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Research article

Isolation and identification of metabolites from ethyl acetate leaf extract of *Solenostemma argel*

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Abstract

Solenostemma argel is well-known in some parts of Sudan as a medicinal plant, where it is used as an anti-spasmodic, antidiabetic and anti-inflammatory because of its many useful secondary metabolites. This study identified and quantified the major metabolites of ethyl acetate extracts of *S. argel* leaves. Dried leaves of *S. argel* were macerated, extracted and subjected to liquid-liquid partitioning. The metabolites of the ethyl acetate extract were purified and isolated using a combination of chromatographic techniques (silica gel column chromatography and preparative thin layer chromatography). The structures of the isolated compounds were identified using infrared and nuclear magnetic resonance spectroscopy. The results showed that the three major metabolites of the ethyl acetate extract of *S. argel* leaves were kaempferol with 0.1% yield, kaempferol-3-O-β-D-glucopyranoside (astragalin) with 6.0% yield and kaempferol-3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl with 16% yield.

Introduction

Solenostemma argel (Hayne) is a perennial herb, belonging to the family Asclepiadaceae, distributed widely in the north of Africa and Arabian Peninsula (Teia, 2018). *S. argel* is available abundantly in the north region in Sudan and is locally known as 'Hargal'. It has been used as an herbal folk medicine for the treatment of bronchitis, measles, suppurating wounds, gastro-intestinal cramps, stomachache, colic, cold, and urinary tract infections (El-Shiekh et al., 2021; Shafek et al., 2012). Several researchers have evaluated the biological activities of *S. argel*, including antispasmodic (El Tahir et al., 1987; Innocenti et al., 2010), anti-inflammatory (Innocenti et al., 2005; Ibrahim et al., 2015; Benmaarouf et al., 2020), antimicrobial and antioxidant (Shafek et al., 2012; Al-Deen and Al-Naqeb, 2014; Shahat et al., 2017), anti-obesity (El-Shiekh et al., 2019), anti-hepatotoxic (Ahmed, 2017), anti-arthritic effects (El-Shiekh et al., 2021) and inhibitory activity of cholinesterase (Demmak et al., 2019). In addition, the methanolic extract of the leaves of *S. argel* had good hypoglycemic potency (Al-Deen and Al-Naqeb,

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2014). *S. argel* has been reported to have high nutritional values and significance in mitigating anemia, as well as reduced hypercholesterolemia (Osman et al., 2013; Osman et al., 2015). Abouzaid et al. (2018) reported on the potency of *S. argel* against lung cancer. In addition, *S. argel* or γ-irradiation or both had antitumor activities (Hanafi and Mansour, 2010). Furthermore, El-Shiekh et al. (2021) reported that the mucilage fraction of *S. argel* had significant gastroprotective effects and could be a promising therapy for ulcer. Therefore, *S. argel* is well known as an important source of many biologically active compounds.

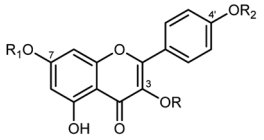
Several secondary metabolites have been isolated from the leaves and stems of *S. argel* and identified as kaempferol (1), and its glucosides: 3-O-glucopyranoside (astragalin) (2), 7-*O*-α-L-arabinoside (3), 3-O-β-D-xyloside (4), 7-O-α-L-rhamnoside (5), 7-O-α-L-arabinoside (6), 3,7-di-O-α-L-glucoside (7), 3,7-di-O-α-L-rhamnoside (8), 3,4'-di-O-β-D-glucoside (9), 7,4'-di-O-β-D-glucoside (10), 3-*O*-glucuronide (11), 3-O-glucopyranosyl (1→6)-rhamnopyranose (3-O-rutinoside) (12), 3-O-α-rhamnopyranosyl(1→2)-β-glucopyranoside (3-O-neohesperidoside) (13), 3-O-α-D-glucopyranosyl(1→2)-β-D-xylopyranoside (14), 3-O-α-L-arabinopyranosyl(1→2)-β-D-galactopyranoside (15). Other flavonoids are also found in *S. argel* such as quercetin-3-β-D-glucopyranosyl (1→6)-α-L-rhamnopyranoside (rutin) (16), quercetin-3-O-α-rhamnopyranosyl(1→2)-β-glucopyranoside (quercetin 3-O-neohesperidoside) (17), isorhamnetin-3-O-β-D-glucoside (18), quercetin -3-O-D-glucopyranoside (19) and luteolin (20) (Michael, 1998; Kamel et al., 2000; El-Askary, 2003; Ahmed, 2005; Heneidak et al., 2006; Shafek et al., 2012; Demmak et al., 2019) (Fig. 1). Other classes of metabolites have been isolated from butanol and chloroform extract obtained from liquid fractionation or directly from the crude extract and identified using ultra-performance liquid chromatography coupled with high resolution mass spectrometry (El-Shiekh et al., 2021).

The isolation and identification of structures of metabolites of Sudanese plants are vital because their active ingredients constitute important resources for pharmaceutical manufacturers to provide useful medicines. Preliminary tests of *S. argel* extracts showed that ethyl acetate extract had the greatest anti-spasmodic activity compared to hexane and chloroform extracts. The current study reported the analysis of one of the important medicinal plants in Sudan namely *S. argel,* and the isolation of compounds by changing the solvent to ethyl acetate with the aim of increasing the efficiency of the extraction.

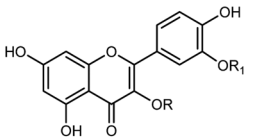
Materials and Methods

General

Methanol (MeOH), ethanol (EtOH), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), *n*-hexane (Hex) and chloroform ($CHCl_3$) were purchased from Kanto Chemical Co., Inc. and used without purification. Thin layer chromatography (TLC) and preparative TLC were performed using Merck-KGaA silica gel 60 F_{254} pre-coated plates (0.25 mm and 0.5 mm, respectively), and visualized using short-wave ultraviolet (UV) light, phosphomolybdic acid stain and Hanessian's stain (cerium molybdate) with heat. Column chromatography was performed using silica gel 60 N (neutral, sphere, particle size 0.063–0.210 mm). Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL 400 YH (400 MHz) instrument and the chemical shifts were reported in δ (parts per million, ppm) relative to deuterated solvent (CD_3OD at 3.31 ppm ^{1}H-NMR and 49.15 ppm ^{13}C-NMR). The following abbreviations were used to explain the ^{1}H-NMR data: chemical shift δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad) and coupling constant (*J*, measured in hertz). Infrared (IR) spectra were recorded on a ThermoFisher Scientific spectrometer Nicolet iS5 and the data were reported in terms of wave number (expressed as per centimeter, cm^{-1}).



	R	R_1	R_2
1	H	H	H
2	Glc	H	H
3	H	Ara	H
4	Xyl	H	H
5	H	Rha	H
6	H	Ara	H
7	Glc	Glc	H
8	Rha	Rha	H
9	Glc	H	Glc
10	H	Glc	Glc
11	Glu	H	H
12	Glc(1→6)Rha	H	H
13	Glc(1→2)Rha	H	H
14	Glc(1→2)Xyl	H	H
15	Glc(1→2)Gal	H	H



	R	R_1
16	Glc(1→6)Rha	H
17	Glc(1→2)Rha	H
18	Glc	CH_3
19	Glc	H

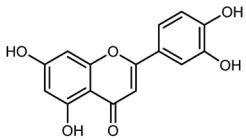


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Fig. 1 Structures of reported compounds from *S. argel*

Plant material

Solenostemma argel was collected from Abu-Hamad, North of Sudan-Sudan, and authenticated at the Herbarium of the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Centre for Research, Khartoum, Sudan.

Extraction

The extraction was performed following the reported method (Handa, 2008) with some modifications. Leaves of the collected plant were cleaned, dried in the shade at room temperature and disintegrated into a coarse powder. The dried powder was placed in a stoppered conical flask with aqueous ethanol (80%) for 3 d at room temperature with frequent agitation. Then, the obtained mixture was filtered and evaporated, and the marc was again macerated in aqueous ethanol 80% for 4 d at room temperature with frequent agitation. This daily filtration and evaporation cycle was repeated until the soluble matter had dissolved. The combined extracts were exposed to air to complete drying.

Liquid-Liquid fractionation

The fractionation was conducted according to Emran et al. (2015) with slight modification. The dried ethanolic extract (33 g) was dissolved in aqueous ethanol (20%); then, hexane was added to the aqueous ethanol in the ratio 2:1 (v/v). The resulting solution was shaken in a separating funnel. The hexane layer was removed; then, hexane was freshly added to the solution. This extraction with hexane was repeated until the hexane soluble materials had been extracted. The combined hexane layer was concentrated under vacuum pressure to obtain the hexane extract. The remaining aqueous ethanol was extracted with $CHCl_3$ and ethyl acetate to obtain the $CHCl_3$ and the ethyl acetate extracts, respectively. The ethanol extract was obtained by concentration of the remaining aqueous ethanol layer under reduced pressure (Fig. 2).

Purification and isolation

The ethyl acetate extract (4 g) was subjected to column chromatography using a column 3×40 cm (diameter × height) packed with silica gel (30 g). Different polarity solvents: 100% EtOAc, 16% MeOH in CH_2Cl_2, 28% MeOH in CH_2Cl_2, 37% MeOH in CH_2Cl_2 and 100% MeOH were used to obtain fraction 1 (F1), fraction 2 (F2), fraction 3 (F3) and fraction 4 (F4), respectively (Fig. 3). F1 (35 mg) was further purified using preparative thin layer chromatography (PLC, 10 cm × 10 cm) and eluted with 100% EtOAc, with the yellow-colored band collected, dissolved in methanol, passed through vacuum filtration and concentrated under vacuum pressure and labelled as compound 1. Using the same procedure, F2 (100 mg) was purified using 16.6% MeOH in CH_2Cl_2 and 28.0% MeOH in CH_2Cl_2 as eluted solvents to obtain compound 2. Finally, F3 (200 mg) was eluted with 37.5% MeOH in CH_2Cl_2 yielding compound 3 (Fig. 3). Compounds 1–3 were characterized and identified using spectroscopic methods (NMR and infrared (IR) spectroscopy).

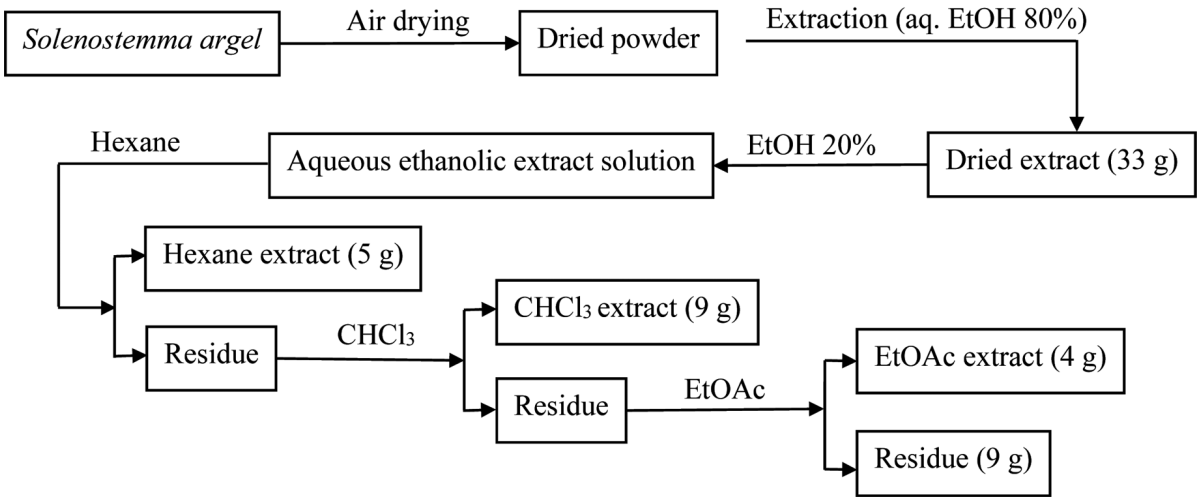

Fig. 2 Flowchart of extraction and fractionation compounds from *S. argel* leaves

Spectral data

Compound 1. IR (MeOH) *v* (3319.9 cm^{-1}, hydroxyl groups), (1652.8 cm^{-1}, α,β-unsaturated ketone) and (1509.6, 1444 cm^{-1}, an aromatic ring); ^{1}H-NMR (400 MHz, CD_3OD, rt) *δ*: 8.08 (2H, d, *J* = 8.0 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.0 Hz, H-3′, H-5′), 6.27 (1H, d, *J* = 2.0 Hz, H-8), 6.09 (1H, d, *J* = 2.0 Hz, H-6) ppm; ^{13}C-NMR (100 MHz, CD_3OD, rt) *δ*: 148.11 (C-2), 137.27 (C-3), 177.49 (C-4), 158.45 (C-5), 99.57 (C-6), 166.24 (C-7), 94.70 (C-8), 162.66 (C-9), 104.56 (C-10), 123.89 (C-1′), 130.82 (C-2′, 6′), 116.45 (C-3′, 5′) and 160.73 (C-4′) ppm.

Compound 2 IR (MeOH) *v* (3323 cm–1, hydroxyl group), (1653 cm^{-1}, α,β-unsaturated ketone) and (1496.8, 1444 cm^{-1}, aromatic ring); ^{1}H-NMR (400 MHz, CD_3OD, rt) *δ*: 8.06 (2H, d, *J* = 8.0 Hz, H-2′, H-6′), 6.89 (2H, d, *J* = 8.0 Hz, H-3′, H-5′), 6.35 (1H, d, *J* = 2.0 Hz, H-8), 6.17 (1H, d, *J* = 4.0 Hz, H-6), 5.22 (1H, d, *J* = 6.4 Hz, H-1″), 3.45 (1H, t, *J* = 8.0 Hz, H-2″), 3.41 (1H, t, *J* = 8.0 Hz, H-3″), 3.19 (1H, ddd, *J* = 9.6, 4.0, 2.4 Hz, H-5″) 3.70 (1H, dd, *J* = 8.0, 4.0 Hz, H-6″a), 3.55 (1H, dd, *J* = 4.0, 8.0 Hz, H-6″b) ppm; ^{13}C-NMR (100 MHz, CD_3OD, rt) *δ*: ppm 158.80 (C-2), 135.51 (C-3), 179.44 (C-4), 163.13 (C-5), 100.66 (C-6), 168.09 (C-7), 95.33 (C-8), 158.94 (C-9), 105.31 (C-10), 122.94 (C-1′), 132.40 (C-2′, 6′), 116.23 (C-3′, 5′), 161.76 (C-4′), 104.32 (C-1″), 75.86 (C-2″), 78.18 (C-3″), 71.44 (C-4″), 78.57 (C-5″), 62.72 (C-6″) ppm.

Compound 3 IR (MeOH) *v* (3295.92 cm^{-1}, hydroxyl group), (1659.6 cm^{-1}, α,β-unsaturated ketone) and (1497.7, 1446.6 cm^{-1}, aromatic ring) functions; ^{1}H-NMR (400 MHz, CD_3OD, rt) *δ*: 8.05 (2H, d, *J* = 8.0 Hz, H-2′, H-6′), 6.89 (2H, d, *J* = 8.0 Hz, H-3′, H-5′), 6.31 (1H, s, H-8), 6.13 (1H, s, H-6), 5.74 (1H, d, *J* = 8.0, H-1″), 3.60 (1H, m, H-2″), 3.55 (1H, m, H-3″), 3.35 (1H, brs, H-4″), 3.23 (1H, m, H-5″), 3.72 (1H, dd, *J* = 4.0 Hz, H-6″a), 3.51 (1H, m, H-6″b), 5.23 (1H, brs, H-1‴), 4.00 (1H, m, H-2‴), 3.77 (1H, dd, *J* = 4.0 Hz, H-3‴), 3.33 (1H, brs, H-4‴), 4.03 (1H, dd, *J* = 8.0 Hz, H-5‴), 0.95 (3H, d, *J* = 8.0 Hz, H-6‴) ppm; ^{13}C-NMR (100 MHz, CD_3OD, rt) *δ*: 161.56 (C-2), 134.26 (C-3), 179.16 (C-4), 163.10 (C-5), 100.36 (C-6), 169.87 (C-7), 95.64 (C-8), 158.05 (C-9), 104.95 (C-10), 123.29 (C-1′), 132.16 (C-2′, 6′), 116.27 (C-3′, 5′), 158.84 (C-4′), 101.18 (C-1″), 80.16 (C-2″), 79.08 (C-3″), 71.89 (C-4″), 78.50 (C-5″), 62.67 (C-6″), 102.75 (C-1‴), 72.52 (C-2‴), 72.38 (C-3‴),74.18 (C-4‴), 70.07 (C-5‴), 17.67 (C-6‴) ppm.

Results and Discussion

The aqueous ethanol extract (33 g) yield from the leaves of *S. argel* was 28%. This dried extract was subjected to liquid-liquid fractionation using hexane, $CHCl_3$ and EtOAc in the sequence. The fatty acids and pigments were removed using hexane and $CHCl_3$ to avoid interference during instrumental analysis. Compared with other extracts, the EtOAc extract had a lower yield (Fig. 2). The ethyl acetate extract was subjected to column chromatographic separation and preparative TLC (PLC) to yield three compounds (1–3). By comparison, there was a high yield (16%) of compound 3, whereas the yield for compound 1 was only 0.1% yield in the EtOAc extract (Fig. 3). Thus, the dried powder of the plant leaves contained compound 1 (0.002%), compound 2 (0.2%) and compound 3 (0.5 %). The structure of these compounds was determined based on their IR and NMR spectra compared with reported data. Compound 1 was identified as kaempferol, compound 2 was kaempferol-3-O-β-D-glycopyronoside, and compound 3 was kaempferol-3-O-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl] (Fig. 4).

Kaempferol (compound 1) was isolated as a faint yellow solid. The TLC chromatogram showed one compound using CH_2Cl_2/MeOH 5/1 (Retention factor, R_f = 0.79) which has a yellow visible color, pink UV 254 nm and an orange color with phosphomolybdic acid (PMA) and Hanessian's stain with heating. Compared with the yield of kaempferol isolated from the stem of *S. argel* (0.009%) reported by Shafek et al. (2012) and from the whole plant (0.0086%) by El-Shiekh et al. (2019), the kaempferol yield from the leaves of *S. argel* was lower (0.002%). The structure of compound 1 was identified based on the comparison of the obtained NMR data with literature values (Wishart et al., 2009; Demmak et al., 2019).

Kaempferol-3-O-β-D-glucopyranoside (compound 2) was obtained as a yellow solid and had one spot based on TLC when developed with CH_2Cl_2/MeOH 5/1 (R_f = 0.32). The spot was pink under UV irradiation (254 nm) and orange using PMA and Hanessian's stain with heating. The yield from the dried powdered of the leaves was 0.2%, which was higher than the amount of kaempferol-3-O-β-D-glucopyranoside (0.04%) isolated from the aerial parts of the plant (Kamel et al., 2000). In the analysis of the NMR spectra, HMQC showed C-4″ that correlated with a proton in the chemical shift region 3.31–3.34 ppm, indicating that H-4″ overlapped with the protons of the solvent used. The analysis of the NMR spectra of compound 2 were used for its identification based on comparisons with reported values (Nowak and Wolbis, 2002; Kazuma et al., 2003; Ebada et al., 2008; Yamasaki et al., 2011; Demmak et al., 2019).

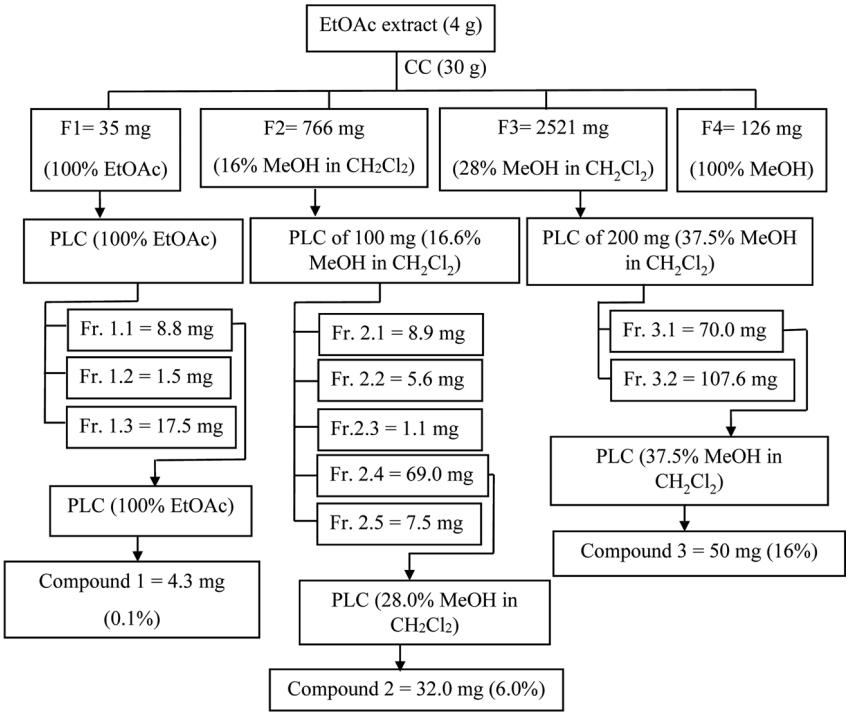

Fig. 3 Flowchart of purification and isolation of compounds from ethyl acetate extract, where CC = Column Chromatography, F1–F4 = Fraction 1–Fraction 4, Fr. = Fraction and PLC = Preparative Thin Layer Chromatography

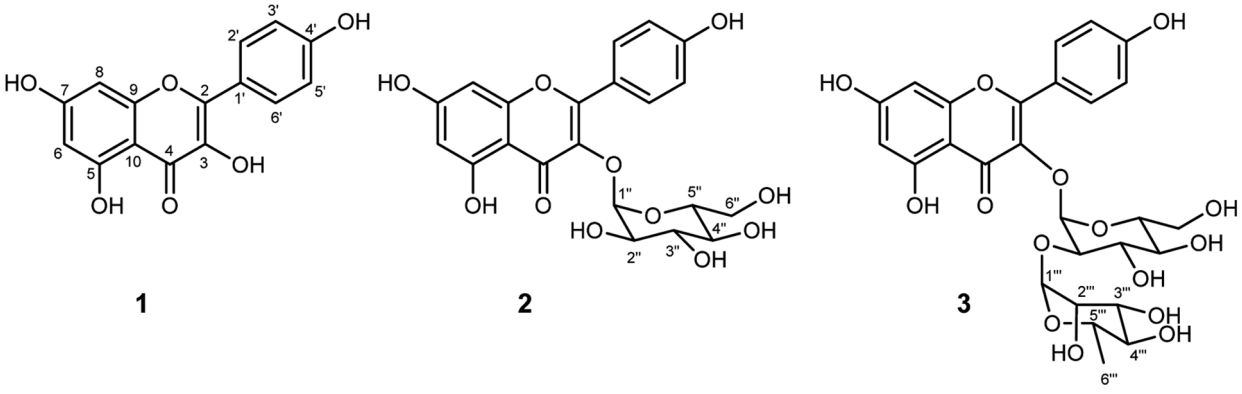

Fig. 4 Structures of isolated compounds (1–3) from ethyl acetate extract of *S. argel*

Kaempferol-3-O-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl (compound 3) was obtained as a yellow solid and appeared as one spot based on TLC when developed with CH_2Cl_2 / MeOH 5/2 (R_f = 0.34). The spot was pink when irradiated by UV 254 nm and orange using PMA and Hanessian's stain with heating. Compared with literature reporting on yields in other plants, the yield (0.5 %) of compound 3 was higher in the dried powdered of the leaves of *S. argel.* The obtained data were compatible with reported values (Kazuma et al., 2003; Yamasaki et al., 2011).

Three major compounds (1–3) of the ethyl acetate extract of the leaves of *S. argel* were successfully purified, isolated and identified. The yields of compounds (1–3) in the ethyl acetate extract and dried of the leaves were determined and the metabolites were identified as kaempferol and its glucosides. Kaempferol and its glucosides have been reported to possess pharmacological properties that can be of therapeutic benefit in treating diseases such as cholinesterase inhibitory activity (Demmak et al., 2019) and compound 3 can used for the treatment of diabetes mellitus (Yamasaki et al., 2011). The extraction methods reported here should be useful for the isolation of these compounds.

Conflict of Interests

The authors declare that there are no conflicts of interest.

Acknowledgements

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