Phytochemical analysis of the roots of Senna didymobotrya

Israel Alemayehu¹, Sisay Tadesse¹, Fikre Mammo¹, Belayhun Kibret² and Milkyas Endale¹*

¹Department of Chemistry, College of Natural and Computational Sciences, Hawassa University, P. O. Box 05, Hawassa, Ethiopia.
²Department of Biochemistry, College of Health Science and Medicine, Hawassa University, P. O. Box 05, Hawassa, Ethiopia.

Received 13 May, 2015; Accepted 27 August, 2015

Senna didymobotrya is a plant traditionally used for treatment of sexually transmitted diseases, purgative, appetizer, skin diseases, insecticidal and antibiotic. Phytochemical screening of the CH₂Cl₂/CH₃OH (1:1) and CH₃OH (100%) roots extract of the plant revealed the presence of phenolic compounds, anthraquinones, tannins, saponins, napthoquinones and terpenes. Silica gel column chromatographic separation of CH₂Cl₂/CH₃OH (1:1) roots extract afforded a stilbenoid derivative (1) and phenylanthraquinone (2). These compounds are reported for the first time from the genus. The structures of the compounds were determined by spectroscopic techniques (UV-Vis, IR, NMR; 1D and 2D).

Key words: Phenylanthraquinones, Phenolic compounds, Senna didymobotrya, stilbenoids.

INTRODUCTION

Natural products have been playing a dominant role in drug discovery efforts for treatment of human and livestock diseases (Newman and Cragg, 2012). The genus Senna L. (Fabaceae, Leguminosae, Caesalpinioideae), commonly known as popcorn cassia, is a large and diverse genus native to tropical Africa found from Congo east to Ethiopia and south to Namibia, Zimbabwe and Mozambique (Erwin and Barneby, 1982, Randall and Barlow, 1998).

In Ethiopia, there are eighteen species belonging to genus Senna. The genus Senna is known to be a rich source of anthraquinones, flavonoids and anthraquinone glycosides and flavonoid glycosides (Singh and Tiwari, 1982). Reports on pharmacological activity of Senna species suggested that the genus is a potential source of enormous bioactive compounds (Anthony et al., 2013; Ngule et al., 2013; Nyaberi et al., 2013). S. didymobotrya (Figure 1), widely used as laxative and purgatives in East
Africa, contains anthraquinones and their glycoside derivatives as main constituent (Spiller et al., 2003). In Ethiopia, the plant is found in dry and moist Kolla and Weyna Dega agro climatic zones of Arsii, Sidama, Wolega, Shewa and in the western part of Welo at 1,400-2,400 meter above sea level (Thulin et al., 1989). *S. didymobotrya* is used to stimulate lactation, induce uterine contraction and abortion when administered as root or leaf decoction (Santos et al., 1990; Jeyaseelan et al., 2010; Khan et al., 2011, Ngule et al., 2013). *S. didymobotrya* was found to contain phytochemicals such as tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and anthraquinones (Anthoney et al., 2013; Anthoney et al., 2014).

In our ongoing study to analyze the chemical constituents of medicinal plants found in Ethiopian flora, we hereby report a comprehensive phytochemical screening, isolation and characterization of two compounds from roots of *S. didymobotrya*.

**MATERIALS AND METHODS**

**Plant botanical description**

*S. didymobotrya* is a semi-deciduous, multi-branching shrub or small tree that can grow up to 25 feet tall, but generally remains much shorter (6 to 10 feet tall). When grown as an annual, it may only get a couple of feet tall. The pinnately compound leaves can grow up to 18 inches long, although container-grown plants generally have smaller leaves. The 8 to 18 paired oval leaflets are up to 3 inches long, each with mucronate apex. Bright green, leathery leaves and rachis are variably pubescent which blooms from spring through fall (but most freely in late summer when temperatures are warm), with dense flower spikes up to one foot tall. The erect axillary racemes have 20 to 30 rounded flower buds. Each unopened bud is enclosed by a brownish green or black bract that opens to allow the five slightly unequal petals to emerge. These bright, buttery yellow petals are incurved at first, but later become more spreading. The plant is common in deciduous bushland, along lake shores, streams, rivers and other damp areas, in grassland and woodland, from sea level up to 2400 m altitude. Sometimes, it is found in old plantations and in hedges near buildings and also grown as an ornamental plant world-wide.

**Plant material collection**

The root of *S. didymobotrya* was collected from natural forest around Wendo Genet, in Southern Nations Nationalities and Peoples region 275 km from the capital city, Addis Ababa in December 2014. The samples were confirmed by botanist Mr. Yadessa Gonfa, Department of Biology, Hawassa University and a voucher specimen (SD-071) was deposited at National Herbarium (ETH), Department of Biology, Addis Ababa University. The root or leaf decoction (Santos et al., 1990; Jeyaseelan et al., 2010; Khan et al., 2011, Ngule et al., 2013) of *S. didymobotrya* was found to contain phytochemicals such as tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and anthraquinones (Anthoney et al., 2013; Anthoney et al., 2014).

**Extraction and isolation**

Shaded dried and grounded roots (473 g) of *S. didymobotrya* were extracted with 4000 ml of MeOH: CH2Cl2 (1:1) (24 h × 3). The marc left was further extracted with 2000 ml methanol (100%) for (24 h × 3). The extracts were combined and concentrated under reduced pressure using rotary evaporator (Model R-205) on water bath at a temperature of 40 to 45 °C which produces reddish brown crude extracts 60.8 g (12.78%) and 9.0 g (1.91) for MeOH:CH2Cl2 (1:1) and methanol (100%), respectively. About 50 g of the crude extract was adsorbed with 30 g silica gel and subjected to silica gel column chromatography (40 cm length and 40 mm diameter, 220 g silica gel) and eluted with increasing gradient of ethyl acetate in n-hexane. A total of sixty two (62) fractions were collected each 100 ml. Based on TLC profile of the fractions (30% EtOAc in n-hexane), further purification was done on fractions 10 to 17 and fractions 38 to 48 to yield compounds 1 and 2, respectively.

**RESULTS**

Preliminary phytochemical screening tests of crude CH2Cl2:CH3OH (1:1) roots extract revealed the presence of anthraquinones, flavonoids, glycosides, terpenes, phenolic compounds, saponins and tannins (Table 1). Column chromatographic separation of the root extract afforded a stilbene derivative (1) and a phenylanthraquinone (2). A comprehensive structural determination of these compounds is discussed below.

**DISCUSSION**

Compound 1 was obtained as white powder (28 mg) with Rf value of 0.53 (30% ethyl acetate in n-hexane) solvent system. The UV-Vis spectrum showed absorbance peaks $\lambda_{max}$ at 305 nm and 315 nm attributed to $\pi$-$\pi^*$ transition of C=C double bond and transitions of lone pair of electrons n-$\pi^*$, respectively. The IR spectrum indicated...
Table 1. Phytochemical screening results.

| Phytochemical constituent | Observation | Inferences |
|---------------------------|-------------|------------|
| Tannins                   | Blue-black colour observed | +          |
| Saponins                  | Stable foam | +          |
| Flavonoids                | Magenta - red colour observed | +          |
| Terpenoid                 | Gray colour observed | +          |
| **Glycosides:**           |             |            |
| Liebermann's test         | Blue to green | +          |
| Salkowsk's test           | Red-brown colour observed | +          |
| Keller-Kilani test color observed | Brown ring at the inter phase | +          |
| Alkaloids                 | No precipitate observed | -          |
| Steroids                  | Blue-green ring was observed | +          |
| Phenols                   | Blue-green colour observed | +          |
| Anthroquinones            | Violet colour at lower phase | ±          |

Table 2. Complete NMR data of compound 1 in DMSO-d$_6$.

| Position | δ$_C$ (ppm) | δ$_H$ (ppm) | COSY | HMBC |
|----------|-------------|-------------|------|------|
| C-1''    | 126.1       | 6.8 (d, J = 16.00) | H$_1''$ ↔ H$_2''$ | H$_1''$ → C$_2''$,$1$,$6'$ |
| C-2''    | 128.3       | 6.9 (d, J = 16.00) | -    | H$_2''$ → C$_1''$,$1$,$6'$ |
| C-1      | 128.5       | -            | -    | -    |
| C-2      | 158.9       | -            | -    | -    |
| C-3      | 104.7       | 6.42 (d, J = 2.02) | -    | H$_3$ → C$_1,2,4,5$ |
| C-4      | 102.2       | 6.12 (t, J = 4.00) | H$_4$ ↔ H$_5$ | H$_4$ → C$_2,3,5,6$ |
| C-5      | 104.7       | 6.42 (d, J = 2.10) | -    | H$_5$ → C$_3,4,6$ |
| C-6      | 158.9       | -            | -    | -    |
| C-1'     | 139.7       | -            | -    | -    |
| C-2'     | 128.5       | 7.45 (d, J = 12.10) | H$_2''$ ↔ H$_3''$ | H$_2''$ → C$_1',3',6',2''$ |
| C-3'     | 115.6       | 6.74 (d, J = 12.00) | -    | H$_3''$ → C$_2',5$ |
| C-4'     | 157.7       | -            | -    | -    |
| C-5'     | 115.6       | 6.74 (d, J = 12.00) | -    | H$_5''$ → C$_6',3$ |
| C-6'     | 128.5       | 7.45 (d, J = 12.10) | -    | H$_6''$ → C$_1',2',5',2''$ |
| 2-OH     | 2'-OH       | 9.21 s       | -    | -    |
| 6-OH     | 6'-OH       | 9.21 s       | -    | -    |
| 4'-OH    | 4'-OH       | 9.61 s       | -    | -    |

vibrations around 3400 cm$^{-1}$ (hydroxyl group), 2936 cm$^{-1}$ (C-H stretching vibrations), 1640 cm$^{-1}$ (C=C) and 1518 cm$^{-1}$ due to the presence of benzene ring. The $^1$H NMR spectrum (DMSO-d$_6$, Table 2) revealed the existence three-aromatic protons with ABC spin system [δ $6.12$ (t, J = 4.0 Hz), δ $6.39$ to $6.41$ (d, J = 1.12 Hz), δ $6.39$ to $6.41$ (d, J = 1.12 Hz)] suggesting a 2,6-dihydroxyphenyl moiety coupled with two vinyl AB-type trans-olefinic protons resonating at [δ $6.82$ (d, J = 16 Hz), δ $6.93$ (d, J = 16 Hz)]. Two sets of symmetrical protons with AA'BB' spin pattern was observed as part of 4-hydroxy phenyl moiety at δ $6.72$ (2H, d, J = 8.0 Hz) and δ $7.43$ (2H, d, J = 8.0 Hz). Three hydroxyl groups at δ $9.25$ (2H, at positions C-2' and C-6') and a 4-hydroxy phenyl moiety at δ $9.65$ (at position C-4') are all evident from the $^1$H NMR spectrum.

The $^{13}$C NMR spectrum showed fourteen carbon signals of which three were oxygenated quaternary carbons at δ $158.9$ (C-2' and C-6') and δ $157.7$ (C-4'), two olefinic carbon atoms at δ $126.1$ and δ $128.3$ (C-1, 2), two quaternary carbons at δ $128.5$ and δ $139.7$ (C-1', 1') and seven aromatic methine (δ $102.2$, δ $104.7$, δ $115.6$, δ $126.1$ and δ $128.3$). The connectivity of aromatic
Table 3. Comparison of spectral data for compound 1 and trans-resveratrol.

| Position | Compound 1 | Resveratrol |
|----------|------------|-------------|
|          | $^1$H ($\delta$ in ppm) | $^{13}$C (ppm) | $^1$H ($\delta$ in ppm) | $^{13}$C (ppm) |
| 1        | -          | 128.5       | -          | 128.1       |
| 2        | 6.37, d, 2.20 | 104.7     | 6.37, d, 2.20 | 104.4       |
| 3        | -          | 158.9       | -          | 158.6       |
| 4        | 6.10, t, 2.20 | 102.2     | 6.10, t, 2.20 | 101.9       |
| 5        | -          | 158.9       | -          | 158.6       |
| 6        | 6.37, d, 2.20 | 104.5     | 6.37, d, 2.20 | 104.5       |
| 1''      | 6.79, d, 16.00 | 126.1     | 6.79, d, 16.20 | 125.7       |
| 2''      | 6.90, d, 16.00 | 128.3     | 6.90, d, 16.20 | 127.9       |
| 1'       | -          | 139.7       | -          | 139.4       |
| 2'       | 7.38, d, 8.30 | 128.5     | 7.38, d, 8.30 | 127.9       |
| 3'       | 6.74, d, 8.30 | 115.6     | 6.74, d, 8.30 | 115.6       |
| 4'       | -          | 157.7       | -          | 157.3       |
| 5'       | 6.74, d, 8.30 | 115.6     | 6.74, d, 8.30 | 115.3       |
| 6'       | 7.38, d, 8.30 | 128.5     | 7.38, d, 8.30 | 127.9       |
| 3'-OH    | 9.20, bs   | -          | 9.30, bs   | -           |
| 5'-OH    | 9.20, bs   | -          | 9.30, bs   | -           |
| 4''-OH   | 9.60, bs   | -          | 9.30, bs   | -           |

The difference between isolated compound 1 and trans-resveratrol (Sivakumar et al., 2013) is the position of the hydroxyl group in 2,6-dihydroxy phenyl moiety. In compound 1, it is situated in 2,4- position while in trans-resveratrol the position assigned is 3,5- and $\delta$-value has varied slightly according to the change in position.

Compound 2 was found as orange crystalline powder with $R_f$ value of 0.62 (30% ethyl acetate in n-hexane as eluent). The $^1$H NMR spectrum (Table 4) of the compound showed anthraquinone moiety with two chelated -OH protons resonating at $\delta$ 12.05 and $\delta$ 12.25 assigned for hydroxyl groups at C-1 and C-8, benzylic methine proton at $\delta$ 7.13 (s, H-2), three aromatic methine protons with ABC spin system [(\(\delta 7.55, d, J = 7.6, CH\)), (7.75, t, \(J = 7.2\) Hz, CH), (\(\delta 7.30, d, J = 7.4\) Hz, CH)] assigned for 5, 6, and 7-positions, respectively on ring C. In addition to the above aromatic protons patterns, methyl protons at $\delta$ 2.45, (s, 3 H, H-3) assigned for methyl groups attached to phenyl ring is observed. The spectrum also revealed the presence of three aromatic ABX type protons signals [(\(\delta 7.55, d, J = 8.4\) Hz, CH), (\(\delta 7.55, dd, J = 12, 8\) Hz, CH), (\(\delta 7.84, d, J = 7.8\) Hz, CH)] on phenyl moiety which are assigned for the 3', 5', and 6' positions, respectively. Phenyl moiety has side methylene $\delta$ 3.78 (2 H, s, C-2'-CH$_2$), hydroxyl group (1 H, s, C-2'-CH$_2$-OH) on carbon C-2' position beside the hydroxyl group at C-4'.

The $^{13}$C NMR spectrum (Table 5) of the compound 2 showed a total of twenty two carbon atoms peaks of which two bands corresponding to chelated carbonyl quaternary carbons at C-1 (\(\delta 128.5\)) from 2, 6 dihydroxyphenyl moiety to C-1'' (\(\delta 126.1\)) of olefinic carbon and that of C-1' (\(\delta 139.7\)) of 4'-hydroxy phenylmoiety to C-1'' (\(\delta 128.3\)) of olefinic carbon was established by gHMBC correlations (Figure 2). The position of hydroxyl groups to C-2 and C-6 in 2,6-dihydroxy phenyl moiety is fixed by using their gHMBC correlation supported by the correlation of triplet proton at $\delta$ 6.12 (H-3) to $\delta$ 102.2 (C-4) (Figure 2) and a doublet of doublet proton resonating at $\delta$ 6.42 (d, H-3 and H-5) on carbon at $\delta$ 104.7 (C-4). Thus, based on the above spectroscopic data, the compound was found to be a resveratrol derivative (2, 6, 4'- trihydroxy-trans-stilbene) (Table 3) isolated for the first time from the genus.

Trans-resveratrol (1, 4'', 6-trihydroxy-trans-stilbene; t-RES) is a polyphenolic compound within the stilbene class. It has been found in high concentrations in a wide variety of plants, including grapes, peanuts, berries, pines and traditional oriental medicine plants (Burns et al., 2002). Presence of this compound in high concentration was reported in grape juice and, especially, in red wine (Bavaresco, 2003; Fremont, 2000). In plants, t-RES is synthesized in response to stress conditions such as trauma, exposure to ozone and fungal infection acting as phytoalexin, a class of antibiotics of plant origin (Schubert et al., 1997; Soleas et al., 1997). Other abiotic elicitors, such as ultraviolet rays and heavy metals, can trigger t-RES production (Bavaresco, 2003).
Table 4. NMR (CDCl₃, 400MHz) spectral data of compound 2.

| Position | DEPT-135 δC (ppm) | δH (ppm) |
|----------|--------------------|-----------|
| **Anthraquinone moiety** | | |
| 1 C-OH | 162.7 | 12.05 s |
| 2 CH | 124.4 | 7.13 s |
| 3 C | 149.4 | - |
| 4 C | 121.4 | - |
| 5 CH | 119.9 | 7.55 (1H, d, J = 8.4 Hz) |
| 6 CH | 137.0 | 7.75 (1H, t, J = 8/ 7.6 Hz) |
| 7 CH | 124.6 | 7.32 (1H, d, J = 8.1 Hz) |
| 8 C-OH | 162.4 | 12.25 (1H s) |
| 9 C | 192.5 | - |
| 10 C | 182.0 | - |
| 4a C | 115.9 | - |
| 8a C | 115.9 | - |
| 9a C | 113.8 | - |
| 10a C | 133.3 | - |
| C3-CH₃ | CH3 | 22.3 | 2.45 (3H,s) |
| **Phenyl moiety** | | |
| 1' C | 133.3 | - |
| 2' C | 133.7 | - |
| 3' CH | 121.8 | 7.65 (1H, d, J = 1.2 Hz) |
| 4' C-OH | 149.4 | 7.54 (1H, s) |
| 5' CH | 124.3 | 7.45 (1H, dd, J = 8.2, 1.2 Hz) |
| 6' CH | 136.9 | 7.84 (1H, d, J = 8.2 Hz) |
| C2'-CH₂OH | CH₂-OH | 68.2 | 3.78 (2H, s) |
| C4'-OH | - | - | 7.28 (1H,s) |

signals at δ 182.0 (C-10) and δ 192.5 (C-9) with hydroxyl groups at positions C-1 and C-8 and non-chelated hydroxyl in position C-10, respectively in agreement with phenyl anthraquinones skeleton where phenyl moiety is linked at either C-4 or C-10 (Bringmann et al., 2008). From ¹³C NMR spectrum the presence of seven benzylicmethine carbons resonating (δ 124.4, δ 119.9, δ 136.9, δ 124.6) assigned to C-2, C-5, C-6 and C-7 together with one methyl carbon (δ 22.3) and (δ 121.8 (C-3'), δ 124.3 (C-5'), δ 136.9 (C-6'), on phenyl moiety, respectively. Five peaks for oxygenated quaternary carbons resonating at δ 162.7, δ 162.4, δ 192.5 and δ 182.0 assigned for positions C-1, C-8, C-9 and C-10 of the anthraquinone moiety were clearly evident. Likewise, one oxygenated quaternary carbon peak, which is part of phenyl moiety, was observed at δ 149.4 (C-4). The spectra also comprises bands for non-oxygenated quaternary carbons (149.4, 121.4, 115.9, 115.8, 113.8 and δ 133.3) assigned on carbon positions C-3, C-4, C-4 a, C-8 a, C-9 a, C-10 a, for anthraquinone moiety and δ 133.3 (C-1) and δ 133.7 (C-2') positions of phenyl moiety to which methylene hydroxide group is (δ 68.2) is linked. From the above data and literatures for anthraquinone moiety (Zhang et al., 2012), the structure of compound 2 (Figure 3) was found to be 4-(2'-oxymethylene-4'-hydroxyphenyl) chrysophanol.

**CONCLUSION AND RECOMMENDATION**

This work is one of the few attempts to analyze the phytochemical constituents of polar extracts of the roots of *S. didymobotrya* from Ethiopian origin. Phytochemical screening of the roots revealed the presence of anthraquinones, terpenoids, flavonoids, phenolic compounds and tannins. Silica gel column chromatographic separation of the CH₂Cl₂/CH₃OH (1:1) yielded a resveratrol derivative (1) and a phenyl anthraquinone derivative (2), reported for the first time from the genus. In agreement with the previous studies, the wide traditional use of the plant may be attributed to its rich anthraquinones, phenolic compounds, flavonoids and resveratrols content. Further work is recommended.
Table 5. Comparison of $^1$H and $^{13}$C NMR spectral data of anthraquinone part of compound 2 and Chrysophanol from literature (Zhang et al., 2012).

| Position | $^1$H (δ in ppm) of compound 2 | Literature (Zhang et al., 2012) | $^{13}$C (δ in ppm) of compound 2 | Literature (Zhang et al., 2012) |
|----------|-------------------------------|---------------------------------|----------------------------------|---------------------------------|
| 1        | -                             | 162.7                           | 162.7                            |                                 |
| 2        | 7.13 (s, 1H)                  | 7.11 (1H, d, J = 1)             | 124.4                            | 124.5                           |
| 3        | -                             | 149.4                           | 149.3                            |                                 |
| 4        | -                             | 7.66 (1H, d, J = 1)             | 121.4                            | 121.4                           |
| 5        | 7.55 (1H, dd, J = 8.1)        | 7.83 (1H, dd, J = 8.5, 1)       | 119.9                            | 119.9                           |
| 6        | 7.75 (1H, t, J = 8.2)         | 7.67 (1H, t, J = 8.5)           | 137.0                            | 136.9                           |
| 7        | 7.32 (1H, dd, J = 8.5)        | 7.30 (1H, dd, J = 8.5, 1)       | 128.6                            | 124.6                           |
| 8        | -                             | 162.4                           | 162.4                            |                                 |
| 9        | -                             | 192.5                           | 192.6                            |                                 |
| 10       | -                             | 182.0                           | 182.0                            |                                 |
| C$_3$-CH$_3$ | 2.45 (3H, s) | 2.47 (3H, s) | 22.3 | 22.2 |
| 4a       | -                             | 115.9                           | 115.9                            |                                 |
| 8a       | -                             | 115.7                           | 115.5                            |                                 |
| 9a       | -                             | 113.8                           | 108.2                            |                                 |
| 11       | -                             | 133.3                           | 135.7                            |                                 |
| 1-OH     | 12.05                         | 12.03                           | -                                | -                               |
| 8-OH     | 12.25                         | 12.13                           | -                                | -                               |

Figure 1. *S.didymobotrya*. (a, b): Flowers with unopened buds; (c): Early forming pods; (d): Matured pods; (e): Dried pods; (f): Dried seeds (Photo taken by Israel Alemayehu on December, 2014).
on various parts of the plant and polar extracts so as to validate the traditional use of the plant and identify more bioactive secondary metabolites in support of its traditional use.

ACKNOWLEDGEMENTS

We are indebted to the Department of Chemistry, Addis Ababa University for allowing us to use NMR (400 MHz), UV-Vis and IR instruments. This research was partly supported by School of Graduate Studies, Hawassa University.

Conflicts of interest

Authors have none to declare.

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