Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Journal of Natural Product and Plant Resources, 2021, 11 (1): 1-12

(http://scholarsresearchlibrary.com/archive.html)

Phytochemical Investigation of the Root Extract of Carduus Chevallieri

Natnael Shamebo, Legesse Adane Bahiru, Tegene Tesfaye Tole

Hawassa University, Department of Chemistry, College of Computational and Natural Sciences, Hawassa University

Corresponding author: Legesse Adane Bahiru; Hawassa University, Department of Chemistry, College of Computational and Natural Sciences, P. O. Box 005; Hawassa, Ethiopia; E-mail: adanelegesse@gmail.com

ABSTRACT

Plant-derived substances have recently become of great interest owing to their versatile applications. The species Carduus chevallieri (C. chevallieri) is a traditional medicinal plant used to treat abdominal disorder, wounds, diabetes and hypertension among the peoples of Southern Nations Nationalities Peoples Regional State, Ethiopia. The chemical constituents of C. chevallieri, however, have not yet investigated although it has a wide traditional use for different ailments. The aim of this study was to carry out phytochemical screening, compound isolation, and structure elucidation of compounds isolated from the root of C. chevallieri.A standard phytochemical screening method was used to investigate the presence or absence of secondary metabolites, in the crude extracts. Compound isolation was performed using chromatographic separation techniques. Spectral data for structure elucidation of the isolated compounds were obtained by using IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic techniques. The structure elucidation of the compounds was performed by interpretation and analyzing the IR, ^{1}H-NMR and ^{13}C NMR spectral data and comparison with literature reports. The root of C. chevallieri was collected from Angacha town, Kembata Tembaro Zone, South Nations and Nationalities Regional State, Ethiopia. The crude extracts were obtained by maceration technique by drying the root (500 g) under shed, finely grinding, and soaking the root powder in solvents n-hexane chloroform, acetone, chloroform/methanol and methanol, sequentially. The solvent was removed by rotary evaporator and the extracts were recovered. The phytochemical screening tests of the chloroform and methanolic extracts revealed the presence of cardiac glycosides, terpenoids, steroids, tannins, alkaloids, flavonoids, anthraquinones, saponins and phenols. Syringin and stigmasterol were isolated from the methanolic extract. Other than the two isolated compounds, its richest bio-resource of several bioactive secondary metabolites that can be used as candidates in drug discovery and development programs. This makes the species a valuable medicinal plant.

Keywords: *Carduus Chevallieri*, Phytochemical Investigation, Stigmasterol, Syringin, Secondary Metabolite

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary

healthcare delivery, not only in Africa but also, in all countries of the world. It is the first choice healthcare treatment for at least 80% of Africans who suffer from high fever and other common ailments [2]. Ethiopians used traditional medicines for many centuries, the use of which has become an integral part of the different cultures. The indigenous peoples of different localities in the country have developed their own specific knowledge of plant resource uses, management and conservation [3].

The genus *Carduus* belongs to the family *Asteraceae,* and consists of approximately 90 species worldwide [4-8]. In this genus, the different species are widely used for medicinal purposes by communities of various countries where the plants are available in abundant. The treatment of various human diseases such as cold, stomachache, and rheumatism are some examples of their medicinal uses [9]. They showed pharmacological activities such as antispasmodic, hypertensive [10], anti-inflammatory, antioxidant, anticancer, antiviral, and antibacterial activities [11,12], antimicrobial, antidiabetic [13], and anti-atherosclerotic effect [14]. The phytochemical screening of the genus *Carduus* [10,11,13] and isolation of compounds [15] have been reported by several research groups.

So far the investigation of phytochemical and biological activities of three *Carduus* species of Ethiopian origin namely *C. macracanthus [15]* and *C. schimperi* [12,16-21] were the only studies reported. Similar to communities elsewhere in the world, these species are being used by the local people to treat different human illnesses. For instance, the root of *C. schimperi* is used for its anti-inflammatory, antinociceptive and antidiabetic activity [25]. *C macracanthus* is used for its anti-hypertension, anti-oxidant and antibacterial activities [15]. The roots of *C. chevallieri* (Figure 1) are used to treat abdominal disorder, wounds, diabetes and hypertension [personal observation]. Though *C. chevallieri* has wide applications in traditional medicine in Southern Ethiopia, no phytochemical investigation of the root of the plant has been performed so far. The aim of this study was to carry out phytochemical screening, compound isolation, and structure elucidation of compounds isolated from the root extract of *C. chevallieri.* The results could contribute to a better understanding of the chemical constituents of the investigated plant.

Figure 1. The aerial par (a) and root part of *C. chevallieri*: (b) photo by Natnael S., February, 2019; Angacha, SNNPR, Ethiopia

MATERIALS AND METODS

Plant material collection, preparation and extraction

The roots of *C. chevallieri* were collected from Angacha town, Kembata Tembaro Zone, South Nations Nationalities Peoples Regional Sate, Ethiopia, in the month of February, 2019. The sample collection site was $7^{0}33'$N latitude, $37^{0}85'$ E longitude and 1831 m above sea level. The species was authenticated by a botanist, and its specimen with voucher number (CC/001) was deposited at Gullele Botanic Center, Addis Ababa, Ethiopia. The collected plant materials (roots) were washed, and dried under shade. The dried roots were ground using a mechanical grinder below 30⁰C. The powdered root (500 g) was soaked in n-hexane (3L) in order to remove fatty and oily substances. The mixture was continuously shaken by orbital shaker (Grant GIS400) at room te

mperature with a speed of 200 rpm for 48 hrs. The solution was then filtrated

And the filtrate was concentrated under reduced pressure using rotary evaporator (LABOROTA 400) at 40°C to get crude extract. Similar procedure was repeated on the marc with chloroform, acetone, chloroform/methanol (50:50% by volume) and methanol, respectively. The resulting crude extracts were placed in refrigerator [26] until used for phytochemical screening and chromatographic separation of compounds.

Phytochemical screening

Qualitative phytochemical analysis of the crude extracts of the *C. chevallieri* roots was performed by standard methods [27-29].

Test for gardiac glycosides (Keller-Kiliani test)

Glacial acetic acid (1 ml) was added in 2 ml of crude extract. Then 1 ml of $FeCl_3$ solution was added into the mixture followed by addition of few drops conc. H_2SO_4. Formation of the green blue color indicates the presence of cardiac glycosides [30,31].

Test for terpenoids (Salkowski test)

Chloroform (10 ml) was added into 5 ml of solution of crude extract. The mixture was filtered, 2 ml of filtrate was added into a test tube holding 2 ml of acetic anhydride. Then 3 ml of concentrated H_2SO_4 acid was added carefully into the mixture. Formation of blue-green ring indicates the presence of terpenoids in the mixture [30].

Test for steroids

Acetic anhydride (10 ml) was added into a test tube containing 2 ml of alcoholic crude extract. Then 1 ml of sulphuric acid was added carefully into the mixture. Formation of violet or blue-green color indicates the presence of steroids [32].

Test for tannins

Small amount (200 mg) of crude extract was boiled with 10 ml of distilled water in a 200 ml beaker. Then the mixture was filtered, and 2 ml of 0.1M $FeCl_3$ solution in 0.1N HCl and 0.8 ml of potassium ferocyanide was added into the filtrate. The formation of blue-black color precipitate indicates the presence of tannins in the plant extracts [33].

Test for alkaloids (Mayer's test)

Alkaloids were tested by adding small amount HCl into the 3 ml of alcoholic solution crude extract in a test tube. The mixture was heated, cooled and filtered. Then the filtrate was tested with 1 ml of Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicates the presence of alkaloids [34,35].

Test for flavonoids (Shinoda test)

Flavonoids were determined by magnesium-hydrochloric acid reduction test. A piece of 1 mg magnesium ribbon (powder) and 1 ml of concentrated hydrochloric acid was added into the 3 ml of alcoholic solution of crude extract. Formation of red color indicates presence of flavonoids [34].

Test for anthraquinones (Borntrager's test)

Detection of anthraquinones was carried out by mixing 200 mg of crude extract with 10 ml of benzene. The mixture was shaken for five minute and filtered. Finally, 10% ammonia solution was added into the filtrate. Formation of pink or red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinones [36].

Test for saponins

Small amount (200 mg) of crude extract was mixed with 10 ml of distilled water in a test tube and was shaken vigorously. The formation of stable foam indicates the presence of saponins [37].

Test for phenols

To test phenols, 2 ml solution of crude extract was treated with 2 ml of 2% $FeCl_3$ solution. Formation of violet color indicates the presence of phenols [29].

Isolation and structure elucidation of compounds

The methanol extract was selected for chromatographic separation of compounds for its good TLC profile (in chloroform:ethanol solvent systems) and its highest yield. The methanolic extract (13.9 g) was dissolved in small amount of methanol, adsorbed onto silica gel (0.063 mm) and then allowed to dry at room temperature. The column was first loaded with n-hexane slurry of 140 g silica gel (0.063 mm) which then was followed by loading the adsorbed methanolic extract on top. The column chromatographic separation was started with chloroform and then mixture of ethanol in chloroform, eluent, with gradual increase in proportion of ethanol. Each fraction (15 ml) was collected and monitored with TLC. The components on the TLC plates were visualized with UV chamber at 254 and 365 nm (LF-2006). Fractions with identical R_f values were combined. Further fractionation of fractions with more than one component was performed to get the pure components. After isolating pure compounds, the samples were kept in refrigerator, to protect further oxidation, until they were sent for spectral analysis. The structures of the isolated compounds were elucidated by interpreting spectroscopic data obtained from ^1H NMR and ^{13}C-NMR (Bruker avance 400 MHz spectrometer), Infrared (IR) (Perk-Elmer BX infrared spectrometer, 4000 - 400 cm^{-1}) and by comparison of the spectroscopic data with literature. All the spectral analyses were carried out at The Department of Chemistry, Addis Ababa University, Ethiopia. Reagents and chemicals were laboratory grade and were purchased from Sigma and Aldrich (Addis Ababa).

RESULTS AND DISCUSSION

Percentage yield of crude extracts

The powdered plant material was extracted in n-hexane, chloroform, acetone, chloroform:methanol (50:50 % by volume), and methanol. The percent yields (calculated using Eq. 1) are presented below (Table 1). The most polar solvent (methanol) extracted the highest yield (3.54%). This indicates that the amount of polar compounds is highest in the plant. According to Cowan [38] methanolic extracts contain the most of the secondary metabolites like

anthocyanins, terpenoids, saponins, tannins, xanthoxyllines, totarol, quasinoids, lactones, flavones, phenones and polyphenols. The relatively small yield of acetone extract reveals the presence of small amount of hydrophilic and lipophilic components [29], more specifically phenols and flavonols [38]. The result is therefore in agreement with literature reports. The most polar solvent (methanol) is therefore the solvent chosen to extract most of the secondary metabolites of this plant. Reports suggest that the extract yield is based on the extent of polarity of the solvent used for extraction which also indicates the plant's pharmacological importance and proves that a particular medicinal plant to possess high potential as source phytochemicals [39,40].

$$\text{The percentage yield} = \frac{\text{mass of the crude extract}}{\text{mass of the plant material used for extraction}} \times 100 \quad \text{Eq.-(1)}$$

Mass of plant material (g)	Extract	Mass of extract (g)	% Yield
500	Chloroform extract	6	1.2
	Acetone extract	2	0.4
	Chloroform/methanol (50:50% by volume) extract	7	1.4
	Methanol extract	17.7	3.54

Table 1. The percentage yield of crude extracts

Phytochemical screening of the crude extracts

Phytochemical screening test of *C. chevallieri* root extracts revealed the presence of secondary metabolites such as cardiac glycosides, terpenoids, steroids, tannins, alkaloids flavonoids, anthraquinones, saponins and phenols. The acetone extract showed positive test for cardiac glycosides, steroids, flavonoids, anthraquinones and phenols whereas the chloroform extract showed positive result for all tests. The chloroform/methanol extracts showed positive test for terpenoids, tannins, alkaloids, anthraquinones and phenols. The methanolic extract showed positive result for most of the secondary metabolites except alkaloids and flavonoids (Table 2). This finding is consistent with literature reports that state a single solvent may not necessarily extract all useful bioactive compounds from a plant suggesting that several solvents need to be used to obtain as many secondary metabolites as possible [41].

Several reports revealed that phytochemicals or secondary metabolites possess several pharmacological activities. Cardiac glycosides are known to lower blood pressure; tannins exhibit antioxidant, antimicrobial and antiviral effects and terpenoids exhibit a potent analgesic as well as anti-inflammatory effects [42]. Alkaloids exhibit antioxidant, anti-inflammatory activities, and flavonoids are used to reduce risk of cancer, heart disease, asthma and stroke. Anthraquinones are known to have anticancer, antimalarial, antileukemic, mutagenicity, anti-inflammatory and antimicrobial activities [43]. Saponins have anti-inflammatory cytotoxicity, antitumor, antimutagenic, antiviral, anti-helmintic and hemolytic activities [44]. Phenolic compounds have the ability to intervene at all stages of cancer development [45]. Steroids are used to reduce the risk of cardiovascular diseases [46]. These facts substantiate the use of *C. chevallieri* in Southern Ethiopia, and it's richest bio-resource of several bioactive compounds that could be used as candidates in drug discovery and development program.

Phytochemical	Extract			
	Chloroform	Acetone	Chloroform/methanol (50:50% by volume)	Methanol
Cardiac glycosides	+	+	-	+

Terpenoids	+	-	+	+
Steroids	+	+	-	+
Tannins	+	-	+	+
Alkaloids	+	-	+	_
Flavonoids	+	+	-	_
Anthraquinones	+	+	+	+
Saponins	+	-	-	+
Phenols	+	+	+	+

"+" denotes presence of phytochemical; "-" denotes absence of phytochemical.

Table 2. The phytochemical screening test results of the crude extracts of root of *C. chevallieri*

Structural elucidations of the isolated compounds

Compound NLT-1 was isolated as a pale yellow solid (43 mg) by combining fractions 31-48 which were obtained by a solvent system of chloroform:ethanol (20:80% by volume). Its R_f was 0.43 (80:20% chloroform:ethanol by volume). Its melting point was 190-193°C. Analysis of IR spectrum of compound NLT-1 showed a broad absorption band at 3379 cm^{-1} indicating O-H stretching of alcohol functionality. The absorption bands at 2923 and 2854 cm^{-1} indicate C-H stretching of CH_3 and CH_2 groups, respectively. The medium band at 1461 cm^{-1} could be attributed to a vinyl group bonded to an aromatic ring.

The ^{1}H-NMR spectrum (DMSO, 400 MHz) (Appendix 2) showed peak at 6.73 pm that could be attributed to aromatic methine (CH). On the other hand, doublet peak 6.5 ppm and triplet peak at 6.3 pm that correspond to proton of a vinyl group bonded to an aromatic ring and methylene group, respectively. The doublet peak at 4.91 ppm could be to glucose moiety methine (CH) group that bonded connected with alpha and anomeric O atoms. On the same spectrum, the doublet peak at 4.15 ppm and an intense singlet peak at 3.74 ppm correspond to aliphatic methylene (CH_2) bonded with –OH group and methoxy group bonded to an aromatic ring (Table 3). The ^{13}C-NMR spectrum (DMSO, 100 MHz, and Appendix 3) showed a peak at 61.3 ppm that can be attributed to the presence of methylene (CH_2) group that bears an OH group. The peaks at 128.9 and 130.6 ppm could indicate aliphatic C=C bond that is bonded to an

aromatic ring. Moreover, the peaks at 104.8, 134.2, 133.08 and 153.1 ppm could be attributed to carbon atoms of benzene ring (Table 3). The peaks in the range of 61.3-103.0 ppm suggest carbon atoms of sugar moiety (Appendix 2). The strong peak at 56.7 ppm indicates a methoxy group bonded to an aromatic/benzene ring. The ^{13}C-NMR spectral data was consistent with the DEPT-135 spectrum (Appendix 4)) of compound NLT-1. The patterns of the spectra of the compound with literature reports suggest that compound NLT-1 is identical to Syringin (Figure 2) [47-50]. The NMR spectral data of compound NLT-1 and that of Syringin are summarized in Table 3. Isolation of Syringing has been reported from the aqueous root extracts *C. schimperi,* and its presence has been attributed to *in vivo* anti-inflammatory and antinociceptive effects of the plant [16].

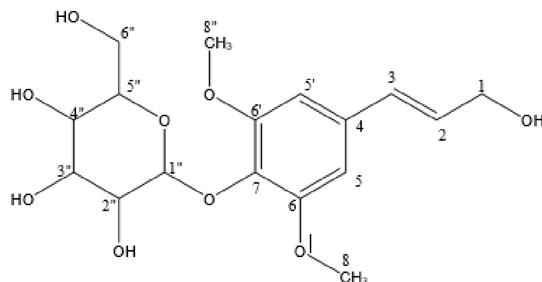

Figure 2. The proposed structure of compound NLT-1 (or Syringin)

Carbon	^{13}C-NMR data of compound NLT1	^{1}H-NMR data of compound NLT1	Reported ^{13}C-NMR data of syringing[47-50]	Reported ^{1}H-NMR data of syringin [47-50]	Nature of carbon
C-1	61.9	4.15 (*dd*, *J*=4.54, 1.2 Hz, 2H)	64.0	4.21 (*dd*, *J*= 5.6, 1.2 Hz, 2H)	CH_2
C-2	128.9	6.3 (*dt*, *J*= 4.8, 5.05 Hz, 1H)	130.5	6.35(*dt*,*J*=15.8,5.6Hz, 1H)	CH
C-3	130.6	6.5(*d*, *J*=16.42 Hz, 1H)	131.7	6.59(*d*,*J*=10.9Hz, 1H)	CH
C-4	134.2		136.3		C
C-5	104.8	6.73 (*s*, 1H)	105.9	6.76 (*s*, 1H)	CH
C-6	153.1		154.7		C
C-7	133.0		135.7		C
C-6’	153.1		154.7		C
C-5’	104.8	6.73 (*s*, 1H)	105.9	6.76 (*s*, 1H)	CH
C-8	56.7	3.73(*s*, 3H)	57.4	3.87 (*s*, 3H)	$-OCH_3$
C-8’	56.7	3.73 (*s*, 3H)	57.4	3.87 (*s*, 3H)	$-OCH_3$
C-1”	103.0	4.91 (*d*, J=6.58 Hz, 1H)	105.8	4.85(*d*, J=7.5Hz, 1H)	CH
C-2”	74.6	3.12 (*m*, 1H)	76.0	3.33 (*m*, 1H)	CH

C-3”	76.9	3.16 (*m*, 1H)	78.1	3.43 (*m*, 1H)	CH
C-4”	70.3	3.25 (*m*, 1H)	71.6	3.50 (*m*, 1H)	CH
C-5”	77.6	3.05 (*m*, 1H)	78.6	3.23 (*m*, 1H)	CH
C-6”	61.3	3.60(*dd*,*J*=11.16Hz, 2H)	63.0	3.69(*dd*,*J*=12.0Hz,2H)	CH_2
OH on 3”and 4”		3.49 (m, 2H)			OH
OH on C-6”		4.35 (s, 1H)			OH
OH on C-1		5.03(s, 1H)			OH
OH on C-2”		3.22 (m, 1H)			OH

Table 3. ^{1}H-NMR and ^{13}C-NMR spectral data of compound NLT-1 and Syringin

from column chromatographic separation that was eluted by a solvent system of chloroform: ethanol (90:10 % by volume). Its R_f value was found to be 0.52 (in 90:10 % chloroform: ethanol by volume). The melting point of this compound was found to be between 139-143°C. The analysis of IR spectrum (Appendix 5) showed a strong absorption band at 3402.20 cm^{-1} indicating the presence of hydroxyl (O-H) group. On the other hand, a band at 1647.10 cm^{-1} could be attributed to unconjugated olefinic (C=C) stretching. The ^{1}H-NMR spectrum (Appendix 6) showed the presence of peaks in the range of 0.69-1.00 ppm and 1.15 - 1.98 ppm revealed the presence of methyl and methylene protons, respectively. On the other hand, peaks at 5.03 ppm and 5.15 ppm revealed the existence ofolefinic protons whereas the multiplet at 3.61 pm may reveal a proton bonded to the carbon that bears OH group.

The ^{13}C-NMR spectrum (DMSO, 100 MHz) indicate existence of methyl and methylene carbon atoms in the range 12-60 ppm. The signals at 141.1, 121.4, 139.1 and 129.2 ppm indicate the presence of olefinic carbon atoms. The signal at 71.1 ppm could be attributed to a carbon atom bearing –OH group. The aforementioned interpretation of the spectral data of NLT-2 and similarity of its spectral data with the spectral data of stigmasterol in the literature [51,52] confirmed that compound NLT-2 is Stigmasterol (Figure 3). The DEPT-135 spectrum is also consistent with the above interpretation. The positive test observed (Table 2) for steroid for methanol crude extract can also support this suggestion. The NMR spectral data of compound NLT-2 and that of Stigmasterol are summarized in Table 4.

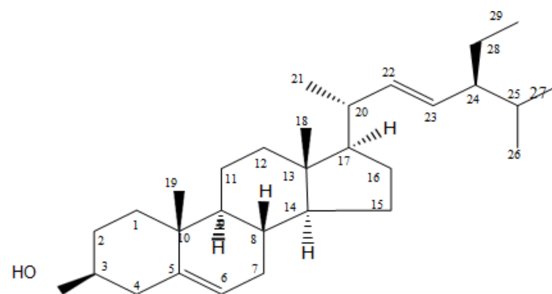

Figure 3. The proposed structure of compound NLT-2 (or Stigmasterol)

Carbon	The ^{13}C-NMR data of compound NLT2	Reported ^{13}C-NMR data of Stigmasterol [51,52]	The ^{1}H-NMR data of compound NLT2	The reported ^{1}H- NMR data of Stigmasterol [51,52]	Nature of Carbon
C-1	37.1	37.15			CH_2
C-2	29.2	31.56			CH_2
C-3	71.1	71.71	3.61 (dtt, 1H)	3.51 (tdd, 1H)	-CHOH
C-4	43.5	42.19			CH_2
C-5	141.1	140.81	-		C=C
C-6	121.4	121.62	5.17 (t, 1H)	5.31 (t, 1H)	C=CH
C-7	31.9	31.56			CH_2
C-8	31.4	31.79			CH
C-9	51.4	50.02			CH
C-10	37.2	36.16			C
C-11	21.1	21.12			CH_2
C-12	39.3	39.57			CH_2
C-13	40.5	42.10			C
C-14	56.4	56.76			CH
C-15	21.6	24.27			CH_2
C-16	31.5	28.83			CH_2
C-17	55.2	55.84			CH
C-18	12.2	12.15	1.00 (s, 3H)	1.03 (s, 3H)	CH_3
C-19	19.1	19.88	0.79 (s, 3H)	0.71 (s, 3H)	CH_3
C-20	43.1	40.51			CH
C-21	21.2	20.99	0.93(d,3H,J=6.5Hz)	0.91 (d, 3H)	CH_3
C-22	139.1	138.23	5.03(m, 1H)	4.98 (m, 1H)	C=CH
C-23	129.2	129.16	5.07(m, 1H)	5.14(m, 1H)	C=CH
C-24	51.4	51.30			CH
C-25	32.1	31.94			CH
C-26	18.9	19.01	1.15 (d, 3H;J=6.6Hz)	0.80 (d, 3H;6.6Hz)	CH_3
C-27	20.9	21.23	1.15 (d, 3H;J=6.6Hz)	0.82 (d, 3H;6.6Hz)	CH_3
C-28	25.5	25.50			CH_2
C-29	12.1	12.25	0.83(t, 3H; 6.9Hz)	0.83 (t, 3H;7.1Hz)	CH_3

Table 4. The ^{1}H-NMR and ^{13}C-NMR spectral data of compound NLT-2 and Stigmasterol

CONCLUSIONS

To the best of our knowledge there is no prior report on the chemical constituents of the root of *C. chevallieri* contrary to its high traditional use among the peoples of South Nations Nationalities Regional State, Ethiopia. Preliminary phytochemical screening of the extracts of the root extract revealed the presence of cardiac glycosides, terpenoids, steroids, tannins, alkaloids, flavonoids, anthraquinones, saponins and phenols. Chromatographic separation of the methanolic extract of the root afforded the glycoside Syringin and the steroid Stigmasterol. These findings substantiate the use of *C. chevallieri* in peoples of Southern Ethiopia, and its potential as the richest bio-resource of several bioactive compounds that can be used as candidates in drug discovery and development program.

ACKNOWLEDGEMENT

The authors kindly grateful to the Department of Chemistry, Addis Ababa University for spectral analyses, and The Department of Chemistry, Hawassa University for material and chemical support.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

1. Ncube, N.S., et al., Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, **2008**. 7(12): p.1797-1806. .
2. Abbink, J., Medicinal and ritual plants of the Ethiopian Southwest. An account of recent research. *Indigenous Knowledge and Development Monitor*, **1995**. 3(2): p.6-8.
3. Grundmann, O., et al., Anti-anxiety effects of Apocynum venetum L. In the elevated plus maze test. *Journal of Ethnopharmacology*, **2007**. 110(3): p.406-411.
4. Rose, F., The wild flower key. *London: Frederick Warne & Co*., **1981**. p.377-80.
5. Chaudhary, S.A., Flora of the Kingdom of Saudi Arabia, vol. II, part 3. Ministry of Agriculture and Water, National Herbarium, National Agriculture and Water Research Center, Riyadh, KSA, **2000**. p.117-202.
6. Mandaville, JP., Flora of eastern Saudi Arabia. Published by Kegan Paul International Limited, London, **1990**. P.284.
7. Butterfield, C., et al., Species abstract of highly disruptive exotic plants. Jamestown. ND: Northern Prairie Wildlife Research Center. **1996**.
8. Beck, K.G. Fact Sheet No. 3.102: Musk thistle. Colorado State University, *Cooperative Extension*, **2004**.
9. Esmaeili, F., et al., Volatile constituents of Centorea depressa and Carduus pycnocephalus L. two compositae herbs growing wild in Iran. *Journal of Essential Oil Research*, **2005**. 17(5): p.539-541.
10. Latifa, AA., Chemical composition and antimicrobial activity of the essential oil and lipid content of Carduus pycnocephalus L. growing in Saudi Arabia. *Journal of Chemical and Pharmaceutical Research*, **2012**. 4(2): p.1281-1287.
11. Slavov, I., et al., Phenolic acids, flavonoid profile and antioxidant activity of Carduus thoermeri Weinm. extract. *Oxidation Communications*, **2014**. 37(1): p.247-253.
12. Conforti, F., et al., The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. *Food Chemistry and Toxicology*, **2009**. 112(3): p.587-594.
13. Shakeel, AH., and AK. Yar. Effect of milk thistle (Silybum marianum) plant parts (seeds and leaves) to control the alloxan induced diabetes in rabbits. *Global Journal of Research on Medicinal Plants and Indian Medicine*, **2014**. 3(1): p.1-7.
14. Białecka, M., The effect of bioflavonoids and lecithin on the course of experimental atherosclerosis in rabbits. *Annales Academiae Medicae Stetinensis*, **1997**. 43, p.41-56.
15. Anza, M., et al., Phytochemical analysis, in vitro antioxidant and antibacterial activities of root extracts of Carduus macracanthus. *Journal of Coastal Life Medicine*, **2007**. 5(11): p. 486-491.
16. Wolde-Mariam, M., et al., Antiinflammatory and antinociceptive activities of extracts and syringin isolated from Carduus schimperi Sch. Bip. ex A. Rich. *Phytopharmacology*, **2012**. 3(2): p.252-262.
17. Tunsag, J., et al., New isoquinoline alkaloid from Carduus crispus L. *Mongolian Journal Chemistry*, **2011**.12(38):p.85-87.
18. Dimitrina, Z., et al., Antioxidant activity of some Carduus species growing in Bulgaria, **2011**. Vol. 1.
19. Iliya, Z., et al., Content of phenolic compounds in the genus Carduus L. from Bulgaria, *Ecologia Balkanica*, **2013**. 5(2): p.13-21.
20. Iliya, Z.S., et al., Phenolic Profile and Antioxidant Activity of Methanolic Extract of Carduus acicularis Bertol. (Asteraceae), *Ecologia Balkanica*, **2016**. 8(1): p.41-46.
21. Dimitrina, Z., et al., Antioxidant activity of some Carduus species growing in Bulgaria, **2015**. Vol. 3.
22. El-Lakany, M., et al., New flavone glycoside with antimicrobial activity from Carduus pycnocephalus. *Journal of Pharmaceutical Science*, **1995**. 9(1): p.41-43.
23. Silva, FM., et al., Roots of the invasive species Carduus nutans L. and C. acanthoides L. produce large amounts of aplotaxene, a possible allelochemical. *Journal of Chemistry and Ecolog,* **2004**. 40(3): p. 276-284.
24. Tadese, M., Asteraceae (Compositae), in: Hedbeng, I., Friis, I. B, Edwards, S. (Eds.), Flora of Ethiopia and Eritrea, Vol 2, Part 2, National Herbarium, Biology Department, Science Faculty, Addis Ababa University, Ethiopia and the Department of Systematic Botany, Uppsala University, Sweden, **2004**: p.23-27.
25. Abebe, E., Ethnobotanical study on medicinal plants used by local communities in Debark wereda, north Gondar zone, Amhara regional state, Ethiopia, M.Sc. Thesis. Addis Ababa University, Ethiopia, **2011**.
26. Harborne, JB., Phytochemical methods. A guide to modern techniques of plant analysis. Chapman and hall Ltd. London, **1973**. P.279.
27. Sofowra, A., Medicinal plants and traditional medicine in Africa. Nigeria: Spectrum Books Ltd., Ibadan **1993**. p.191-289.
28. Harborne, JB., Phytochemical methods: A guide to modern techniques of plant analysis, 3rd edition, Chapman and Hall, London **2007**. p.75-125.
29. Prashant, T., et al., Phytochemical screening and extraction: A review, *Internationale Pharmaceutica Sciencia*, **2011**. 1(1):p.98-106.
30. Ajiboye, B.O., et al., Qualitative and quantitative analysis of phytochemicals in Senecionbiafrae leaf. *International Journal of Invention and Pharmaceutical Science*, **2013**. 1(5): p.428-432.
31. Jaradat, N., et al., Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata Dencne. *Journal of Materials and Environmental Science*, **2015**. 6(6): p.1771-1778.
32. Bargah, R.K., Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringapterygsperma Gaertn. *Journal of Pharmacgnosy and Phytochemistry*, **2015**. 4(1): p.7-9.

33. Edeoga H.O., et al., Phytochemical constituent of some Nigeria medicinal plants. *African Journal of Biotechnology*, **2005**. 4(7): p.685-688.
34. Pandey, C.N., Gujarat ecological education and research foundation; medicinal plants of Gujarat, India, **2005**. p.387.
35. Liu, Y., et al., Methylalpinumisoflavone Inhibits Hypoxia-inducible Factor (HIF) activation by simultaneously targeting multiple pathways. *Journal of Biological Chemistry*, **2009**. 284 (9): p.5859- 5868.
36. Geetha, T.S., et al., Phytochemical screening, quantitative analysis of primary and secondary metabolites of Cymbopogan citratus (DC) stapf leaves from Kodaikanal hills, Tamilnadu. *International Journal of PharmTech Research*, **2014**. 6(2): p.521-529.
37. Devmurari V P., Phytochemical screening study and antibacterial evaluation of Symplocos racemosa. *Archives of Applied Science Research*, **2010**. 2 (1): p.354-359.
38. Cowan, M.M., Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **1999**. 12(4): p.564-582.
39. Sultana, B., et al., Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, **2009**. 14(6): p.2167-2180.
40. Ibrahim, R., et al., Percentage yield and acute toxicity of the plant extracts of Ceiba pentandra grown in Bauchi State, North Eastern Nigeria. *Journal of Pharmacognosy and Phytochemistry*, **2017**. 6(5): p.1777-1779.
41. Madike, L.N., et al., Preliminary phytochemical screening of crude extracts from the leaves, stems, and roots of Tulbaghia violacea. *International Journal of Pharmacognosy and Phytochemical Research*, **2017**. 9(10): p.1300-1308.
42. Armelle, T.M., et al., Toxcological survey of African Medicinal plants, **2014**.
43. Siti, M.M.N., et al., Synthesis of new cytotoxic aminoanthraquinone derivatives via nucleophilic substitution reactions, *Molecules*, **2013**. 18(7): p.8046-8062.
44. Jin, D., et al., Plant phenolics: extraction, analysis and their antioxidant and anticancer properties, *Molecules*, **2010**. 15(10): p.7313-7352.
45. Kareru, P.G., et al., Direct detection of triterpenoid saponins in medicinal plants. *African Journal of Traditional and Complement Alternative Medicine*, **2008**. 5(1): p.56- 60.
46. Cabral, CE., et al., Phytosterolsin the treatment of hypercholesterolemia and prevention of cardiovascular diseases. *Arquivos Brasileirosde Cardiologia,* **2017**. 109(5): p.475-482.
47. Agrawal, P.K., NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Journal of Phytochemistry*, **1992**. 31(10): p.3307-3330.
48. Ahmad, R.G., et al., Phytochemical and chemotaxonomic investigation of Stelleropsis iranica. *Australian Journal of Basic and Applied Sciences*, **2009**. 3(4): p.3423-3427.
49. Yan, J., et al., Preparative isolation and purification of syringin and edgeworoside C from Edgeworthia chrysantha Lindl by high-speed counter current chromatography. *Journal of Chromatography*, **2004**. 1043(2): p.329-332.
50. Mahadeva, R.U., et al., Chemistry and Pharmacology of Syringin, a novel bioglycoside: A Review. *Asian Journal of Pharm Clinical Research*, **2015**. 8(3): p.20- 25.
51. Luhata, L.P., et al., Isolation and characterisation of stigmasterol and β- Sitosterol from Odontonema strictum (Acanthaceae). *Journal of Innovations in Pharmaceuticals and Biological Sciences*, **2015**. 2 (1): p.88-95.
52. Venkata, S., et al., Isolation of Stigmasterol and ß-Sitosterol from the dichloromethane extract of Rubus suavissimus. *International Current Pharmaceutical Journal*, **2012**. 1(9): p.239-242.