

Full Length Research Paper

Antioxidant activity of extracts from *Schinus molle* L. and *Gleditsia triacanthos* L.

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Methanolic and chloroform extracts obtained from various parts of *Schinus molle* and *Gleditsia triacanthos* were evaluated by DPPH radical scavenging assay. The methanolic extracts from *S. molle* showed scavenging activity ranging from 35.97±1.02 to 83.38±2.74%, while the chloroform seed extract showed relatively weak scavenging activity ranging from 8.20±1.02 to 68.82±3.17%. The IC₅₀ values of *S. molle* methanolic leaves extract, *S. molle* methanolic stem-bark extract and *S. molle* chloroform seed extract (SMMEELS, SMMESB and SMCHSD) were found to be 476.43, <250 and ~3000 µg mL⁻¹, respectively. The methanolic extracts from *G. triacanthos* showed scavenging activity ranging from 35.97±1.02 to 92.36±0.11%, while the chloroform seed extract showed relatively very weak scavenging activity ranging from 3.74±1.04 to 15.47±4.57%. The IC₅₀ values for *G. triacanthos* methanolic leaves extract, *G. triacanthos* methanolic stem-bark extract, *G. triacanthos* methanolic thorns extract and *G. triacanthos* chloroform seed extract (GTMEELS, GTMESB, GTMETS and GTCHSD) were found to be 452.32, 720.56, <250 and >3000 µg mL⁻¹, respectively. The positive controls, GAMEOH and GAETAC, showed an IC₅₀ value <250 µg mL⁻¹ each. From this study, we concluded that the extracts from these two medicinal plants, *S. molle* and *G. triacanthos*, showed promising antioxidant activity. Therefore, further investigations, such as bioactive guided isolation of pure compounds, antioxidant activity of pure compounds, application of these extracts or pure compounds in culinary, etc., are required.

Key words: Antioxidant, chloroform extract, gallic acid, *Schinus molle*, *Gleditsia triacanthos*, radical scavenging assay, methanolic extract.

INTRODUCTION

The species *Schinus molle* L. also known as American pepper, peruvian pepper, pepper tree, aguaribay, peppercorn tree, etc., belongs to the Anacardiaceae

family (Mehani and Segni, 2013; Pedro et al., 2012; Trevor et al., 2013). *S. molle* is a medicinal plant used in traditional medicine (Abderrahim et al., 2018). *S. molle* is

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a tree that grows to 7 to 10 m height; it is native to South and Central America and has been cultivated in Southern African countries. The fruits (seeds) of *S. molle* are edible and they are about 5 mm diameter (Trevor et al., 2013). The seeds of *S. molle* are reddish pink and have a taste similar to pepper. It has been reported that *S. molle* has antibacterial, analgesic, cytotoxic, anti-inflammatory, antifungal, antiseptic, insecticidal, and antioxidant activities (Deveci et al., 2010; Diaz et al., 2008; Ferrero et al., 2006; Ruffa et al., 2002; Yueqin et al., 2003; Abir et al., 2016; Abderrahim et al., 2018; Bendaoud et al., 2010; Mohamed et al., 2016).

The species of *Gleditsia triacanthos* L. is a deciduous tree belonging to the Fabaceae family. *G. triacanthos* is also known by other names such as honey locust and thorny locust. *G. triacanthos* is native to Asia and North America (Benhamiche et al., 2016; Mohammed et al., 2014). *G. triacanthos* grows to 15 to 30 m height (Stubbeniek and Conard, 1989). The yellow flowers of *G. triacanthos* have strong pleasant smell. *G. triacanthos* has thorns which are single or branched and grow to 3 to 10 cm. The edible fruits of *G. triacanthos* are about 15 to 40 cm long and about 2.5 to 3.5 cm wide (Blair, 1990). GreenTech S.A. uses extract from seeds of *G. triacanthos* and has been sold in the name of *Gleditschia* as cosmetic product (Miguel et al., 2010).

Gleditsia species have been used for personal care and medicinal applications that include hair protection, local medicine for smallpox, skin diseases, whooping, measles, asthma and difficult labour in the Native American (Miyase et al., 2010). It has been reported that *G. triacanthos* has anti-inflammatory, analgesic, hepatoprotective, antimicrobial and antioxidant activities (Tahia et al., 2013; Mohammed et al., 2014; Miguel, 2010).

The antioxidant study of essential oils and extracts from *S. molle* and *G. triacanthos* were reported previously (Abir et al., 2016; Abderrahim et al., 2018; Bendaoud et al., 2010; Mohamed et al., 2016; Mohammed et al., 2014; Miguel, 2010). However, the scavenging activity of methanolic and chloroform extracts of various parts of *S. molle* and *G. triacanthos* has not been reported previously, particularly the plant species gathered from the Kingdom of Lesotho. Therefore, the aforementioned plant extracts were screened for their DPPH radical scavenging assay and the results thus obtained are reported.

MATERIALS AND METHODS

Plant materials

The plant materials, *S. molle* and *G. triacanthos*, were collected from Botanical Garden, Roma Campus, National University of Lesotho. Both plant materials were collected in August 2017 and were identified by Mr. Moretloa Polaki, Lecturer, Department of Biology, Faculty of Science and Technology, National University of Lesotho. From *S. molle*, the following parts of plant materials were

used for this study: leaves (596.744 g), stem-bark (241.246 g) and seeds (115.126 g). From *G. triacanthos*, the following parts were used for this study: leaves (337.000 g), stem-bark (247.271 g), thorns (165.169 g) and seeds (217.705 g). A voucher specimen for each part of the plants is kept at Organic Chemistry Laboratory, Department of Chemistry and Chemical Technology, Faculty of Science and Technology, National University of Lesotho. The following labelling was used to represent various parts of plant materials: KMSMLS, KMSMSB and KMSMSD for leaves, stem-bark and seeds of *S. molle*, respectively; KMGTLS, KMGTSB, KMGTTs and KMGTSd for leaves, stem-bark, thorns and seeds of *G. triacanthos*, respectively.

Processing of materials

The plant materials were allowed to air dry at room temperature for two weeks. The air dried leaves and seeds of *S. molle* were ground into powder using a commercial blender (Waring Blender, Blender 80119, Model HGB2WT93, 240V AC, 50-80 Hz, 3.6 AMPs, Laboratory and Analytical Supplier). The air dried stem-bark of *S. molle* was chopped into small pieces and then crushed using a Woodworking Table Saw 250 mm machine (Serial Number: JFD1412109-13, Model Number: SAWLD001, Motor, 1500W, 220 V, 50 Hz, Blade Rising Range: 0-80 mm, Motor Speed: 4500 rpm, Max. Depth of Cutting (90° and 45°): 80 mm and 5 mm). The crushed materials were further ground into powder using the aforementioned Waring Blender. The same procedures mentioned were repeated to get powder from the air-dried leaves, seeds, thorns and stem-bark of *G. triacanthos*.

Preparation of plant extracts

The powdered leaves of *S. molle* were extracted first with methanol at room temperature for two days by shaking manually and occasionally. The solution was filtered using Whatman No.1 filter paper and the solvent methanol was removed using water bath and/or Buchi rota-vapour. The same procedure was repeated once again. Finally, the material was extracted with methanol at reflux condition for 5 h. All three filtrates were combined and 63.00 g of methanolic extract was obtained. The same procedure was followed for the powdered stem-bark of *S. molle* and 7.19 g of methanolic extract was obtained. The powdered seeds of *S. molle* was extracted first with chloroform with at room temperature by shaking manually and occasionally followed by at reflux condition for 5 h. The extracts were combined and 12.91 g of chloroform extract was obtained after removal of solvent using water bath and/or Buchi rota-vapour. The same methanolic extraction procedure was followed for leaves, stem-bark and thorns of *G. triacanthos*, respectively, 47.51, 8.44 and 7.51 g of methanolic extracts were obtained. Similarly, the same chloroform procedure was followed for seeds of *G. triacanthos* and 8.94 g of resinous chloroform extract was obtained after removal of solvent using water bath and/or Buchi rota-vapour.

Chemicals and solvents used

Gallic acid, DPPH, DMSO (AR grade, 99.5%), methanol (AR grade, 99.5%), ethyl acetate (AR grade, 99.5%), and chloroform (AR grade, 99.5%) were all purchased from Sigma-Aldrich.

DPPH radical scavenging assay and determination of IC₅₀ values

DPPH radical scavenging activity of various extracts of *S. molle* and

Table 1. The percentage radical scavenging activity of various extracts from *S. molle* and *G. triacanthos* at various concentrations.

| Extract | Concentrations ($\mu\text{g mL}^{-1}$) | | | | |
|---------|--|------------|------------|------------|------------|
| | 250 | 500 | 1000 | 2000 | 3000 |
| SMMELS | 39.41±3.19 | 71.62±1.41 | 77.96±5.10 | 82.53±5.13 | 83.38±2.74 |
| SMMESB | 51.04±2.74 | 51.81±0.39 | 54.22±4.57 | 54.86±3.19 | 56.68±5.13 |
| SMCHSD | 8.20±1.02 | 21.37±4.57 | 22.23±2.29 | 63.40±2.69 | 68.82±3.17 |
| GTMELS | 39.50±3.49 | 62.47±5.18 | 66.43±5.14 | 84.04±0.61 | 86.29±2.27 |
| GTMESB | 35.97±1.02 | 44.87±3.29 | 59.94±2.72 | 60.05±4.40 | 63.40±2.69 |
| GTMETS | 67.10±7.06 | 89.31±0.88 | 90.15±0.81 | 92.00±0.35 | 92.36±0.11 |
| GTCHSD | 3.74±1.04 | 6.14±1.39 | 8.26±0.99 | 8.34±0.11 | 15.47±4.57 |
| GAMEOH | 52.53±4.64 | 54.30±0.82 | 56.29±2.64 | 64.44±2.04 | 69.42±7.25 |
| GAETAC | 76.96±1.50 | 92.49±5.18 | 93.12±0.39 | 93.67±4.06 | 94.57±1.02 |

SMMELS: *S. molle* methanolic leaves extract; SMMESB: *S. molle* methanolic stem-bark extract; SMCHSD: *S. molle* chloroform seed extract; GTMELS: *G. triacanthos* methanolic leaves extract; GTMESB: *G. triacanthos* methanolic stem-bark extract; GTMETS: *G. triacanthos* methanolic thorns extract; GTCHSD: *G. triacanthos* chloroform seed extract; GAMEOH: gallic acid in 50% methanol served as positive control for GTMESB, GTMESB, GTMETS, SMMELS and SMMESB; GAETAC: gallic acid in ethyl acetate served as positive control for GTCHSD and SMCHSD. All experiments were conducted in triplicate (n = 3) and reported as mean of three values together with standard deviation, ±SD.

G. triacanthos was conducted according to the method described in the literature (Sasidharan et al., 2007) with slight modification. Briefly, stock solutions of methanolic extracts were prepared at a concentration of 3.0 mg of extract in 1 mL of 50% methanol (v/v). Further dilutions were made from these stock solutions such that solution was obtained with concentrations of 3000, 2000, 1000, 500 and 250 μL for each extract. 50 μL of each one of them was mixed with 1 mL of 0.1 mM solution of DPPH in 50% methanol (v/v). The mixture without extract sample was used as blank and just spiked with 50 μL of 50% methanol (v/v). A stock solution of commercial antioxidant, gallic acid, of the same concentration in 50% methanol (v/v) was prepared and further dilutions were made as previously and served as positive control for methanolic extracts. Similarly, stock solutions of chloroform extracts were prepared at a concentration of 3.0 mg of extract in 1 mL of ethyl acetate.

Further dilutions were made from these stock solutions such that extract solution was obtained with concentrations of 3000, 2000, 1000, 500 and 250 μL for each extract. 50 μL of each one of them was mixed with 1 mL of 0.1 mM solution of DPPH in ethyl acetate. The mixture without extract sample was used as blank and just spiked with 50 μL of ethyl acetate. A stock solution of gallic acid of the same concentration in ethyl acetate was prepared and further dilutions were made as previously and served as positive control for chloroform extracts. The mixtures were incubated for 30 min and their optical density was measured at 517 nm. The IC_{50} values were calculated from graphs by plotting extract concentrations versus percentage inhibition of DPPH radical using Microsoft Excel.

The extract concentration that causes 50% reduction in the initial concentration of DPPH is defined as the IC_{50} value of extract which is important measure of potency for a given extract. Each experiment was carried out in triplicate and the averages of the three values were used to calculate IC_{50} values. Standard deviation was calculated for each concentration from the three values of the experiment. The ability to scavenge DPPH radical was calculated by Equation 1:

$$\text{DPPH radical scavenging activity (\%)} = ((A_0 - A_1) / A_0) \times 100 \quad (1)$$

Where:

A_0 = optical density of solution of DPPH radical and A_1 = optical

density of solution of DPPH radical + solution of extract (or optical density of solution of DPPH radical + solution of Gallic acid).

Statistical analysis

Results were expressed as means of three determinations. One way analysis of variance (ANOVA) was used to compare means at the significance level $p < 0.05$. All analysis were performed by Microsoft Excel software.

RESULTS AND DISCUSSION

Table 1 shows the DPPH radical scavenging activity of various extracts of *S. molle* and *G. triacanthos*. *S. molle* methanolic leaves extract (SMMELS) showed 39.41±3.19, 71.62±1.41, 77.96±5.10, 82.53±5.13 and 83.38±2.74% of scavenging activity at concentrations of 250, 500, 1000, 2000 and 3000 $\mu\text{g mL}^{-1}$, respectively. While the positive control (GAMEOH), showed 52.53±4.64, 54.30±0.82, 56.29±2.64, 64.44±2.04 and 69.42±7.25% of scavenging activity at concentrations of 250, 500, 1000, 2000 and 3000 $\mu\text{g mL}^{-1}$, respectively. Thus, SMMELS exhibited higher radical scavenging activity than positive control at all concentrations except at concentration 250 $\mu\text{g mL}^{-1}$. At concentration 250 $\mu\text{g mL}^{-1}$, SMMELS showed only 39.41±3.19% of scavenging activity while GAMEOH showed 52.53±4.64% of scavenging activity. *S. molle* methanolic stem-bark extract (SMMESB) showed 51.04±2.74, 51.81±0.39, 54.22±4.57, 54.86±3.19 and 56.68±5.13% of scavenging activity at concentrations 250, 500, 1000, 2000 and 3000 $\mu\text{g mL}^{-1}$, respectively. This result showed that SMMESB has comparable activity as that of positive control at low concentrations and at high concentrations; the scavenging

Table 2. The IC₅₀ values of various extracts of *S. molle* and *G. triacanthos* based on DPPH radical scavenging assay.

| S/N | Extracts | IC ₅₀ (µg mL ⁻¹) |
|-----|----------|---|
| 1 | SMMELS | 476.43 |
| 2 | SMMESB | <250 |
| 3 | SMCHSD | ~3000 |
| 4 | GTMELS | 452.32 |
| 5 | GTMESB | 720.56 |
| 6 | GTMETS | <250 |
| 7 | GTCHSD | >3000 |
| 8 | GAMEOH | <250 |
| 9 | GAETAC | <250 |

SMMELS: *S. molle* methanolic leaves extract; SMMESB: *S. molle* methanolic stem-bark extract; SMCHSD: *S. molle* chloroform seed extract; GTMELS: *G. triacanthos* methanolic leaves extract; GTMESB: *G. triacanthos* methanolic stem-bark extract; GTMETS: *G. triacanthos* methanolic thorns extract; GTCHSD: *G. triacanthos* chloroform seed extract; GAMEOH: gallic acid in 50% methanol served as positive control for GTMESB, GTMESB, GTMETS, SMMELS and SMMESB; GAETAC: gallic acid in ethyl acetate served as positive control for GTCHSD and SMCHSD. All experiments were conducted in triplicate (n = 3) and reported as mean of three values together with standard deviation, ±SD.

activity was slightly higher. *S. molle* chloroform seed extract (SMCHSD) showed 8.20±1.02, 21.37±4.57, 22.23±2.29, 63.40±2.69 and 68.82±3.17% of scavenging activity at concentrations 250, 500, 1000, 2000 and 3000 µg mL⁻¹, respectively. In this case, the positive control (GAETAC) showed 76.96±1.50, 92.49±5.18, 93.12±0.39, 93.67±4.06 and 94.57±1.02% of scavenging activity at concentrations of 250, 500, 1000, 2000 and 3000 µg mL⁻¹, respectively. This result showed that SMCHSD exhibited very weak activity at low concentrations relative to positive control, GAETAC. However, at high concentrations such as 2000 and 3000 µg mL⁻¹, it showed higher scavenging activity of 63.40±2.69 and 68.82±3.17%, respectively. Among the three extracts (SMMELS, SMMESB and SMCHSD) from *S. molle*, SMMELS showed highest scavenging activity (refer to Table 1).

G. triacanthos methanolic leaves extract (GTMELS) showed 39.50±3.49, 62.47±5.18, 66.43±5.14, 84.04±0.61 and 86.29±2.27% of scavenging activity at concentrations 250, 500, 1000, 2000 and 3000 µg mL⁻¹, respectively. Thus, GTMELS exhibited higher radical scavenging activity than positive control (GAMEOH) at all concentrations except at concentration 250 µg mL⁻¹. At concentration 250 µg mL⁻¹, GTMELS showed only 39.50±3.49% of scavenging activity while GAMEOH showed higher scavenging activity of 52.53±4.64%. *G. triacanthos* methanolic stem-bark extract (GTMESB) showed 35.97±1.02, 44.87±3.29, 59.94±2.72, 60.05±4.40 and 63.40±2.69% of radical scavenging activity at concentrations 250, 500, 1000, 2000 and 3000 µg mL⁻¹, respectively. Therefore, GTMESB showed low scavenging activity of 35.97±1.02 and 44.87±3.29% at

concentrations 250 and 500 µg mL⁻¹, respectively, while GAMEOH showed scavenging activity of 52.53±4.64 and 54.30±0.82%, respectively. However, at concentrations 1000, 2000 and 3000 µg mL⁻¹, both GTMESB and GAMEOH showed comparable scavenging activity (Table 1). *G. triacanthos* methanolic thorns extract (GTMETS) showed scavenging activity of 67.10±7.06, 89.31±0.88, 90.15±0.81, 92.00±0.35 and 92.36±0.11% at concentrations 250, 500, 1000, 2000 and 3000 µg mL⁻¹, respectively. This result showed that GTMETS showed remarkably high scavenging activity at all concentrations compared to positive control, GAMEOH. *G. triacanthos* chloroform seed extract (GTCHSD) showed very weak activity of 3.74±1.04, 6.14±1.39, 8.26±0.99, 8.34±0.11 and 15.47±4.57% of scavenging activity at concentrations 250, 500, 1000, 2000 and 3000 µg mL⁻¹, respectively. Among the four extracts (GTMELS, GTMESB, GTMETS and GTCHSD) from *G. triacanthos*, GTMETS showed highest scavenging activity (Table 1).

The IC₅₀ values of various extracts of *S. molle*, and *G. triacanthos* are shown in Table 2. SMMELS, SMMESB and SMCHSD exhibited IC₅₀ values of 476.43, <250 and ~3000 µg mL⁻¹, respectively. SMMESB is the most potent with IC₅₀ value <250 µg mL⁻¹. GTMELS, GTMESB, GTMETS and GTCHSD exhibited IC₅₀ values of 452.32, 720.56, <250 and >3000 µg mL⁻¹, respectively. Of the four extracts from *G. triacanthos*, GTMETS was found to be the most potent with IC₅₀ value <250 µg mL⁻¹. The positive controls, GAMEOH and GAETAC both showed IC₅₀ value <250 µg mL⁻¹.

The radical scavenging activity of hexane, ethyl acetate, ethanol and methanol extracts from fruits of *S. molle* have previously been evaluated and their IC₅₀

values were found to be 539.4 ± 13.3 , 30.7 ± 0.9 , 12.5 ± 0.4 and $4.4 \pm 0.2 \mu\text{g mL}^{-1}$, respectively (Abir et al., 2016). The essential oils from leaves, stems and fruits of *S. molle* showed IC_{50} values 3586 ± 119.0 , 3559.2 ± 122.0 and $>10000 \mu\text{g mL}^{-1}$, respectively (Abir et al., 2016). However, methanolic extracts were obtained from leaves and stem-bark (SMMELS and SMMESB) and their IC_{50} values were found to be 476.43 and $<250 \mu\text{g mL}^{-1}$, respectively. This means that these two extracts exhibited much higher scavenging activity than essential oils from leaves and stems. The essential oils from leaves and fruits of *S. molle* collected from two different regions in Algeria showed IC_{50} values ranging from 6900 to $8600 \mu\text{g mL}^{-1}$ (Abderrahim et al., 2018). However, in the present study, SMMELS and SMCHSD showed lower IC_{50} values of 476.43 and $3000 \mu\text{g mL}^{-1}$, respectively.

The essential oil from fruits of *S. molle* collected from Sfax, Tunisia showed IC_{50} values $3607.6 \pm 104.0 \mu\text{g mL}^{-1}$ in the DPPH assay and $257 \pm 10.3 \mu\text{g mL}^{-1}$ in the ABTS assay (Bendaoud et al., 2010). However, the chloroform seed extract (SMCHSD) from the present study showed slightly lower IC_{50} value of about $3000 \mu\text{g mL}^{-1}$ in the DPPH assay. The essential oils from fruits of *S. molle* collected from Mograne, Tunisia showed scavenging activity ranging from ~3 to ~28% in the DPPH assay (Hosni et al., 2011). The essential oil from a branch of *S. molle* collected from Alexandria, Egypt and its methanol, methylene chloride and water extracts showed IC_{50} values 13.11 ± 3.00 , 228.66 ± 1.12 , 334.11 ± 1.53 and $12.66 \pm 2.15 \mu\text{g mL}^{-1}$, respectively (Mohamed et al., 2016). The methanolic stem-bark extract (SMMESB) from the present study also showed a comparable IC_{50} value of $<250 \mu\text{g mL}^{-1}$.

The ethanolic extract from leaves of *G. triacanthos* exhibited 97.89% antioxidant activity in the *in-vivo* assay (Mohammed et al., 2014). Luteolin-7-O- β -glucopyranoside, a pure compound, isolated from aqueous ethanol fraction exhibited 91.80% scavenging activity (Mohammed et al., 2014). The ethanolic extract from seeds of *G. triacanthos* collected from Porto, Portugal showed 18.77% scavenging activity and showed IC_{50} value of $13310 \pm 0.67 \mu\text{g mL}^{-1}$ (Miguel, 2010). Some fractions from this ethanolic extract showed scavenging activity ranging from 61.88 to 71.59% and showed IC_{50} values ranging from 1400 ± 0.37 to $4170 \pm 0.32 \mu\text{g mL}^{-1}$ (Miguel, 2010).

Conclusion

DPPH radical scavenging activity of methanolic and chloroform extracts obtained from various parts of two medicinal plants *viz.* *S. molle* and *G. triacanthos* collected from the Kingdom of Lesotho have been evaluated. The methanolic extracts from *S. molle* showed scavenging activity ranging from 35.97 ± 1.02 to $83.38 \pm 2.74\%$, while the chloroform seed extract showed scavenging activity

ranging from 8.20 ± 1.02 to $68.82 \pm 3.17\%$. The methanolic extracts from *G. triacanthos* showed scavenging activity ranging from 35.97 ± 1.02 to $92.36 \pm 0.11\%$, while the chloroform seed extract showed scavenging activity ranging from 3.74 ± 1.04 to $15.47 \pm 4.57\%$. From this study, it was concluded that the extracts from these two medicinal plants, *S. molle* and *G. triacanthos*, showed promising antioxidant activity. The IC_{50} values of these extracts were also determined and found to be between <250 and $3000 \mu\text{g mL}^{-1}$.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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