Regular Article

Journal of Medicinal Herbs and Ethnomedicine 2015, 1: 79-83
http://scienceflora.org/journals/index.php/jmhe/
doi: 10.5455/jmhe.2015-08-016

Androgenic steroid composition of the hexane/ methanol whole plant extract of *Solanecio tuberosus* (Selbilla) around Lake Tana Northwest Ethiopia

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Received: 17.08.2015
Revised: 02.09.2015
Accepted: 10.09.2015
Published: 28.09.2015

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ABSTRACT

The plant kingdom is a treasure house of potential drugs. Steroids are very important molecules that mediate a wide range of biological functions in the human body. Today, there is no study reporting the androgenic steroid content of *Solanecio tuberosus* extract. In this study, the androgenic steroid content of hexane/methanol extract of *S. tuberosus* was analyzed by Fourier transform (FT)-infrared spectroscopy, ultraviolet-visible spectroscopy, FT-magnetic resonance spectroscopy, and thin layer chromatography. Androgenic steroid derivatives such as predinsolone, testosterone cypionate, and methyltestostrone were identified. The present study showed that *S. tuberosus* can be considered as a potential remedy to improve the sexual and physical health of men as they go aged and in testosterone deficient condition if further studies are conducted on safety and efficacy of the plant material.

KEY WORDS: Medicinal plants, *Solanecio tuberosus*, steroids

INTRODUCTION

The plants are a very important potential source for drugs and drug discovery. In recent years, there has been an increasing awareness about the importance of medicinal plants. Drugs from plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, and anti-hepatotoxic compounds (Tadesse and Demissew 1992; Giday *et al.*, 2009). About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants (Tadesse, 1986; Giday *et al.*, 2009).

Medicinal plants contain organic compounds which provide definite physiological action in the human body. These bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for the synthesis of complex chemical substances (Tadesse and Demissew, 1992; Giday *et al.*, 2009 Bathori *et at.*,2008; Vasudeva *et al.*,2008).

The genus *Solanecio* (Asteraceae) contains 17 species confined to tropical Africa, Yemen, and Madagascar. In Ethiopian traditional medicine chewing of the leaves of one of the *Solanecio* species called *Solanecio angulatus* is said to give an instant relief of tooth pain (Tadesse, 2004; Asres *et al.*, 2008). In Tanzania, it is one of the most common used plants for the treatment of dermatological and gastrointestinal problems (Schlage *et al.*, 1999). In Cameroon, a handful of young leaves of *Solanecio mannii* mixed with other plants is used as an enema for the treatment of epilepsy (Noumi and Fozi, 2003).

Solanecio tuberosus is an erect perennial herb, which grows up to only 30-60 cm in height. Its florets are bright yellow or orange. Unlike the other members of the genus, it has a tuber, which is brown on the outside and whitish inside having a peppery smell (Tadesse, 2004). In Ethiopia, both

the aerial parts and the tubers of the *S. tuberosus* are used for wound healing and also for stomach problems (Asres *et al.*, 2008). There is one study about the pyrrolizidine alkaloids content of *S. tuberosus* by Asres *et al.* (2008). However, no report could be found regarding the androgenic steroids content of *S. tuberosus*.

MATERIALS AND METHODS

Study Area and Plant Material Collection

S. tuberosus was harvested during its growing season from the Lake Tana near the Abay River and from Chagni district located about 200 km away from Lake Tana in the Amhara region, Northwest Ethiopia. The area is found at 10°57'N and 36°30'E with an altitude of 1583 m above sea level. The average rain fall of the area is 1300-1800 mm/year with a temperature range of 22-37°C. The taxonomical classification was identified and confirmed by Dr. Ali Seid, Bahir Dar University, College of Science, Biology Department, and the species has been found deposited in the National herbarium, Department of biology Addis Ababa University by Asres *et al.* (2008).

Preparation of the Hexane Plant Extracts

The plant material (stem, leaves, and root) was washed with tap water to remove adherent material and oven dried at 70°C until all dry for 4 days. The dry plant material was then grinded to fine power. An amount of 40.0 g of the plant powder was weighed in an Erlenmeyer flask of 250 ml to which 140 ml of hexane (purity grade 99.9%) was added (the plant sample was submerged with solvent) and placed in water bath incubated at 40°C for 30 min stirred continuously for pre-extraction. The mixture was filtered using Wattman filter paper (0.8 mm thickness), followed by washing the Erlenmeyer flak with 40 ml of hexane and then with 100 ml of hexane. The filtrate was poured into a round-bottomed flask and concentrated in vacuum (at about 11 mmHg) up to 5-10 ml by means of rotary evaporator (2 rpm), utilizing a water bath at 40°C. This residue was brought in a 30 ml vessel and left overnight in a well-ventilated hood to evaporate the last traces of the solvent in the hexane pre-extract (Chiong *et al.*, 1992; Vaghasiya *et al.*, 2011; Saraswathi *et al.*, 2010; Sajjadi *et al* ., 2013).

Preparation of the Methanol Extract

The dried hexane extract was dissolved with 70 ml methanol-water (90:10) stirred continuously in water bath incubated at 40°C. The mixture was sonicated at 40°C for 30 min and filtered followed by washing the Erlenmeyer flask with 20 ml of 99% methanol. The filtrate was poured into a round-bottomed flask, and the solvent was evaporated by means of a rotary evaporator (2 rpm) in a vacuum completely. The dried 99% methanol extract was dissolved in a little methanol (99%) by using the sonicator-bath and poured into a 30 ml vessel to let it evaporate overnight in the hood (Chiong *et al.*, 1992; Vaghasiya *et al.*, 2011).

Instrumental Analysis

A part of crude extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise (Yadav and Agarwala, 2011). Another test was also performed by mixing crude extract with 2 ml of chloroform by adding 2 ml of each of concentrated H_2SO_4 and acetic acid into the mixture (Yadav and Agarwala, 2011; Saxena *et al.*, 2012). The thin layer chromatography plate with 20 cm × 20 cm dimension coated with silica gel as stationary phase, chloroform as mobile phase and cholesterol, 5α-cholestane, and highly refined testosterone reference standards were used for chromatographic analysis (Bhawani *et al.*, 2010; Dinan *et al.*, 2001). The spots were detected by the ultraviolet (UV) lamp (LF-206-LS, 6w, 365 nm tube). The retention time was measured, and retention factor was calculated. 0.5 mg of the extract was diluted with methanol for UV-visible (UV-VIS) spectrophotometric analysis by Agilent 8453 UV-VIS spectrophotometer in the Institute of Technology Laboratory, Bahir Dar University. The absorption spectrum of the sample was measured in the range 190-400 nm against the blank solution cholesterol as standard with similar preparation. The solid sample was analyzed by Spectrum 65 Fourier transform-infrared (FT-IR) spectrometer (PerkinElmer) in the range 4000-400/cm using KBr pellets (1.5 mm KBr micropellet) as standard. A total of two scan was performed with optimum resolution and data was obtained in ASCII files form that is analyzed by Origin version 8 software. Moreover, the sample was also analyzed by the nuclear magnetic resonance (NMR) instrument by using tetramethylsilane (TMS) $(CH_3)_4Si$, as an internal standard in Addis Ababa University, Department of Chemistry. The sample was prepared by using dimethyl sulfoxide (d_6) as solvent with optimum concentration for HNMR and C^{13} NMR analysis in such a way that NMR spectrum of sufficient signal-to-noise and resolution in a reasonable amount of time can be obtained. Proton NMR spectra were recorded. Proton chemical shifts were reported in ppm (δ) relative to internal TMS (d 0.0 ppm). Carbon NMR spectra were recorded, and carbon chemical shifts were reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard. All NMR spectra were acquired at ambient temperature

(Kasal *et al*., 2010; Abdulmalik *et al*., 2011). The results were compared with standards.

RESULT AND DISCUSSION

The hexane crude extract of the plant material containing steroid and related molecules that are soluble in hexane was weighed. Similarly, the crude methanol extract of the plant material containing steroids and their derivatives was weighed. The yields of both solvents plant crude extract were calculated as a percent (%) of the dried plant material used [Table 1].

In the lower chloroform layer of the sulfuric acid test, a red was produced. Similarly, in the concentrated H_2SO_4 and acetic acid test (Liebermann burchard test) greenish color development was observed. Characteristic color development in both tests indicated the presence of steroids in the plant crude extract. A study done by Yadav and Agarwala (2011) and Saxena *et al*. (2012) on steroid analysis indicated similar result with the current study for the steroids.

The thin layer chromatographic analysis showed that three major components are present in the sample. The retention factor of sample component X was 0.16 which is comparable with the predinsolone ($R_f = 0.19$) [Table 2]. The retention factor of sample component Y was 0.56 which is comparable with the methyltestostrone, and the retention factor of sample component Z was 0.94 which is comparable with testosterone cypionate. The study done by Chiong *et al*. (1992) in the American Laboratory Standards also reported that the retention factor of methyltestostorone and testosterone cypionate is 0.56 and 0.94, respectively, which were comparable with the results of this study.

The UV-VIS spectrum of the sample showed high or strong absorption in between 200 and 300 nm wavelength [Figure 1]. This data is similar with the data reported by Kasal *et al*. (2010).

The IR spectrum of the sample shows peaks in between 1600 and 1300/cm. This band is the characteristic peak for aromatics and hetroaromatics. Broad band is also observed above 3000/cm that indicate the existence of -C=C-H stretch in the steroid structure. It also has peaks at 2970 and 2850/cm, these peaks indicate the existence of -C-H- stretch in the steroid. The IR spectral

Table 1: Total steroid (calculated as percent of dried plant material) content of *S. tuberosus*

Species	Steroid content (%)	
	Hexane extract	Methanol extract
S. tuberosus	0.225	0.138

S. tuberosus: Solanecio tuberosus

Table 2: Distance moved by sample components and their retention factor of chromatographic result by using TLC plate with 20 cm×20 cm dimension coated with silica gel as stationary phase and chloroform as mobile phase (cholesterol, 5α-cholestane, and highly refined testosterone were used as reference standards)

Steroids	Distance moved by the steroids (in cm)	Distance moved by the mobile phase (in cm)	R_f value
Sample			
Component X (derivative of predinsolone)	2	12.8	0.16
Component Y (derivative of methyltestostrone)	7	12.8	0.55
Component Z (derivative of testosterone cypionate)	12	12.8	0.94

TLC: Thin layer chromatography, R_f: Retention factor

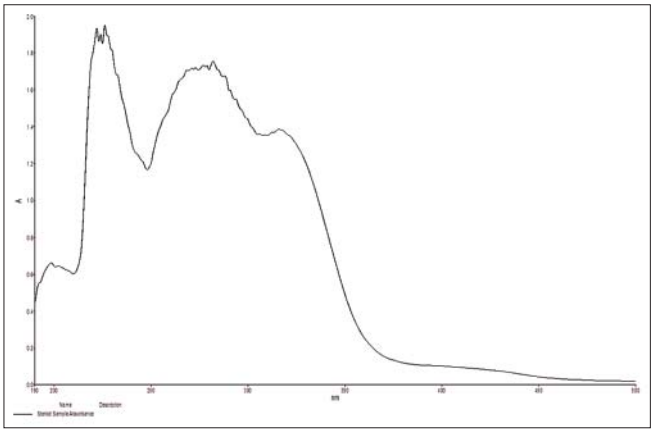

Figure 1: Ultraviolet-visible (UV-VIS) chromatogram of the sample done by using standard with similar preparation and wave length 190-400 nm in Agilent 8453 UV-VIS spectrophotometer

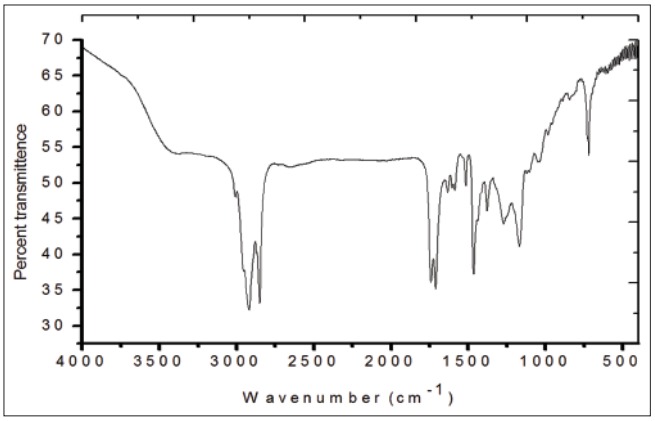

Figure 2: Infrared spectrum of sample analyzed by 65 Fourier transform-infrared spectrometer (Perkin Elmer) in the range 4000-400/cm using KBr pellets (1.5 mm KBr micropellet), as standard

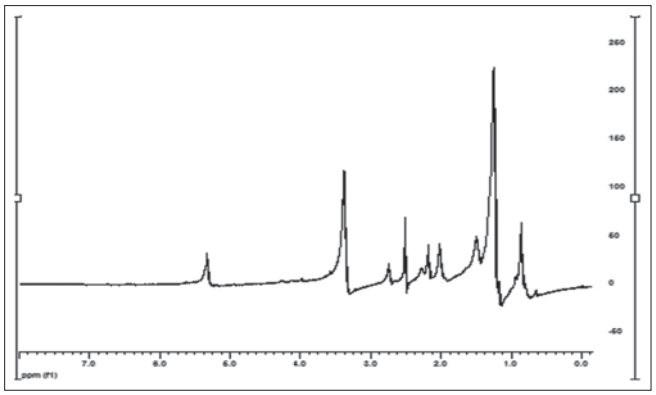

Figure 3: H nuclear magnetic resonance (NMR) spectrum of the sample describing the nature of CH_2 and CH_3 analyzed by NMR instrument by using tetramethylsilane $(CH_3)_4Si$, as internal standard

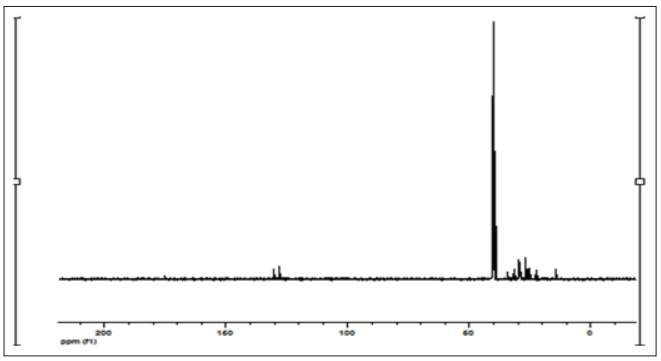

Figure 4: C^{13} nuclear magnetic resonance (NMR) spectrum of sample describing the nature of C-C bond analyzed by NMR instrument by using tetramethylsilane $(CH_3)_4Si$, as internal standard

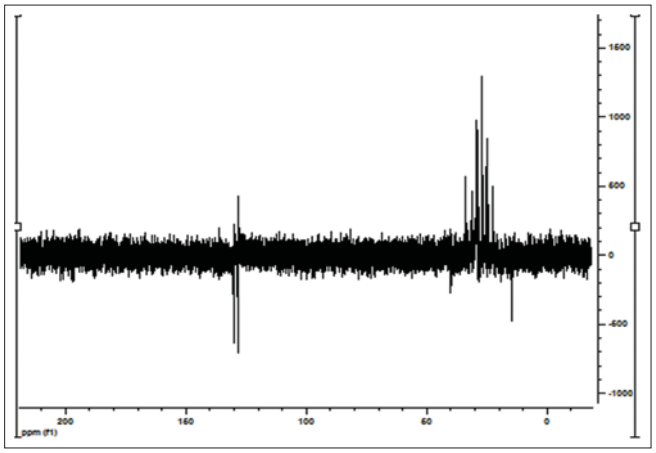

Figure 5: C^{13} nuclear magnetic resonance (NMR) showing the nature of CH_2-CH_3 interaction analyzed by NMR instrument by using tetramethylsilane $(CH_3)_4Si$, as internal standard

analysis also revealed a broad peak at 3426.3/cm for OH group. Two peaks are formed, one at 1635/cm and the another at 1605/cm. The peaks are the characteristic peak for steroidal-1, 4-diene-3-ones. There are also peaks that lie between 1500 and 1300/cm, these peaks are the main spectral region affected by the skeletal structure [Figure 2]. These data are similar with the data reported by Chiong *et al.* (1992) for the testosterone and other steroid derivatives.

The proton NMR spectra of the sample shows signals of alkane hydrogen ($\delta = 6.5$), aliphatic hydrogens ($\delta = 1.1$, $\delta = 1.3$), the hydroxyl group ($\delta = 3.5$), and aromatic hydrogens ($\delta = 7.2$). The natures of the carbon-carbon bond are also illustrated with the existence of spectra signal on ($\delta = 130$) for aromatic-carbon, ($\delta = 40$) for aliphatic C-C as shown on the Figures 3-5. These spectral results are similar with most of the NMR spectral signals done for the steroid by different researchers such as Kasal *et al.* (2010).

CONCLUSION

The present Study determined the androgenic steroid content of hexane/methanolic extract of *S. tuberosus*. The study also indicated that *S. tuberosus* plant contains derivatives of predinsolone, methyltestostrone, and testosterone cypionate. If further safety and efficacy studies are conducted, the methanol extract of the *S. tuberosus* can be used as important biological active component playing important role in increasing the sexual and physical health of men as they go aged and in testosterone deficient condition.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Biotechnology Research Institute of Bahir Dar University for funding the research. We are also grateful to the staff member of Bahir Dar University, Mr. Fasika kebede and Mr. Addisu Wondimu, for their unreserved support during the laboratory work.

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