Trends Phytochem. Res. 7(2) 2023 110-116

Trends in Phytochemical Research (TPR)

Journal Homepage: https://tpr.shahrood.iau.ir/

Original Research Article

Triterpenes from *Urtica dioica* L. roots of Ethiopian origin

Tegene Tesfaye Tole[1]*

Chemistry Department, College of Natural and Computational Sciences, Hawassa University, P.O. Box 5, Hawassa, Ethiopia

ABSTRACT

Urtica dioica L. (family Urticaceae) is an herbaceous shrub originally from the colder regions of northern Europe and Asia grows today all over the world. It has a long history of use in the household home remedies and nutritious diet. This study was conducted to investigate the chemical constituents of the root of *Urtica dioica* L. grown in Ethiopia. The secondary metabolite class screening test of *n*-hexane extract revealed the presence of alkaloids, saponins and terpenoids, whereas the chloroform crude extract showed the presence of phenols, flavonoids, and tannins in addition to the classes of secondary metabolites present in the *n*-hexane extract. The methanolic extract revealed the presence of alkaloids, glycosides, phenols, saponins, steroids, tannins, flavonoids, and terpenoids. The column chromatographic separation of the methanolic extract afforded two ursane-type pentacyclic triterpenes namely 3*β*-hydroxy-urs-12-ene-28-oic acid commonly known as ursolic acid and 3,7,24-trihydroxyl-urs-12-en-28-oic acid which is also known as 7,24 dihydroxy ursolic acid.

ARTICLE HISTORY

Received: 27 March 2023
Revised: 10 June 2023
Accepted: 23 June 2023
ePublished: 28 June 2023

KEYWORDS

Antidiabetic
Dihydroxy ursolic acid
Hepato-protective
Ursane type
Ursolic acid
Urtica dioica L.

dor: 20.1001.1.25883623.2023.7.2.4.9

1. Introduction

Considering their incomparable chemical diversity and novel mechanisms of action, medicinal plants have continued to play a pivotal role in many drug development and research programs. Several natural products have the ability to interact with numerous, varied biological targets, and become the most important drugs in health care system. For example, plants, microorganisms, and animals manufacture small molecules known as secondary metabolites, which have played a major role in drug discovery (Li et al., 2009; Kinghorn et al., 2011, Mohammadhosseini et al., 2019; Silva et al., 2021; Mohammadhosseini et al., 2022; Olaoluwa et al., 2022). The search for plants as a source of potential candidate for drug development still needs more efforts. Out of 250,000 to 500,000 species available on the earth, only 1-10% is being potentially used (Newman et al., 2003). Among 69 small molecules new drugs approved from 2005 to 2007 worldwide, 13 of them were natural products or originated from natural products. These facts underline the importance of such products in drug research and development (WHO 2002; Ngo et al., 2013; Anees et al., 2014).

These days, developed countries are turning to the use of traditional medicinal systems that involves the use of herbal drugs and remedies. It is often noted that 25% of all drugs prescribed today come from plants (Barrett, 2002; Bhattaram, 2002). This estimate suggests that plant-derived drugs make up a significant segment of natural product-based pharmaceuticals. It is, therefore, crucial to consider proven agro-industrial technologies that need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines (Cordell, 2002).

Urtica dioica L., family *Urticaceae,* is a perennial plant which is commonly known as stinging nettle. It is widely distributed throughout the temperate and tropical

Corresponding author: Tegene Tesfaye Tole
Tel: +251 462 205311; Fax: +251 462 204 975
E-mail address: tegenetesfaye19@gmail.com, **doi: 10.30495/tpr.2023.1982911.1331**

areas around the world (Harwood et al., 2007). Although there are differences on the number of subspecies in this plant, there are at least six subspecies of *U. dioica* L., some of which formerly were classified as separate *Urtica* species. *Urtica dioica* subspecies *galeopsifolia* is the only one of the six subspecies that does not have stinging hairs (Wagner et al., 1989; Joshi and Pandey, 2007; Bisht et al., 2012). The stinging action is due to the liquid contained in nettle's hairs. This liquid contains at least three compounds that could be the cause of its allergic reactions: acetylcholine, histamine and serotonin (Bhuwan et al., 2014).

U. dioica L. is considered to be native to Europe, Asia, Northern Africa, and North America and is the best-known member of the nettle genus *Urtica* (Pashazadeh, 2013). It is abundant in Northern Europe and many parts of Asia, usually found in the countryside. It is less widespread in Southern Europe and North Africa. It has been introduced to many other parts of the world. In North America, it is widely distributed in Canada and the United States where it is found in every province (Lukesova, 2017).

U. dioica L. has a long history of use in the household home remedies and nutritious diet. The powered leaf extract was used as anti-hemorrhagic agent to reduce excessive menstrual flow, nose bleeding, in the treatment of arthritis, anemia, hay fever, as diuretics, astringent and blood builder in folk medicine (Manganelli et al., 2005). Traditionally, a tea made from the leaves of *U. dioica* L. has been used as a cleansing tonic and blood purifier (Gulcin et al., 2004; Joshi and Pandey, 2007). The juice of nettle leaf has been used as a hair rinse to control dandruff and to stimulate hair growth, and is a functional ingredient in modern European hair-care formulations. It is used as a vegetarian source of rennet in cheese-making and is still included among Passover herbs (Joshi and Pandey, 2007). The nettle leaves are used as a nutritious supplement and as weight loss aid. Nettle leaf has been used safely in large food-like doses (up to 100 g daily) for thousands of years (Upton, 2013). Externally, this plant is used to treat skin complaints, gout, sciatica, neuralgia, hemorrhoids, anemia, eczema, hair problems, nasal and menstrual haemorrhagia etc (Ji, 2009). Nicholas Culpeper recommended a nettle-honey preparation for wounds and skin infections, worms in children, as an antidote to venomous stings, and as a gargle for throat and mouth infections (Joshi and Pandey, 2007).

The root of *U. dioica* L. has a beneficial effect upon enlarged prostate glands, in the treatment of rheumatic gout, nettle rash and chickenpox (Riehemann and Behnke, 1999). The root of *U. dioica* L. has antiviral, antioxidant, antimicrobial, antiulcer, analgesic, anticancer, immunostimulant, diuretic, and anti-inflammatory effects (*Urtica dioica* L., 2018).

Chemical interest in *U. dioica* L. was stimulated by reports that they cause irritation when they come in contact with skin. Many compounds were previously isolated by different researchers on *U. dioica* L. These are phytosterols, lignans (Baytop, 1989), carotenoids (Wetherilt, 1989), fatty acids (Does, et al. 1999), phenolics (Le Moal and Truffa-Bachi, 1988), etc. The seeds and leaves of *U. dioica* L. contain vitamins, minerals and amino acids (Akgeul, 1993). The leaves were reported to contain caffeic acid and chlorogenic acid (Wagner et al., 1989). The roots of *U. dioica* L. showed the presence of *trans* neo-olivil, a lignans glycoside (Bradley, 1992; Chrubasik, et al. 1997; Ernst and Chrubasik, 2000; Farag, et al. 2013). To date there is no phytochemical investigation that has been done on root of *U. dioica* L. of Ethiopian origin. Therefore, this study is aimed to determine the phytochemical constituents of the roots extract of *U. dioica* L. of Ethiopian origin.

2. Experimental

2.1. Reagents and materials

Column chromatography (CC) was performed with column size 3 cm × 30 cm packed with silica gel 60, size 0.063-0.200 mm (70-230 mesh ASTM) and thin layer chromatography (TLC) on aluminum sheets, silica gel 60 F_{254}, and layer thickness 0.2 mm (Merck). Spot detection on TLC was performed by using UV (254 nm, 365 nm). Melting point of the isolated compounds was determined with Bűchi B-540 melting point apparatus. NMR data (TMS as internal standard and DMSO solvent) was generated with 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The IR spectra were obtained by SHIMADZU Fourier Transform Infrared (FTIR 1800) spectrometer. The FT-IR samples were prepared using spectral grade KBr and made into pellets and spectral analysis was made in the range between 4000 and 400 cm^{-1}.

2.2. General procedures

2.2.1. Plant material collection and preparation

U. dioica 'Sama' (Amharic) or 'Doobbii' (Afan Oromo) roots were collected on 15th January, 2018 from Guder, Toke Kutaye Wereda, West Shewa Zone, Oromia Region, Ethiopia. Latitude: 8.97 North; Longitude: 37.76 East; Altitude: 1995.00 m/6545.28 ft. The seed, leaf, and stem of the plant were collected for botanical identification. The plant was authenticated by botanist Dr. Getachew Fata, Department of Biology, Addis Ababa University. The plant specimen was stored in the National Herbarium of Addis Ababa University, with voucher no: TT1-5/0068. The plant material was prepared in such a way that one kilogram of the root of *U. dioica* L. was washed with tap water to remove soil particles and other foreign materials, and air dried in a shade for three weeks. The air-dried root was pulverized into powder using Mortar and Pestle. The pulverized plant material was kept in sealed plastic container and put on a dry cup board until used for extraction.

2.2.2. Extraction

The under shade air dried powdered root (500 g) of *U. dioica* L. was soaked in *n*-hexane (1.5 L) in a round bottom flask, at room temperature. The round bottom flask was put on orbital shaker and shaken for 72 h. After 72 h the solution was filtered using Whatman filter paper. It was then concentrated using rotary evaporator

at reduced pressure and a temperature of 36 to 38 °C. The marc was further extracted with chloroform and methanol solvents (1.5 L each) consecutively, likewise. The crude extracts (*n*-hexane = 1.80 g; $CHCl_3$ = 2.60 g; MeOH = 24.70 g) were stored separately in refrigerator until used for further analysis.

2.2.3. Phytochemical screening

Phytochemical screening tests of secondary metabolites were carried out on the *n*-hexane, chloroform, and methanol crude extracts of *U. dioica* L. roots following the standard procedures of Ganesh and Vennila (2011), Prashant et al. (2011) and Pakrashi et al. (1968). To be more specific Wagner's and Dragendorff's tests for alkaloids, Keller-Kiliani test for glycosides, froth and foam tests for saponins, Salkowski test for terpenoids, ferric chloride (0.1%) test for phenols and tannins, Liebermann Buchard reaction for steroids, and alkaline reagent test was used for flavonoid detection.

2.2.4. Compound isolation

Solvent selection for the chromatographic separation was performed by various proportions of *n*-hexane, chloroform, and methanol and chloroform showed better TLC profile. It is, therefore, necessary to use *n*-hexane, chloroform, and methanol solvent mixtures of various proportions in the course of column chromatography in order to maximize the number of compounds isolated. The *n*-hexane (1.80 g) and chloroform (2.60 g) extracts were small for performing both phytochemical screening and compound isolation. The column was packed by preparing slurry of silica gel (250 g) with *n*-hexane to achieve least polarity to the mobile phase. The amount of methanolic crude extract (24.7 g), however, was sufficient for compound isolation and therefore about half of it (12 g) was adsorbed on silica gel and subjected to the aforementioned column. Chromatographic separation of the components was conducted with gradient elusion beginning from *n*-hexane, *n*-hexane:chloroform, chloroform, chloroform:methanol, and methanol solvent systems of various proportions. Several fractions (102) of each 20 ml were collected and the components in each fraction were analyzed by using UV visualization of the spots on TLC plates. Fractions with elution solvent chloroform:methanol (9:1 v/v) showed single spot on TLC plate with Rf = 0.80 in chloroform:methanol solvent system. This compound was named compound **1**. Fractions with elution solvent chloroform:methanol (8:1 v/v) also showed single spot on TLC plate with R_f = 0.57 in chloroform:methanol (8:1 v/v) solvent system and the compound was named compound **2**. Single spot containing fractions (on TLC plate) were combined and concentrated using rotary evaporator. The concentrated and dried compounds (compound **1** = 67.3 mg, white crystalline solid; compound **2** = 74 mg, yellow crystalline solid) were packed in a clean dry small vial and stored in a refrigerator until sent for spectral analysis.

2.2.5 Compound characterization

Compound **1** (Ursolic acid): White crystalline solid (67.3 mg); R_f = 0.80 in $CHCl_3$:CH_3OH (9:1); mp 284-286 °C; IR (KBr) ν_{max} 3363, 3321, 2924, 2855, 1712, 1655, 1461, 1377, 941, 780 cm^{-1}; ^1H NMR (DMSO, 400 MHz) ppm 5.41(1H, d *J* = 6Hz, H-12), 3.43(1H, d *J* = 6.8 Hz, H-3), 2.61(1H, d *J* = 6.5 Hz, H-18), 2.23(1H, t *J* = 6.7 Hz, H-15), 2.21(1H, t *J* = 7Hz, H-16), 1.95(1H, t *J* = 6.9 Hz, H-22), 1.91(1H, d *J* = 7.2 Hz, H-11), 1.82-1.79(1H, m, H-2), 1.61(2H, t *J* = 6.5 Hz, H-7 & H-9), 1.54(2H, t *J* = 6.66 Hz, H-1 & H-6), 1.50-1.47(1H, m, H-19), 1.49-1.46(1H, m, H-21), 1.22(2H, s, H-23 & H-27), 1.06(1H, s, H-26), 1.04-1.02(1H, m, H-20), 1.03-1.01(1H, m, H-24 & H-29), 0.94(1H, d *J* = 6.5 Hz, H-30), 0.92(1H, s, H-25); ^{13}C NMR (DMSO, 100 MHz) ppm 179.4(C-28), 139.0(C-13), 127.2(C-12), 78.1(C-3), 53.6(C-5 & C-18), 48.1(C-17), 47.3(C-9), 42.0(C-14), 39.7(C-20), 39.5(C-19), 39.3(C-1 & C-4), 38.4(C-8), 38.2(C-22), 37.7(C-10), 33.0(C-7), 31.1(C-21), 28.4(C-2 & C-23), 26.8(C-15), 24.5(C-16 & C-27), 23.6(C-11), 22.3(C-30), 18.1(C-6 & C-9), 17.0(C-24), 16.7(C-26), 16.5(C-25).

Compound **2** (3,7,24-trihydroxyl-urs-12-en-28-oic acid): yellow crystalline solid (74 mg); R_f = 0.57 in $CHCl_3$:CH_3OH (8:1); mp 293-295 °C; IR (KBr) ν_{max} 3362-3321, 2924, 2855, 1654, 1461, 1377, 721cm^{-1}; ^1H NMR (DMSO, 400 MHz) ppm 5.41(1H, d *J* = 6 Hz, H-12), 3.45(1H, t *J* = 7.1 Hz, H-7), 3.44-3.42(1H, m, H-3), 3.33(1H, s, H-24), 2.61(1H, d *J* = 7.0 Hz, H-18), 2.23(1H, t *J* = 6.8 Hz, H-15), 2.21(1H, t *J* = 6.7 Hz, H-16), 1.95(1H, t *J* = 7.2 Hz, H-22), 1.92-1.90(1H, m, H-11), 1.90-1.70(1H, m, H-2), 1.61(1H, t *J* = 7.0 Hz, H-9), 1.54(2H, t *J* = 6.9 Hz, H-1 & H-6), 1.50-1.48(1H, m, H-19), 1.48-1.46(1H, m, H-21), 1.22(2H, s, H-23 & H-27), 1.06(1H, s, H-26), 1.04-1.02(1H, m, H-20), 1.02(1H, d J = 7.2 Hz, H-29), 0.94(1H, d J = 6.7 Hz, H-30), 0.92(1H, s, H-25); ^{13}C NMR (DMSO, 100 MHz) ppm 179.4(C-28), 139.0(C-13), 127.2(C-12), 77.4(C-3), 66.2(C-7), 65.2(C-24), 55.2(C-18), 47.4(C-5), 47.3(C-9), 47.2(C-17), 45.8(C-4), 42.1(C-1 & C-14), 40.5(C-8), 40.3(C-19), 40.1(C-20), 39.3(C-10), 38.0(C-22), 33.3(C-2), 31.7(C-21), 29.2(C-15), 26.0(C-16), 24.1(C-27), 23.8(C-11), 23.7(C-23), 21.5(C-26), 18.5(C-6), 17.5(C-29 & C-30), 17.4(C-25).

3. Results and Discussion

3.1. Extraction

Phytochemical investigation of specimens of plant origin is essentially required to optimize the concentration of constituents and also to maintain their activities (Aziz et al., 2003). Extraction is an important step in the route of phytochemical investigation and for the detection of bioactive constituents from plant materials. Choice of a suitable extraction technique is also important for the tuning of phytochemical constituents leaving out unnecessary materials with the aid of the solvents. Furthermore, selection of suitable extraction process and optimization of various parameters are critical for up scaling purposes, *i.e.*, from bench scale to large scale phytochemical analysis. The most commonly used extraction techniques include conventional techniques such as maceration, percolation, infusion, decoction, hot continuous extraction, etc. In this study cold maceration technique is used.

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. The percentage yield of crude extract in respective solvent was calculated as percent yield and the result is presented in Table 1.

Table 1
Percentage yield of the crude extracts.

Extract	Mass of extract (g)	Yield (%)
n-hexane	1.80	0.36
Chloroform	2.60	0.52
Methanol	24.70	4.94

Extraction solvent choice needs to be based on sample matrix properties, chemical properties of the analytes, matrix-analyte interaction, efficiency and desired properties (Ishida et al., 2001; Hayouni et al., 2007). The extractability of solvents depends mainly on the solubility of the compound in the solvent, the mass transfer kinetics of the product and the strength of solute/matrix interaction with corresponding limitations on heat and mass diffusion rate (Dhanani et al., 2017). The extraction solvent choice also depends on what natural compounds or classes of natural compounds one is looking for. Generally, the choice of solvent is influenced by what is intended with the extract. In this case, however, we are not interested in a specific natural product because we are performing total phytochemical analysis of the plant roots. The high yield of the methanolic extract suggests that constituents in the plant specimen are mainly polar.

3.2. Phytochemical screening

The secondary metabolite test of the *n*-hexane extract revealed the presence of alkaloids and terpenoids, whereas the chloroform crude extract revealed the presence of phenols, flavonoids and tannins in addition to the secondary metabolites present in the *n*-hexane extract. The methanolic extract, however, revealed the presence of alkaloids, glycosides, saponins, terpenoids, phenols, tannins, flavonoids, and steroids. The methanolic extract was found to be rich in secondary metabolites compared to *n*-hexane and chloroform extracts.
The classes of secondary metabolites found in the plant have the following biological activities. Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities (Kumar and Panday, 2013). Saponins are amphiphilic molecules consisting of carbohydrate and either triterpenoid or steroid aglycone moieties and are noted for their multiple biological activities such as fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant and immunomodulatory effects (Juang and Liang, 2020). Several glycosides have therapeutic uses, with others known to possess pharmacological activities with remarkable therapeutic potential. The pharmacological activities include analgesic, antiinflammatory, cardiotonic, antibacterial, antifungal, antiviral, and anticancer effects (Soto-Blanco, 2022). The pharmacological activities of tannins include antioxidant, radical scavenging, antimicrobial, antiviral, antimutagenic, and antinutrient (Serrano et al., 2009). Steroids have antidiarrhoeal; terpenoids, antimicrobial, and antidiarrhoeal activities (Prashant et al., 2011). The presence of phenols could confer antibiotic, antimicrobial, and antidiarrhoeal activities (Jacob and Burri, 1996; Prashant et al., 2011). Alkaloids demonstrate a broad spectrum of activities. In nature, they not only are produced against herbivores but also reduce bacterial or fungal infestation (Adamski et al., 2020). Therefore, they are substances that possess high potential in medicine, plant protection, veterinary, or toxicology.

3.3. Structure elucidation

After having appropriate extraction and fractionation of the methanolic extract of the root of *U. dioica* L., two pure compounds, namely compound **1** and compound **2** were obtained. The IR, ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data were generated for structure elucidation. Characterization of the compounds was performed on the basis of melting point and comparison of the experimental spectral data with literature (Seebacher et al., 2003; Gnoatto et al., 2008; Venditti et al. 2015; 2016) for compound **1** and (Mazumder et al., 2011) for compound **2**.
The experimental NMR data of compound **1** corresponds to that of the reported 3β-hydroxy-urs-12-ene-28-oic acid commonly known as ursolic acid (Fig. 1). In addition to this the experimental melting point (284-286 °C) is not far from the actual melting point of ursolic acid (285-288 °C).

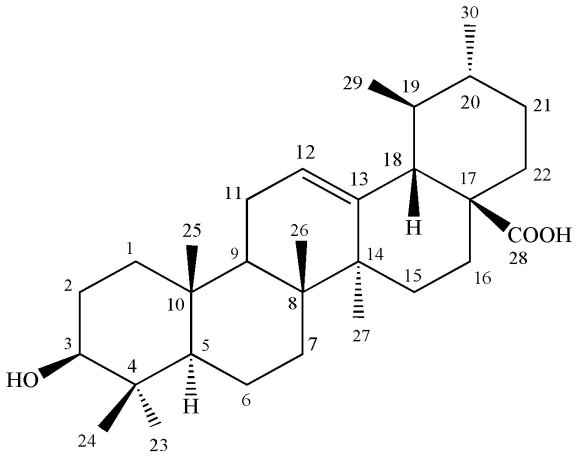

Fig. 1. Proposed structure of compound **1** (Ursolic acid).

Ursane-type pentacyclic triterpenoids from medicinal plants have been identified as one class of secondary metabolites that could play a critical role in the treatment and management of several non-cummunicable diseases. Ursolic acid (UA), a natural pentacyclic triterpenoid, is widely present in medicinal plants such

as fructus ligustri lucidi, hawthorn and bear fruit. In recent years it has created extensive interest because of several pharmacological activities, such as anti-inflammatory, anticancer, antidiabetic, antioxidative, antibacterial, antitumor, and hepato-protective activity (Mazumder et al., 2013; Mlala et al., 2019).

The experimental NMR data of compound **2** corresponds to that of the reported 3,7,24-trihydroxyl-urs-12-en-28-oic acid which is ursane-type pentacyclic triterpene 7,24-dihydroxy ursolic acid (Mazumder et al., 2011) (Fig. 2).

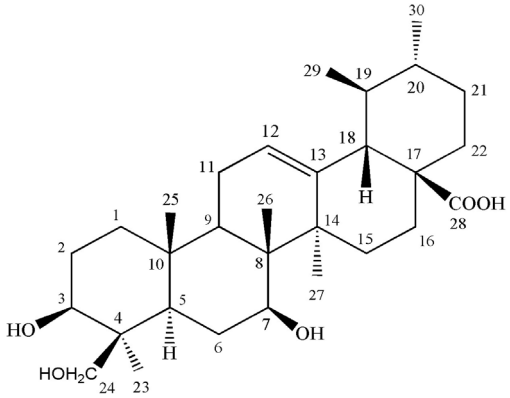

Fig. 2. Proposed structure of compound **2** (3,7,24-Trihydroxyl-urs-12-en-28-oic acid).

This ursane-type pentacyclic triterpene is isolated for the first time in 2011 from the methanolic extract of the leaves of the Bangladeshi medicinal plant, *Saurauia roxburghii* Wall (Mazumder et al., 2011, 2013). The 7,24-dihydroxy ursolic acid is used as a potential precursor for producing cytotoxicity in ursolic acid against A431 tumor cell (Mazumder et al., 2011).

Lignans, sterols, flavonoids, fatty acids, phenols, terpenoids, alcohols, alkaloids, benzopyranoids, and other compounds were isolated from genus *Urtica* (Taheri et al., 2022). *Urtica pilulifera* and *U. dioica* L. essential oil compositions have been investigated and consist mainly of hexahydrofarnesyl acetone, 1,8-cineole, *α*-ionone, *β*-ionone, farnesylacetone, methylbenzene, (−)-limonene, 3-carene, (+)-limonene, gamma-terpinene, vanillin, butyl acetate, 1,2-benzenedicarboxylic acid, and 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin (Ilies, et al., 2012; Sitrallah and Merza, 2018). The seeds, leaves, and roots of *U. dioica* L. have been investigated and the classes of compounds obtained were phytosterols, lignans (Baytop, 1989), carotenoids (Wetherilt, 1989), fatty acids (Does, et al. 1999), phenolics (Le Moal and Truffa-Bachi, 1988), vitamins, minerals, and amino acids (Akgeul, 1993). Caffeic acid and chlorogenic acid are isolated from the leaf (Wagner et al., 1989). The roots of *U. dioica* L. showed the presence of *trans* neo-olivil, a lignans glycoside (Bradley, 1992; Chrubasik, et al., 1997; Ernst and Chrubasik, 2000; Farag, et al. 2013). Although the presence of terpenoids is reported in the genus *Urtica* only the presence of monoterpenes was reported in *Urtica dioica* L. leaves (Đurović, et al., 2017). So this is the first report on the isolation of the ursane-type pentacyclic terpenoids from *Urtica dioica* L. in general and on the roots in particular.

4. Concluding remarks

Compound isolation of the root of *Urtica dioica* L. of Ethiopian origin was performed for the first time in Ethiopia (Ahmed, 2021). Phytochemical screening of the *n*-hexane, chloroform and methanolic extracts has shown the presence of alkaloids, glycosides, phenols, saponins, steroids, tannins, flavonoids, and terpenoids. The presence of these secondary metabolites in the plant is a scientific validation of the plants' traditional use in the treatment of various diseases affecting living organisms. Compound isolation of the methanolic extract resulted in 3*β*-hydroxy-urs-12-ene-28-oic acid commonly known as ursolic acid and 3,7,24-trihydroxyl-urs-12-en-28-oic acid which is ursane-type pentacyclic triterpene 7,24 dihydroxy ursolic acid. Literatures show that the pharmacological activity of 7,24-dihydroxy ursolic acid is not known very well. Ursolic acid on the other hand has created extensive interest in the scientific community because of its pharmacological activities such as anti-inflammatory, anticancer, antidiabetic, antioxidative, antibacterial, antitumor, and hepato-protective activities. This is the first time that the isolation of ursane-type pentacyclic triterpenes took place from the roots of *U. dioica* L. species of Ethiopian origin.

Acknowledgement

The author acknowledges Hawassa University, School of Graduate Studies for financial support.

Conflict of interest

The author declares that there is no conflict of interest.

References

Adamski, Z., Blythe, L.L., Milella, L., Bufo, S.A., 2020. Biological activities of alkaloids: From toxicology to pharmacology. Toxins (Basel) 12(4), 210-213.

Ahmed, M.I., 2021. Phytochemical Screening and Antimicrobial Activities of Stinging Nettle (*Urtica dioica* L.) Leaf, Fruit and Root Extacts. MSc Thesis, Haramaya University, Haramaya, Ethiopia.

Akgeul, A., 1993. Spies Science and Technology. Association Food Technology, Publ. No: 15. Ankara, Turkey.

Anees, A.S., Farah, I., Seemi, S., Kapendra, S., 2014. Role of natural products in drug discovery process. Int. J. Drug. Dev. Res. 6(2), 172-204.

Aziz, R.A., Sarmidi, M.R., Kumaresan, S., 2003. Phytocehemical processing: The next emerging field in chemical engineering aspects and opportunities. J. Kejurut. Kim Malay 3, 45-60.

Barrett, D., 2002. From natural products to clinically useful antifungals. Biochim. Biophys. Acta. 1587(2-3), 224-233.

Baytop, T., 1989. Therapy with Plant in Turkey. Istanbul

University, Faculty of Pharmacy (Second Press). Nobel Medicine Bookstores, Istanbul, Turkey.
Bhattaram, V.A., Graefe, U., Kohlert, C., Veit, M., Derendorf, H., 2002. Pharmacokinetics and bioavailability of herbal medicinal products. Phytomedicine 9(Suppl 3), 1-33.
Bhuwan, C.J, Minky, M., Ajudhia, N.K.. 2014. Pharmacognostical review of *Urtica dioica* L. Int. J. Green Pharm. 8, 201-209.
Bisht, S., Bhandari, S., Bisht, N.S., 2012. *Urtica dioica* (L): An undervalued, economically important plant. Int. Res. J. 2(5), 250-252.
Bradley, P., 1992. Ed. British Herbal Compendium. Dorset, England: British Herbal Medicine Association; 1, 166-167.
Brodal, P., 2004. The Central Nervous System: Structure and Function. Oxford University Press, pp., 170.
Chrubasik, S., Enderlein, W., Bauer, R., Grabner, W., 1997. Evidence for antirheumatic effectiveness of Herba *Urticae dioica* in acute arthritis: A pilot study. Phytomedicine 4(2), 105-108.
Cordell, G.A., 2002. Recent developments in the study of biologically active natural products. ACGC Chem. Commun. 14, 31-63.
Dhanani, T., Shah, S., Gajbhiye, N.A., Kumar, S., 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. Arab. J. Chem. 10(Suppl 1), 1193-1199.
Does, M.P., Ng, D.K., Dekker, H.L., Peumans, W.J., Houterman, P.M., Van Damme, E.J., Cornelissen, B.J., 1999. Characterization of *Urtica dioica* agglutinin isolectins and the encoding gene family. Plant Mol. Biol. 39(2), 335-347.
Đurović, S., Pavlić, B., Šorgić, S., Popov, S., Savić, S., Petronijević, M., Radojković, M., Cvetanović, A., Zeković, Z., 2017. Chemical composition of stinging nettle leaves obtained by different analytical approaches. J. Funct. Foods 32, 18-26.
Ernst, E., Chrubasik, S., 2000. Phyto-anti-inflammatories: A systematic review of randomized, placebo-controlled, double-blind trials. Rheum. Dis. Clin. North Am. 26(1), 13-27.
Farag, M.A., Weigend, M., Luebert, F., Brokamp, G., Wessjohann, L.A., 2013. Phytochemical, phylogenetic, and anti-inflammatory evaluation of 43 *Urtica* accessions (stinging nettle) based on UPLC-Q-TOF-MS metabolomic profiles. Phytochemistry 96, 170-183.
Fischer, C., 1997. Nettles-an aid to the treatment of allergic rhinitis. Eur. Herbal Med. 3(2), 34-35.
Ganesh, S., Vennila, J., 2011. Phytochemical analysis of *Acanthus ilicifolius* and *Avicennia ofcinalis* by GC-MS. Res. J. Phytochem. 5(1), 60-65.
Gnoatto, S.C.B., Dassonville-Klimpt, A., Da Nascimento, S., Galera, P., Boumediene, K., Gosmann, G., Sonnet, P., Moslemi, S., 2008. Evaluation of Ursolic acid isolated from *Ilex paraguariensis* and derivatives on aromatase inhibition. Eur. J. Med. Chem. 43(9), 1865-1877.
Gulcin, I., Kufre, O., Oktay, M., Buyuko, E., 2004. Antioxidant, antimicrobial, anticancer and analgesic activities of *U. dioica*. J. Ethnopharmacol. 90(2-3), 205-215.
Harwood, M.B., Danielewska, J.F., Borzelleca, G.W., Flamm, G.M., Williams, T.C., 2007. A critical review of the data related to the safety of quercetin and lack of evidence of *in vivo* toxicity, including lack of genotoxic/carcinogenic properties. Food Chem. Toxicol. 45(11), 2179-2205.
Hayouni, E.A., Abedrabba, M., Bouix, M., Hamdi, M., 2007. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera L.* and *Juniperus phoenica L.* fruit extracts. Food Chem. 105(3), 1126-1134.
Ilies, D.C., Tudor, I., Radulescu, V., 2012. Chemical composition of the essential oil of *Urtica dioica*. Chem. Nat. Comp. 48(3), 506-507.
Ishida, B.K., Ma, J., Bock, C., 2001. A simple rapid method for HPLC analysis of lycopene isomers. Phytochem. Anal. 12(3), 194-198.
Jacob, R.A., Burri, B.J., 1996. Oxidative damage and defense. Am. J. Clin. Nutr. 63(6), 985S-990S.
Ji, H.F., Li, X.J., Zhang, H.Y., 2009. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO Rep. 10(3), 194-200.
Joshi, N., Pandey, S.T., 2007. Stinging nettle (*Urtica dioica*)-history and its medicinal uses. Asian Agrihist. 21(2), 133-138.
Juang, Y.P., Liang, P.H., 2020. Biological and pharmacological effects of synthetic saponins. Molecules 25(21), 4974.
Kinghorn, A.D., Pan, L., Fletcher, J.N., Chai, H., 2011. The relevance of higher plants in lead compound discovery programs. J. Nat. Prod. 74(6), 1539-1555.
Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: An overview. Sci. World J. 2013, 162750.
Le Moal, M.A., Truffa-Bachi, P., 1988. *Urtica dioica agglutinin*, a new mitogen for murine T lymphocytes: Unaltered interleukin-1 production but late interleukin 2-mediated proliferation. Cell Immunol. 115(1), 24-35.
Li, J.W., Vederas, J.C., 2009. Drug discovery and natural products: End of an era or an endless frontier? J. Sci. 325 (5937), 161-165.
Lukesova, H., 2017. Identifying plant fiber textiles from Norwegian Merovingian Period and Viking Age graves: The Late Iron Age Collection of the University Museum of Bergen. J. Archaeol. Sci. Reports. 13, 281-285.
Manganelli, R.E., Uncini, L.Z., Tomei, P.E., 2005. Antiviral activity *in vitro* of *Urtica dioica* L., *Parietariadiffusa* M. et K. and *Sambucus nigra* L. J. Ehnopharmacol. 98(3), 323-327.
Mazumder, K., Siwu, E.R.O., Nozaki, S., Watanabe, Y., Tanaka, K., Fukase, K., 2011. Ursolinic acid derivatives from Bangladeshi medicinal plant, *Saurauja roxburghii*: Isolation and cytotoxic activity against A431 and C6 glioma cell lines. Phytochem. Lett. 4(3), 287-291.
Mazumder, K., Tanaka, K., Fukase, K., 2013. Cytotoxic activity of ursolic acid derivatives obtained by isolation and oxidative derivatization. Molecules 18(8), 8929-8944.
Mlala, S., Oyedeji, A.O., Gondwe, M., Oyedeji, O.O., 2019. Ursolic acid and its derivatives as bioactive agents. Molecules 24(15), 2751-2775.
Mohammadhosseini, M., Frezza, C., Venditti, A., Akbarzadeh, A., 2019a. Ethnobotany and phytochemistry of the genus *Eremostachys* Bunge. Curr. Org. Chem. 23, 1828-1842.
Mohammadhosseini, M., Venditti, A., Flamini, G., Sarker, S.D., Kalaee, M., 2022. The genus *Micromeria* Benth.: An overview on ethnobotany, chemotaxonomy and

phytochemistry. Trends Phytochem. Res. 6(3), 155-186.
Olaoluwa, O., Taiwo, O., Nahar, L., Sarker, S.D., 2022. Ethnopharmacology, phytochemistry and biological activities of the African species of the genus *Ficus* L. Trends Phytochem. Res. 6(1), 46-69.
Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod. 66(7), 1022-1037.
Ngo, L.T., Okogun, J.I., Folk, W.R., 2013. 21st Century natural product research and drug development and traditional medicines. J. Nat. Prod. Rep. 30(4), 584-592.
Pakrashi, C.S., Datta, S., Ghosh-Dastidar, P.P., 1968. Indian medicinal plants-XVII.3 Phytochemical examination of *Carissa SPP*. Phytochemistry 7(3), 495-496.
Pashazadeh, M., Rahbani, M., Zakhireh, S., 2013. Effect of Nettle oil (*Urtica dioica*) consumption on levels of blood glucose and lipids in alloxan-induced diabetic rats. Bull. Env. Pharmacol. Life Sci, 2(10), 84-89.
Prashant, T., Bimlesh, K., Mandeep, K., Gurpreet, K., Harleen, K., 2011. Phytochemical screening and extraction: A review. Int. Pharm. Sci. 1(1), 98-106.
Riehemann, K., Behnke, B., Schulze-Osthoff, K., 1999. Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-κB. FEBS Lett. 442(1), 89-94.
Rout, S.P., Choudary, K.A., Kar, D.M., Das, L., Jain, A., 2009. Plants in traditional medicinal system-future source of new drugs. Int. J. Pharm. Pharm. Sci. 1(1), 1-23.
Seebacher, W., Simic, N., Weis, R., Saf, R., Kunert, O., 2003. Complete assignments of ^{1}H and ^{13}C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives. Magn. Reson. Chem. 41(8), 636-638.
Serrano, J., Puupponen-Pimi, R., Dauer, A., Aura, A.M., Saura-Calixto, F., 2009. Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. Mol. Nutr. Food Res. 53, 310-329.
Silva, I.F., Oliveira, F.F.d., Oliveira, R.A.d., 2021. *Siparuna* Aublet genus (Siparunaceae): From folk medicine to chemical composition and biological activity. Trends Phytochem. Res. 5(4), 168-189.
Sitrallah, S., Merza, J., 2018. Chemical composition of essential oil extracted from *Urtica pilulifera* and evaluation of its biological activity. Food Sci. Qual. Manag. 78, 44-48.
Soto-Blanco, B., 2022. Chapter 12-Herbal Glycosides in Healthcare, Editor(s): Subhash C. Mandal, Amit Kumar Nayak, Amal Kumar Dhara, Herbal Biomolecules in Healthcare Applications, Academic Press, pp. 239-282.
Taheri, Y., Quispe, C., Herrera-Bravo, J., Sharifi-Rad, J., Ezzat, S.M., Merghany, R.M., Shaheen, S., Azmi, L., Prakash Mishra, A., Sener, B., Kılıç, M., Sen, S., Acharya, K., Nasiri, A., Cruz-Martins, N., Tsouh Fokou, P.V., Ydyrys, A., Bassygarayev, Z., Daştan, S.D., Alshehri, M.M., Calina, D., Cho, W.C., 2022. *Urtica dioica*-derived phytochemicals for pharmacological and therapeutic applications. Evid. Based Complement. Alternat. Med. 4024331.
Upton, R., 2013. Stinging nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine. J. Herb. Med. 3, 9-38.
Urtica dioica L. Plants of the World Online: Kew Royal Botanic Gardens. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:260630-2_accession date 12:29/2022.
Venditti, A., Guarcini, L., Ballero, M., Bianco, A., 2015. Iridoid glucosides from *Pentas lanceolata* (Forssk.) Deflers growing on the Island of Sardinia. Plant Syst. Evol. 301, 685-690.
Venditti, A., Lattanzi, C., Ornano, L., Maggi, F., Sanna, C., Ballero, Alvino, A., Serafini, M., Bianco, A., 2016. A new glucosidic phthalide from *Helichrysum microphyllum* subsp. *tyrrhenicum* from La Maddalena Island (Sardinia, Italy). Nat. Prod. Res. 30(7), 789-795.
Wagner, H., Willer, F., Kreher, B., 1989. Biologically active compounds from the aqueous extract of *Urtica dioica*. Planta Med. 55(5), 452-454.
Wetherilt, H., 1989. Isırgan Otu Yaprak Ve Tohumlarının Besleyici €ozelliklerive Antit €um €ore Etkileri. PhD Thesis, (Turkish). Hacettepe University. Graduate Institute of Health Science, Ankara, Turkey.
World Health Organization, 2002. National Cancer Control Programmes: Policies and Managerial Guidelines, 2nd Edn. Geneva, World Health Organization.