond, WA, USA).

RESULTS

Total phenolic content

The reagent, Folin-Ciocalteu, was used to determine the total polyphenol content in root extracts. Folin-Ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions, which are formed from phosphomolybdic and phosphotungstic heteropoly acids [11]. The total phenolic content in the various organic and water extracts was determined and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry plant. The total phenolic content from the different solvents ranged from 0.3 ± 0.43 to 83.4 ± 0.89 mg GAE/g. The water extract exhibited the highest phenolic content, whereas the lowest content was from the n-propanol extract. The total phenolic contents from each of the different extracts are presented in Table 1.

Total flavonoid content

Flavonoids are the most common and widely distributed group of plant phenolic compounds, ever-present in fruits and vegetables. The total flavonoid values of the extracts ranged from 0.4 ± 0.12 to 91.9 ± 0.14 mg catechin equivalent (CE)/100 g. The highest value was observed in the water extract, whereas the lowest values were observed in the toluene, n-butanol, n-propanol, and isopropanol extracts. Total flavonoid content analysis is shown in Table 1.

Table 1: Total phenolic and flavonoid contents of *Mentha spicata* root extracts

Solvent Extract	Total phenolic (mg GAE/100g of dry mass)	Total flavonoid (mg CE/100g of dry mass)
Hexane	18.5 ± 1.07	14.7 ± 2.12
Chloroform	32.0 ± 0.76	34.0 ± 0.67
Ethyl acetate	45.9 ± 0.18	44.8 ± 0.89
Methanol	12.8 ± 0.34	11.2 ± 0.56
Ethanol	10.8 ± 0.23	10.1 ± 0.23
Toluene	0.7 ± 0.18	0.8 ± 0.14
n-butanol	0.8 ± 0.11	0.4 ± 0.12
n-Propanol	0.3 ± 0.43	0.6 ± 0.76
Isopropanol	0.6 ± 0.21	0.4 ± 0.45
Water	83.4 ± 0.89	91.9 ± 0.14

GAE, gallic acid equivalents; CE, catechin equivalents. Value is expressed as mean ± standard deviation (n = 3)

Phenolics are secondary metabolites, which are major components of plants that scavenge free radicals and perform important biological activities [12,13]. Flavonoids are a group of polyphenolic compounds with various chemical structures and characteristics that exhibit numerous biological activities, including anti-inflammatory, antiviral, and antibacterial activity,

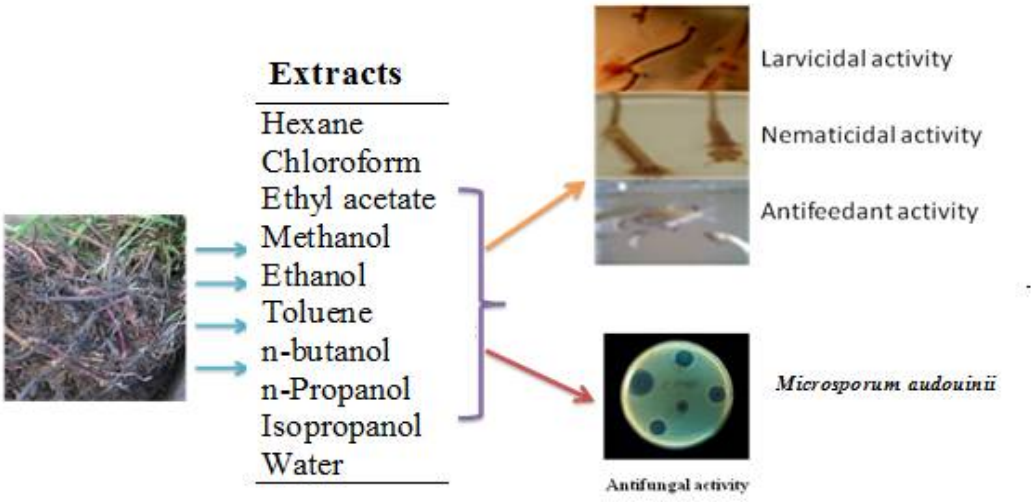

Figure 2: Total biological screening for various *Mentha spicata* root extract

Table 2: Larvicidal profile of *Mentha spicata* root extract on second instar larvae of *Culex* sp. at room temperature

M. spicata extract	Mortality (%)				LD_{50}
	*10*µg/mL	*20*µg/mL	*30*µg/mL	*40*µg/mL	
Hexane	22 ± 1.0	47 ± 1.0	68 ± 1.0	72 ± 3.0	26.37
Chloroform	32 ± 3.0	69 ± 2.0	82 ± 2.0	100 ± 1.0	15.78
Ethyl acetate	28 ± 1.0	40 ± 1.0	67 ± 3.0	87 ± 1.0	21.56
Methanol	35 ± 1.0	67 ± 1.0	78 ± 1.0	100 ± 3.0	15.04
Ethanol	37 ± 3.0	61 ± 2.0	76 ± 2.0	100 ± 1.0	14.74
Toluene	0.4 ± 1.0	10 ± 1.0	27 ± 3.0	38 ± 1.0	>100
n-butanol	19 ± 3.0	39 ± 2.0	52 ± 2.0	69 ± 1.0	29.52
n-Propanol	18 ± 1.0	30 ± 1.0	47 ± 3.0	60 ± 1.0	32.67
Isopropanol	12 ± 1.0	31 ± 1.0	41 ± 2.0	52 ± 1.0	38.23
Water	40 ± 3.0	84 ± 2.0	100 ± 0.0	-	11.77

[a] Values are the mean ± SD (n = 3)

Table 3: Nematicidial activity of *Mentha spicata* root extract

M. spicata extract	Mortality (%)				LD_{50}
	10µg/mL	*20µg/mL*	*30µg/mL*	*40µg/mL*	
Hexane	28 ± 1.0	34 ± 3.0	49 ± 3.0	55 ± 2.0	36.45
Chloroform	32 ± 2.0	69 ± 1.0	82 ± 1.0	100 ± 0.0	13.07
Ethyl acetate	48 ± 2.0	60 ± 3.0	76 ± 1.0	100 ± 0.0	12.56
Methanol	35 ± 1.0	67 ± 1.0	75 ± 1.0	100 ± 0.0	19.91
Ethanol	37 ± 3.0	61 ± 2.0	81 ± 2.0	100 ± 0.0	18.60
Toluene	0.0 ± 1.0	0.6 ± 1.0	15 ± 3.0	25 ± 1.0	>100
n-butanol	0.9 ± 3.0	19 ± 2.0	38 ± 2.0	45 ± 1.0	>100
n-Propanol	0.7 ± 1.0	17 ± 1.0	32 ± 3.0	50 ± 1.0	40.0
Isopropanol	0.0 ± 1.0	11 ± 1.0	26 ± 2.0	32 ± 1.0	>100
Water	43 ± 1.0	63 ± 1.0	100 ± 0.0	-	11.78

[a] Values are the mean ± SD (n = 3)

in addition to their antioxidant properties [14]. The total phenolic content of the *M. spicata* root extracts was determined from a regression equation of the calibration curve (y = 0.5181x – 0.5953) and expressed in GAE. Values were 83.4, 45.9, 32.0, 18.5, 12.8, 10.8, 0.8, 0.7, 0.6, and 0.3 mg GAE/100 g dry mass for water, ethyl acetate, chloroform, hexane, methanol, ethanol, n-butanol, toluene, isopropanol, and n-propanol extracts respectively. Total flavonoid content was determined from the regression equation of the calibration curve (y = 0.244x + 0.2849) and expressed in catechin equivalents (CE). The water, ethyl acetate, chloroform, hexane, methanol, ethanol, toluene, n-butanol, and isopropanol extracts of *M. spicata* root exhibited total flavonoid contents of 91.9, 44., 34.0, 14.7, 11.2, 10.1, 0.,0.6, 0.4, and 0.4 mg CE/100 g dry mass, respectively. The water extract contained greater phenolic and flavonoid contents than did any of the other solvent extracts.

Biological profile

Figure 2 shows the total biological screening outline of *M. spicata* root extract compounds. The water extract exhibited the highest levels of larvicidal (LD_{50} = 11.77 µg/mL), nematicidal (LD_{50} = 11.78 µg/mL), antifeedant (LD_{50} ≥ 40 µg/mL), and antifungal activities (28 mm at minimum inhibitory concentration [MIC]: 16 µg/mL) against *Microsporum audouinii*.

DISCUSSION

M. spicata root extract was screened for larvicidal activity against second instar larvae for 24 h at room temperature. Water extract, at 30 µg/mL, induced 100 % mortality while hexane extract exhibited low toxicity compared with the other solvent extracts. The extracts induced 50 % mortality of second instar larvae.

The larvicidal activities of various plant components, natural products, and secondary metabolites of plant origin have been evaluated against mosquitoes, with varying results [15].

M. spicata essential oil exhibits a significant toxic effect against early third-stage larvae of *C. quinquefasciatus*, with LC_{50} values of 62.62 µg/mL [16]. The water extract of the *Mentha spicata* root showed high levels of larvicidal activity. Toluene extract was less active than the other solvent extracts in larvicidal screening, reaching 38 % mortality at 40 µg/mL, with an LD_{50} value of > 100.37 µg/mL. The chloroform extract was slightly more active compared with

Table 4: Antifeedant activity of *Mentha spicata* root extracts

M. spicata extract	Mortality (%)				Time of death (h)of death	LD_{50} (µg/mL)
	10µg/mL	*20µg/mL*	*30µg/mL*	*40µg/mL*		
Hexane	41 ± 1.0	68 ± 2.0	82 ± 3.0	100 ±0.0	5	13.08
Chloroform	48 ± 3.0	63 ± 1.0	76 ± 3.0	100 ± 0.0	4	12.13
Ethyl acetate	61 ± 3.0	74 ± 3.0	87 ± 2.0	100 ± 0.0	3	08.71
Methanol	35 ± 1.0	67 ± 1.0	78 ± 1.0	100 ±0.0	5	17.66
Ethanol	37 ± 3.0	61 ± 2.0	76 ± 2.0	100 ± 0.0	5	15.67
Toluene	84 ± 1.0	91 ± 1.0	100 ± 0.0	-	4	02.45
n-butanol	49 ± 3.0	66 ± 2.0	78 ± 2.0	100 ±0.0	4	11.23
n-Propanol	58 ± 1.0	70 ± 1.0	87 ± 3.0	100 ± 0.0	4	09.55
Isopropanol	62 ± 1.0	81 ± 1.0	100 ± 0.0	-	5	08.02
Water	12 ± 2.0	34 ± 2.0	42 ± 3.0	48 ± 1.0	6	>40

[a] Values are the mean ± SD (n = 3)

Table 5: Antifungal activity of *Mentha spicata* root extracts, Minimum Inhibitory Concentration (MIC; µg/mL)

M. spicata extract	*A. niger*	*C. albicans*	*C. neoformans*	*M. audouinii*
Hexane	>356	>356	>356	32
Chloroform	>356	>356	64	64
Ethyl acetate	128	128	128	32
Methanol	64	128	128	>356
Ethanol	64	128	128	>356
Toluene	>356	64	>356	128
n-butanol	128	64	>356	128
n-Propanol	64	64	32	128
Isopropanol	128	128	>356	>356
Water	32	64	32	16

the hexane and ethyl acetate extracts, reaching 100 % mortality at 40 µg/mL, with an LD_{50} of 15.78 µg/mL. The water extract was highly active compared with the other solvents, with an LD_{50} of 11.77 µg/mL. The remarkably toxic effects exhibited by the *M. spicata* root extracts against the third and fourth instar larvae of *C. quinquefasiatus* indicate its potential use as a natural larvicide.

The water extract at 30 µg/mL induced 100 % mortality, whereas the toluene, n-butanol, and isopropanol extracts exhibited lower toxicity. Chloroform and ethyl acetate extracts showed even lower toxicity.

Many investigations have addressed the effects of root solvent extracts of various plants against several plant parasitic nematode species. Aqueous root extract samples of the marigold *Tagetes erecta*, the dusty miller Cineraria maritime, and the common rue *Ruta graveolens* exhibit high nematicidal activity against *Heterodera schachtii* and against the root knot nematodes *Meloidogyne arenaria, Meloidogyne hapla, Meloidogyne incognita,* and *Meloidogyne javanica* [17]. However, the root extract of *M. spicata* exhibited greater nematicidal activity than that of its leaf extracts against the juvenile nematodes of *M. javanica*. Previous studies reported an LC_{50} value of 0.293 mg/mL for 100–200 mg/kg for *M. spicata* and *M. javanica* [18]. The hexane extract was found to be less active than the water solvent extracts in nematicidal screening, with 55 % mortality induced by the 40 µg/mL concentration and an LD_{50} of 36.45 µg/mL. Chloroform and ethyl acetate extracts were equally active compared with the water extract, while the water extract was highly active compared with the other solvents, with an LD_{50} of 11.78 µg/mL.

Antifeedant activity was measured to evaluate the ichthyotoxicity profile of *M. spicata* root extracts of various solvents. Water extract exhibited low toxicity with 48 % mortality induced at 6 h at a concentration of 40 µg/mL while the other solvent extracts were highly toxic, with 100 % mortality induced at 3 to 5 h at the same extract concentration.

Feeding deterrent activity of plant extracts and plant products against *P. xylostella* has been reported by many investigators [19]. Koul *et al* and Chen *et al* stated that *Aglaia elaeagnoidea* extract induced maximum antifeedant and larvicidal activities against *Helicoverpa armigera* and *Pieris rapae* [20].

Ling et al reported that an ethanol extract of *Mimordica charantia* had both feeding deterrent and larvicidal activities against *P. xylostella* [21].

Using the same method to screen marine acclimated *Oreochromis mossambicus*, we evaluated the ichthyotoxic potential of *M. spicata* root extracts and found that the toluene extract was less active than were the other solvent extracts evaluated.

The water extract was highly active against *M. audouinii* assay (MIC: 16 µg/mL) and also showed a significant response against the fungal species, *Aspergillus niger, Candida albicans*, and *Cryptococcus neoformans*.
There is great demand for more efficient and safer treatments. Medicinal herbs could provide an alternative source for the treatment of dermatophytosis. Herbal medicines are generally safer and free from side effects. Moreover, the effectiveness of many traditional medicines is now accepted [22]. Some Indian plants have gained reputation for their treatment of fungal infections [23], but no systematic study has been conducted to investigate their efficacy. Few studies on *M. spicata* extracts have comprehensively investigated their antifungal activity against various microorganisms. One study, however, showed that the crude extracts (MIC: 0.75 mg/mL) are highly active against *C. albicans* compared with water extracts (MIC: 3.5 mg/mL) [24]. The present study showed that while the hexane and chloroform extracts did not show significant activity against the fungal strains evaluated, the water extract of the *M. spicata* root (MIC: 16 µg/mL) exhibits significant fungicidal activity against *M. audouinii*.

CONCLUSION

The findings of this study demonstrate that the *M. spicata* root water extract exhibits higher phenolic and flavonoid contents and it has higher larvicidal and nematicidal activity but not antifeedant activity compared with the other extracts evaluated. It also showed high antifungal activity against *M. audouinii*. Further investigation will be required to determine the active constituents of the extract as well as the mechanism of action to develop potent larvicidal, nematicidal, and antifungal agents.

DECLARATIONS

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no. RGP-1435-042. They are also grateful to Saudi Biological Society and Prince Sultan Research Chair for Environment and Wildlife, Department of Botany & Microbiology, College of Sciences, King Saud University (KSU), Riyadh, Saudi Arabia for encouragement and support.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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