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Research Article

Isolation, Characterization and Structural Elucidation of the Roots of *Rumex nervosus*

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ABSTRACT

Rumex nervosus belongs to the family of Polygonaceae, which is traditionally used in Ethiopia to treat various diseases. This prompted us to isolate bioactive compounds from the root of this plant. Ground root parts of *Rumex nervosus* were subjected to exhaustive extraction successively with petroleum ether and methanol. The solvent from each extract was evaporated under reduced pressure using rotavapour to obtain petroleum ether and methanol extract. Chromatographic purification of the methanol extracts by Column chromatography followed by Preparative Thin layer Chromatography using Chloroform: methanol (9.5:0.5) ratio gave a compound coded as RN-6. The structure of this compound 4-ethylheptyl benzoate was characterized as by means of ^{1}H NMR, ^{13}C NMR, UV and IR spectral data.

Keywords: Ethiopia; Medicinal plant; Polygonaceae; *Rumex nervosus*

INTRODUCTION

Plants have been used to treat a wide range of diseases throughout the history of human beings and this practice continues to date. This is mainly because most of these herbals are accessible, affordable and the extracted chemicals have little or no side effects as compared to drugs synthesized in the laboratory. Plants comprise the largest component of the diverse therapeutic elements of traditional health care practices both in humans and animals. The medicinal values of plants are due to the chemical substances that produce a definite physiological action on human body and are called phytochemicals. They are chemicals extracted from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants [1,2]. Naturally occurring compounds may be divided into two broad categories. The first class of compounds is known as primary metabolites. They occur in all cells and play a central role in the metabolism and reproduction of those cells. Primary metabolites include the nucleic acids, the common amino acids, sugars and the high molecular weight polymeric materials such as cellulose, lignins and proteins which form the cellular structures. Most primary metabolites exert their biological effect within the cell or organism that is responsible for their production. The second class of compounds is secondary metabolites. Such compounds are characteristic of a limited range of species and occur in plants in a high structural diversity. The major classes of secondary metabolites include tannins, glycosides, flavonoids, alkaloids, terpenoids, steroids, quinones and saponins are among others and play significant role in drug discovery. Secondary metabolites have often attracted interest of researchers because of their biological effect on other organisms [3,4].

The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites. Natural products often have an ecological role in regulating the interactions between plants, micro-organisms, insects and animals. They can be defensive substances, anti-feedants, and attractants. Natural products from plants remain vital in drug discovery where they can be used directly as drugs or serve as leads to new drugs by providing chemical entities [5]. The currently accepted modern medicines have gradually developed over the years by scientific and observational efforts of scientists. However, the basis of their development remains rooted in traditional medicine and therapies. The approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs [6].

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The *Rumex* species, belonging in the Polygonaceae family, comprise about 200 species widely distributed around the World. The name *Rumex* originated from the Latin word for dart, alluding to the shape of the leaves [7]. There have been numerous ethno botanical and ethno pharmacological literature reports dealing with the occurrence and traditional uses of *Rumex* species [8-10]. In some regions, the leaves of *Rumex* species (e.g. *R. acetosa, R. acetosella, R. abyssinicus, R. crispus, R. sanguineus, R. tuberosus* and *R. thyrsiflorus, R. vesicarius*) are utilized as foods, mainly in the forms of sour soups (usually in milk), sauces and salads [11,12]. Traditional names for several species used as food reflect their gustatory characteristics, taste and aroma, e.g. sour weed in the case of *Rumex*. The roots of many species belonging in the *Rumex* genus have been used in medicine from ancient times because of their gentle laxative effect. *R. acetosa* is officially listed in the Korean Food Code (Korea Food & Drug Administration) as one of the main food materials and has been used in folk medicine as a mild purgative and also for the treatment of cutaneous diseases [13]. Some of the species are cultivated, e.g. *R. acetosa and R. vesicarius* [14]. On the other hand, the members of this genus include many invasive weeds (e.g. *R. obtusifolius* and *R. crispus*) [15]. Plants belonging to the Polygonaceae are known to produce a large number of biologically important secondary metabolites, such as anthraquinones, naphthalenes, stilbenoids, steroids, flavonoid glycosides, leucoanthocyanidins and phenolic acids [16-20]. The aerial parts, leaves and roots of the plants are used in traditional medicine for the treatment of several health disorders such as infections, diarrhoea, constipation, mild diabetes, oedema, jaundice, and as an antihypertensive, diuretic and analgesic and in case of skin, liver and gallbladder disorders, and inflammation. The genus *Rumex* has attracted the attention of many researchers because of its phytoconstituents and medicinal properties. The extracts of these plants, and compounds isolated from them, have been demonstrated to possess various pharmacological activities, including anti-inflammatory, antioxidant, antitumour, antibacterial, antiviral and antifungal properties *in vitro* and *in vivo* [13,18,21-26].

Rumex nervosus

Rumex nervosus is commonly found near and around the terraces of high altitude areas (above 1000m.). Genus *Rumex* is a genus of about 200 species of annual, biennial and perennial herbs in the buckwheat family Polygonaceae. Members of this family are very common perennial herbs growing mainly in the northern hemisphere, but various species have been introduced almost everywhere. *Rumex nervosus* Vahl. is a perennial herb mainly distributed in Yemen, Saudi Arabia, Ethiopia, Somalia, Kenya, Tanzania and Eritrea [27,28].

Use in ethnomedicine

Rumex nervosus locally called “Embuacho” in Amharic, “Huhot” in Tigrigna and “Dhangaggoo” in Afan Oromo in Ethiopia. The juice of *Rumex nervosus* is used in Ethiopia to seizure bleeding during male circumcision [29]. *Rumex nervous* leaves are an edible, consumed by some people in Saudi Arabia. *Rumex* species are used as food plants by the larvae of a number of Lepidoptera species [28]. The leaves of the plant are usually boiled with water, filtered and the water extract is consumed to reduce non-specific diarrhea1. The roots and aerial parts of *Rumex nervosus* have been used traditionally for a variety of therapeutic uses, such as antioxidant, cytotoxic, antifertility, anti-inflammatory, antimicrobial, antidiarrheal and antiviral activities [30]. Leaves of *Rumex nervosus* crushed and its paste applied on affected area can prevent Brest Cancer diseases [31]. The use of this plant as anti-dysentery, cure for stomach ache, and effective treatment of warts [32]. The roots of *Rumex nervosus* used as anti-microbial and anti-inflammatory activity [33].

R. nervosus is used as a cure for acne, a hypoglycemic agent, and an ophthalmic antiseptic [34]. It also shares the uses of *R. abyssinicus* for the treatment of wounds, eczema, typhus and rabies [35]. In Eritrea the leaves and stem of this herb is used for traditional medicine by the practitioners mostly on highland and on the villages it is used for purifying the body by women (traditionally known ’tish’) as substituent of olive tree, to do this, the leaves are put on fire then they cover the patient body with that hot leaves and blanket so that the vapours and smoke surround all the body [28]. Leaf of *Rumex nervosus* used to treat skin disorders, leaves are crushed and mixed with butter, and then it is applied on the affected area [36]. Eat or chew and swallow the fluid of leaf and steam part of *R. nervosus* used to treat for Ascariasis, leaf of *R. nervosus* Soak it in water together with whole part of *Withania somnifera* and fruit of *Citrus aurantifolia* and wash body with it for the treatment of Michi and leaf of *R. nervosus* Crush and mixing with leaves of *Withania somnifera*, seeds of *Lepidium sativum* and bulbs of *Allium sativum*, soak it in water and wash body with it for the treatment of Itching /skin rash [37]. Traditionally in Eritrea, the leaves, stems and sometimes roots of *Rumex nervosus* are used as traditional medicines, for the eye disease, taeniacapitis, haemorrhoids, infected wounds, arthritis, eczema, abscess and gynecological disorders [28].

Biological Activities of *Rumex nervosus*

Rumex species contains anthracene derivatives like chrysophanol, physcion, emodin, aloe-emodin, rhein; which are the main biologically active compounds responsible for anti-cancer, cytotoxic, genotoxic and mutagenicity properties [38]. Some reports in literature about the biological activities of *Rumex nervosus* Vahl. ; Analgesic [39], anti-inflammatory and anti-microbial activity [33], urease enzyme inhibition [40], anthelmintic [41], anti-diarrheal activity [42], anti-bacterial activity [28], anti-oxidant activity [43], acute-toxicity and analgestic activity [44], anti-leishmanial, insecticidal and phytotoxic potential [45] and *in vitro* anticancer, antimicrobial and antioxidant activities [46]. The methanol, water and chloroform extracts of the leaf, bark, stem and root parts of *R. nervosus* and the root of *R. abyssinicus* were reported to possess antibacterial activity against several bacteria including *S. aureus* and *P. aeruginosa* [35].

Phytochemistry of *Rumex nervosus*

Previously isolated classes of constituents of *R. nervosus* was flavonoids, steroids, tannins, tartaric and citric acids [47]. Recently the biologically active components of the plant

reported and characterized 19 flavonoids for the first time in its flowers; namely (epi)catechin O-gallate, quercetin O-pentoside, luteolin 6-C-glucoside isomers (two), apigenin 8-C-glucoside, apigenin 6-C-glucoside, quercetin 3-O-glucoside, quercetin acetyl glycoside isomers (three), quercetin 3-O-rhamnoside, quercetin 3-O-rutinoside isomers (two), quercetin 3-acetylrhamnoside, hesperetin, naringenin, apigenin 6-C-glucoside 7-O-glucoside isomers (two), and liquiritin. These flavonoid components showed effective inhibition of pro-inflammatory mediators in mouse macrophage RAW 264.7 cells, such as inducible nitric oxide synthase, cyclooxigenase-2, inhibitor of kappa B, and interleukin-1 β [48]. Studies showed that four compounds, viz. chlorogenic acid, catechin, orientin, and apigenin-O-acetylglycoside were characterized for the first time in *Rumex nervosus* leaves and stems by using liquid chromatography with electrospray ionization tandemmass spectrometry [43]. Studies although showed that essential oil obtained from ethyl acetate fraction of leaves of *R. nervosus* was subjected to GC-MS analysis and identified seven saturated and unsaturated fatty acid. All the compounds were identified as: C16:0; Palmitic acid, methyl ester, C16:1c; Palmitoleic Acid Methyl ester, C17:1; Heptadecenoic Acid, Methyl ester, (E)-, C18:0; Stearic acid, Methyl ester, C18:2c; Linoleic acid, Methyl ester, C18:2T; Octadecadienoic acid, Methyl ester, C18:1c; Oleic acid, the Methyl ester with retention times as: 16.364, 17.376, 19.440, 19.569, 19.826, 19.892 and 20.345 minutes respectively. The major fatty acids obtained as the their methyl esters were C16:1c; Palmitoleic Acid (28.35%) followed by C16:0; Palmitic acid, (25.37%), C18:0; Stearic acid (20.25%), while C18:2c; Linoleic acid, C17:1; Heptadecenoic Acid, (E)- and C18:1c; Oleic acid, were as (9.18%), (8.99%) and (7.24%) respectively. The lower fatty acid obtained was C18:2T; Octadecadienoic acid, Methyl ester with (0.62%) [46]. A recent review by Vasas et al. [49] showed that detail information on Phytochemistry of *Rumex* species. However, to the best of our knowledge there is no published scientific report on the isolation and characterization of the roots extracts of this plant. So, since such medicinal herbs are widely distributed in different regions of Ethiopia and are traditionally used in the treatment of different varieties of diseases, the researcher took a big interest in conducting this research for chemical investigation of the roots extracts of the plant which could be important to generate adequate knowledge to the societies.

MATERIALS AND METHODS

Instruments and chemicals

IR spectrum was obtained as pellets on Perkin-Elmer Bx infrared spectrometer in the range 4000-400 cm^{-1}, 1H NMR, 13C NMR spectra was recorded on a Bruker advance 400 MHz spectrometer with TMS as internal standard. The Ultra-Violet and Visible (UV-Vis) spectra was taken on GENESY'S 2PC UV-Vis scanning spectrometer (200-800 nm). Melting points was recorded digital melting point apparatus. Silica gel with fluorescent indicator at 254 nm and aluminum cards with layer thickness 0.2 mm was used for TLC. Silica gel 60 (Merck), particle size 0.063-0.200 (70-230 mesh ASTM) was used for column chromatography. Compound on TLC was detected using eye protects by UV-Vis. PTLC (Preparative Thin Layer Chromatography) was used in the separation of analytes from small quantities of sample often it is used in conjunction with column chromatography as a final purification step of relatively less complex mixtures. Rotary evaporator was used to concentrate the samples. Petroleum ether, chloroform and methanol were used as solvent.

Sample collection

The plant *Rumex nervosus* was collected from Deber Berhan,Amhara Region in the local distinct of Debersina and identified by Prof. Sebsibie Demissew of the National herbarium, Department of Biology, Addis Ababa University.

Scientific Classification of *Rumex nervosus*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Polygonaceae
Genus	*Rumex* L.
Species	*Rumex nervosus* Vahl

Figure 1: The plant *Rumex nervosus*.

Extraction and Isolation

After collection, plant material was dried to constant weight at room temperature in open air in the laboratory away from direct sunlight. The dry plant material was then ground to a fine powder using a Warring commercial blender. The powdered plant material was stored in tightly closed glass bottles in the dark at room temperature. The drying of plant material makes handling, working on and storing of plant material much easier. It also improves extraction efficiency as some membranes of some organelles containing phytochemicals are destroyed during drying. However, labile or volatile compounds can be lost and some undesirable artifacts may be formed so caution is taken to dry plant material at ambient temperatures away from direct sunlight. The dry powdered plant material was sequentially extracted with solvents of increasing polarity: petroleum ether (PE) and methanol (MeOH).Two hundred grams (200 g) of root

samples were extracted sequentially with one liter petroleum ether. The mixtures were shaken gently in a mechanical shaker for about one hour to increase extraction efficiency and left to stand at room temperature for 36 hours. Extraction solutions were filtered through filter paper onto pre-weighed flasks and organic solvents were removed through evaporation under a stream of air at room temperature in the fume hood. Similarly the solvent free marc was then soaked with 1.5 L methanol for 72 hours and the extract was collected. This filtrate was evaporated under the reduced pressure using the Rota vapor and afforded 17 g brown gummy extract. Dry extracts were kept in the refrigerator in tightly closed vials and used for the TLC and CC analysis. The methanol extract (6.5) was applied on a column chromatography packed with 200 g silica gel. Isolation was carried out using the solvents chloroform and methanol with increasing polarity (Table 1).

The Column was eluted using the following solvent system and 54 fractions were collected.

Table 1: Methanol extract fraction.

Solvent System	Ratio	Volume(ml)	Fractions
Chloroform	100%	100	01-Dec
Chloroform- Methanol	09:01	100	13-20
Chloroform- Methanol	08:02	100	21-30
Chloroform- Methanol	07:03	100	31-40
Chloroform- Methanol	06:04	100	41-49
Chloroform- Methanol	01:01	100	50-54

Based on TLC analysis, fraction that showed the same characteristics of spots were combined. Fraction 1-6 (75.3 mg) was combined and have four spots on TLC showed under UV. The series combined fraction was subjected to Preparative Thin Layer Chromatography (PTLC) developed in chloroform-methanol (9.5: 0.5) mixture which yielded one spot with a white fluorescence under UV. The extraction of the plant and isolation of the compound was described in detail in the Figure 2 below.

RESULTS AND DISCUSSION

Yield of solvent extract and isolation of roots of *Rumex nervosus*

The dried and powdered Roots (200 g) of *Rumex nervosus* subjected to exhaustive extraction successively with petroleum ether and methanol. The solvent from each extract was recovered under reduced pressure using rotavapour to obtain a methanol extract (17 g). Chromatographic purification of the methanol extract (6.5 g) yielded a compound coded; RN-6. The structure of this compound has been elucidated on the basis of spectroscopic evidence as described in the following section.

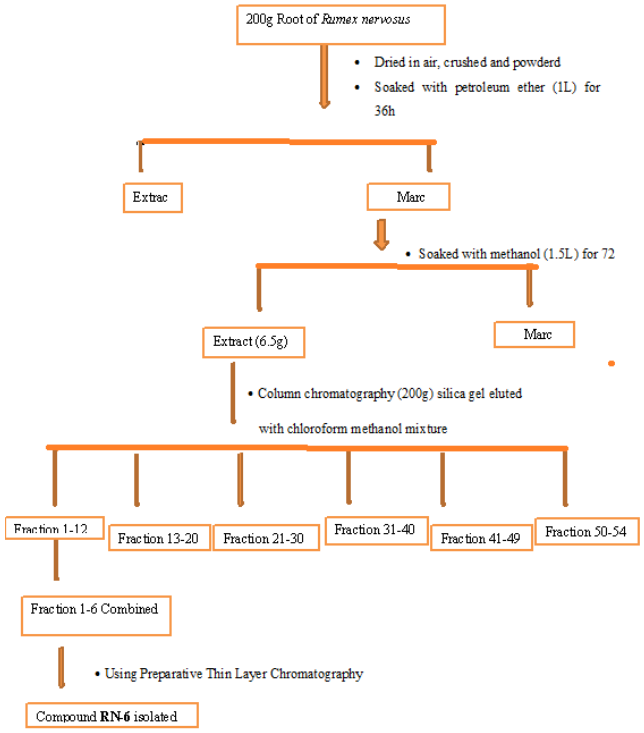

Figure 2: Method of extraction of the plant material.

Characterization of fraction RN-6

The UV spectrum of RN-6 (Figure 3) shows absorbance peaks at 276 nm which indicate the presence of a carbonyl substituted aromatic ring.

In the IR (KBr) spectrum (Figure 4) of the compound displayed the absorption band at δ 3439 cm^{-1} may be due to moisture. The absorption band at δ 2925 cm^{-1} indicates -C-H stretching. The absorption band at δ 1728 cm^{-1}indicates the presence of ester carbonyl group attached to aromatic ring. The absorption Band at δ 1276 cm^{-1} indicates C-O stretching. The absorption band at δ 1125 cm^{-1} indicates –C-C- stretching of the compound.

The ^{1}H-NMR Spectrum (Figure 5, Table 2) of the compound showed Signals at δ 0.96 indicates the presence of two methyl group. The proton signal at δ 1.25, 1.29, 1.33, and 1.75 shows the presence of five methylene groups. The signal at δ 1.47 multiplate indicate methine group. The signal at δ 4.25 triplets was due to oxygenated methylene carbon protons. The signals at δ 7.73-7.79 indicate protons of aromatic carbon.

Table 2: ^{1}H-NMR spectra data of compound RN-6.

Hydrogen Number	δ (PPm)
1	4.25 (2H, t)
2	1.75 (2H, t)
3	1.25 (2H, m)
4	1.47 (1H, m)

5	1.25 (2H, m)
6	1.33 (2H, m)
7	0.96 (3H, d)
8	1.29 (2H, m)
9	0.96 (3H, d)
3',7'	7.97 (2H, dd)
5'	7.47 (1H, dd)
4',6'	7.37 (2H, dd)

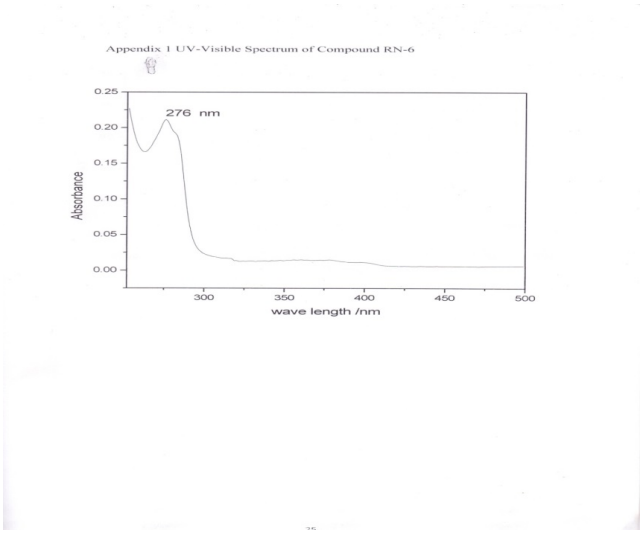

Figure 3: UV-visible spectrum of compound RN-6.

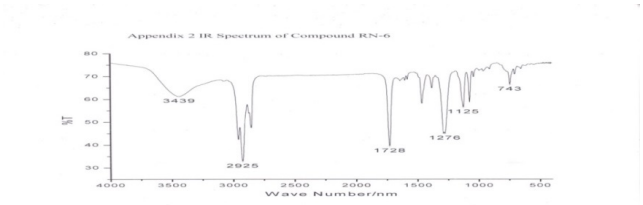

Figure 4: IR spectrum of compound RN-6.

The ^{13}C NMR and DEPT-135 (Figures 6 and 7, Table 3) indicate the compound RN-6 has 14 carbons. The spectra show two methyl carbons at δ 10.97 and 14.7. Five methylene carbon at δ 23, 23.74, 28.93, 29.75 and 30.4. One methylene carbon that is attached with oxygen at δ 68.16. Two quaternary carbons at δ 167.79 and 132.45. Four methane carbons at δ 38.72, 130.9, 130.87.128.81. Additionally the ^{13}C NMR spectrum of compound RN-6, indicate the presence of aromatic ring.

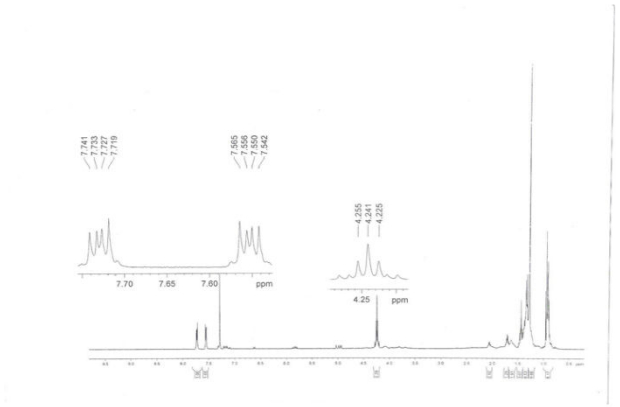

Figure 5: ^{1}H-NMR spectrum of compound RN-6.

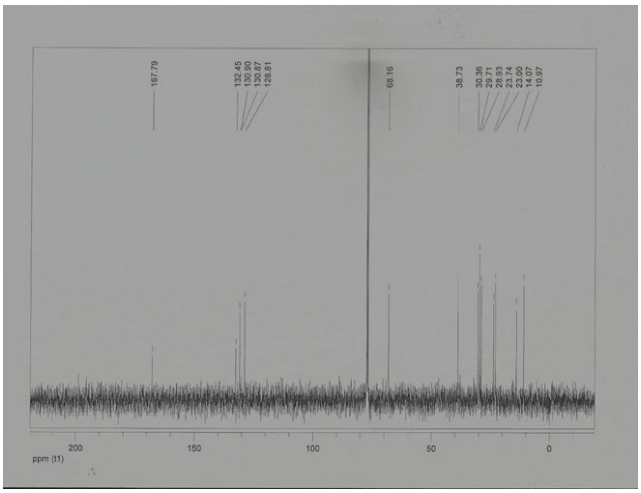

Figure 6: ^{13}C NMR spectrum of compound RN-6.

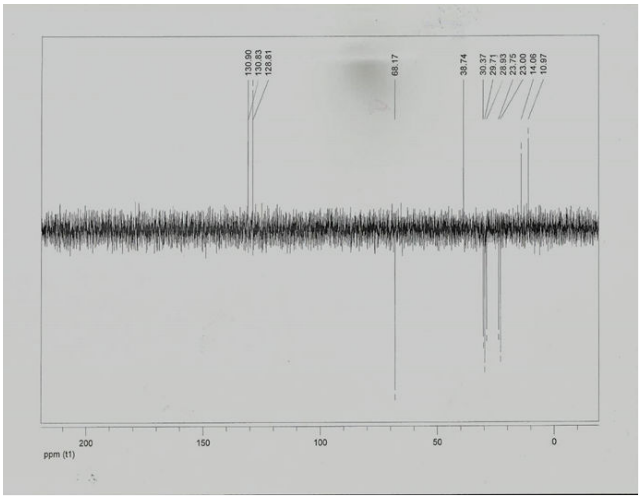

Figure 7: DEPT-135 spectrum of compound RN-6.

Table 3: ^{13}C NMR and DEPT-135 spectra data of compound RN-6.

1	68.16	Down	CH_2
2	23.75	Down	CH_2
3	29.71	Down	CH_2

4	38.74	Up	CH_2
5	30.37	Down	CH_2
6	23	Down	CH_2
7	14.07	Up	CH_3
8	28.93	Down	CH_2
9	10.97	Up	CH_3
1'	167.79	-	Quaternary
2'	132.45	-	Quaternary
5'	130.9	Up	CH
3',7'	130.87	Up	CH
4',6'	128.81	Up	CH

From comparison of phytochemistry of *Rumex* species with literature, compound RN-6 (4-ethyl heptyl Benzoate) closely resembles (Figure 8) [46,49].

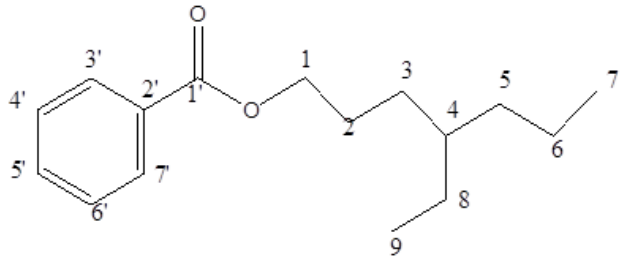

Figure 8: Proposed structure of compound RN-6 (4-ethyl heptyl Benzoate).

CONCLUSION AND RECOMMENDATIONS

The Chloroform-methanol (9.5: 0.5) extract of *Rumex nervosus* affords 13 mg of compound RN-6. Compound RN-6 (4-ethyl heptyl Benzoate) was isolated and further purified by chromatographic methods such as Column Chromatography, Thin layer Chromatography, Preparative thin layer Chromatography and the structural elucidation of this compound was accomplished by means of a combination of spectroscopic methods. To the best of our Knowledge there was no report isolation and characterization of compounds from the root part of *Rumex nervosus*. Compound RN-6 was reported here for the first time from this species on their root parts.

In this study the extraction, isolation and structure elucidation of compound RN-6 (4-ethyl heptyl Benzoate) was accomplished using chromatographic and spectroscopic method. The researcher recommended that advanced chromatographic techniques such as HPLC should be used to isolate more compounds from different extracts of the plants. 2D-NMR techniques are also required to elucidate structures of novel compounds isolated from the plant. Additionally bioassay tests should be conducted on crude extracts fractions and isolated compounds from the plant.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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