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Chemical constituents of *Solanum mauense* (Solanaceae) and *Dovyalis abyssinica* (Salicaceae)

K. Denis CHIRCHIR^{1,2*}, K. Peter CHEPLOGOI², O. Josiah OMOLO² and
K. Moses LANGAT³

¹Department of Physical Sciences, University of Kabianga, P.O Box 2030-20200, Kericho, Kenya.

²Department of Chemistry, Egerton University, 536-20115, Egerton, Kenya; ³Department of Chemistry, Faculty of Engineering and Physical Sciences, University of Surrey, GU2 7XH, United Kingdom.

*Corresponding author; E-mail: chir07@yahoo.com

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ABSTRACT

Profiling the chemical constituents of medicinal plants used in folk medicine is vital in enhancing their full exploitation and utilization in modern medicine. The objective of the study was to characterize anticancer compounds from two medicinal plants, *Dovyalis abyssinica* and *Solanum mauense* that are used in folk medicine by the Kipsigis community, in Kenya, in managing various ailments. There is unvalidated folk medicine claim that the decoctions from the roots of *D. abyssinica* and fruits of *S. mauense* have anticancer property among other uses. This study reports on phytochemistry of *D. abyssinica* and *S. mauense*. Cold extraction method via soaking in solvents was used to prepare the dichloromethane crude extracts, which were later fractionated and purified using chromatographic techniques. Betulinic acid, benzoylated triterpenoid and fatty acids were isolated from dichloromethane extract of *S. mauense*. Similarly, two compounds, benzoic acid and tremulacin, were isolated from *D. abyssinica* alongside fatty acid. Anticancer property of betulinic acid, benzoic acid derivative and tremulacin has been reported previously in literature. This study gives the scientific basis for the use of these medicinal plants in folk medicine.

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Keywords: Medicinal plants, *Solanum mauense*, *Dovyalis abyssinica*, Betulinic acid and tremulacin.

INTRODUCTION

Cancer is a general term applied to a series of malignant diseases that may affect different parts of a body (Sakarkar and Deshmukh, 2011). These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating

abnormal growth at other sites. Cancers may be caused in one of three ways, namely incorrect diet, genetic predisposition, and via the environment (Bhanot et al., 2011). The major treatment regime for cancer includes chemotherapy, radiotherapy and surgery (Owoeye et al., 2010). The synthetic anticancer remedies are beyond the reach of common man because of cost factors. Higher

plants are ancient sources of medicinal agents (Tatsimo et al., 2017). Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical though the effectiveness in crude forms is subject to discussion (Sakarkar and Deshmukh, 2011). Gandonou et al. (2017) also highlighted the usage of medicinal plants in improving traditional drugs/medicines.

Plants have a long history of use in the treatment of cancer and it is significant that over 60% of currently used anti-cancer agents come from natural sources (Bhanot et al., 2011). According to World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as primary healthcare system (Sakarkar and Deshmukh, 2011). The history of plants as sources of anti-cancer agents started in earnest in the 1950s with the discovery and development of the vinca alkaloids (vinblastine and vincristine) and the isolation of the cytotoxic podophyllotoxins. Vinca alkaloids were responsible for an increase in the cure rates for Hodgkin's disease and some forms of leukemia (Bhanot et al., 2011). Anticancer drugs discovered from herbal medicines have a long history and plant-derived compounds have been an important source of several useful anti-cancer agents in clinical practice (Saisri, 2015). Some herbs protect the body from cancer by enhancing detoxification functions of the body whereas others reduce toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer (Sakarkar and Deshmukh, 2011).

The Solanaceae family comprises a large number of species with both toxic and pharmacological properties (Vieira et al., 2013). The Solanaceae are the third most important plant taxon economically and the most valuable in terms of vegetable crops, and are the most variable of crops species in terms of agricultural utility (Saisri, 2015). A number of *Solanum* species have previously

been investigated for their cytotoxicity, antioxidant and antiviral activities, and treatment of protozoal infections. Some of the anticancer compounds isolated from Solanaceae family are capsaicin, withnolide, nicotine and solasodine. The ripe fruits, orange or reddish brown in colour, of *S. maunse* are used traditionally by the Kipsigis community to manage bacterial diseases and cancer. It is also reported that decoction from the ground seeds are used by the Ogiek community in Kenya to expel worms, as purgative, manage tuberculosis and to treat chest ailments (Amuka et al., 2014).

Salicaceae family is known to yield metabolites, which have bacteriocidal effects (Amuka et al., 2014). *Dovyalis abyssinica* (A. Rich), commonly called African gooseberry is native to Africa (De Rosso and Mercadante, 2007) and belongs to the small genus *Dovyalis*, formerly of the Flacourtiaceae family, which comprises 16 species. Cyanogenic tribes of the Flacourtiaceae were separated in the family Achariaceae, and the noncyanogenic tribes, including *Dovyalis* and *Homalium*, were united with the Salicaceae (Rasmussen et al., 2006). *D. abyssinica* occur naturally from Ethiopia, Eritrea and Somalia in the North through Kenya and Tanzania to Malawi in the South and grows in upland rainforest, dry evergreen forest, on riverbanks and sometimes in more open woodland (Kiamba et al., 2009).

Ethnobotanical uses of *D. abyssinica* have been reported in literature. Jeruto et al., (2011) reported that it is used traditionally to treat gonorrhoea, brucellosis and teeth problems in humans and to treat mastitis in animals. In addition decoction from the roots is reported to be used in managing typhoid and diarrhea among the Maasai community in Kenya (Omwoyo et al., 2017). The aqueous extracts of the roots of *D. abyssinica* are used by the Kipsigis Community, in folk medicine, as anticancer agent and in managing other ailments. However the anticancer property of the two medicinal plants under study has not been validated. As part of our ongoing quest on secondary metabolite profiling of

indigenous medicinal plants, this study was conducted in a view to characterizing scientifically the two medicinal plants.

MATERIALS AND METHODS

Sample collection

Information of the medicinal plant was obtained through direct interview with the local healers and field observations. Details of the parts used, traditional mode of preparation, route of administration, dose given, duration of the treatment and other plants used together were gathered. The roots of a mature *D. abyssinica* and fruits of *S. mauense* were collected based on the interview. Leaves of the two medicinal plants were also collected to aid in authentication. The samples were collected from southwest Mau forest, Nakuru County and taken to Chemistry Research Laboratory, Egerton University. The plant identification was performed in the Department of Biological Sciences by a botany specialist. The voucher specimens were labeled with scientific and vernacular names and stored.

Sample preparation

Fresh root samples of *D. abyssinica* and ripe fruits of *S. mauense* were collected from the Southwest Mau forest. The samples were cleaned, chopped to small pieces and air dried inside the research laboratory to avoid direct sunlight that could degrade some of the compounds in the samples. They were then spread out and regularly turned over to avoid fermenting and rotting. This was done for four weeks till they dried. The dried samples were ground to fine powder using electrical grinder. The powder was then weighed, packed and labeled in sample bags and stored at room temperature.

Extract preparation

The dried, ground samples were then re-weighed and about 1.5 Kg was soaked in 1.5 L hexane in a 2.5 L bottle for 72 hours at room temperature with frequent shaking. The solvent-containing extracts were then decanted and filtered in a 500 mL beaker through

cotton wool to remove coarse particles and lastly through a filter paper (Whatmann No.1) to obtain crude hexane extract. This was followed by serial extraction using dichloromethane (1.5 L), followed by ethyl acetate (1.5 L) and lastly methanol (1.5 L) in the order of increasing polarity of the solvents for 72 hours each with frequent shaking. It was filtered to obtain the crude extracts in each step. The solutions containing crude extracts were then concentrated under reduced pressure to a minimum volume using a rotavapor (Büchi Labortechnik AG, Switzerland). The concentrated crude extracts were allowed to dry to constant weight at room temperature. The dichloromethane extracts were purified using repeated column chromatography and TLC plates.

The mobile phase system that was used was hexane/ethyl acetate in the ratio of 5:4 and hexane/dichloromethane in the ratio of 1:4. The retention factors of visualized compounds on the TLC were established. NMR analysis of the pure compounds was performed on an Avance Bruker 500 MHz NMR spectrophotometer and spectra were recorded in CDCl₃ at the University of Surrey. Structures of compounds isolated were confirmed by comparison of NMR data against literature values.

RESULTS

Repeated column chromatography and TLC analysis of solanum species resulted in five pure compounds; SF1, SF2, SF3, SF4 and SF5. Interpretation of NMR spectral data of the pure compounds led to the proposal of their chemical structures. Compound SF1 was isolated as white crystal. The ¹³C NMR spectrum of compound SF1 established a lupeol-type triterpene derivative. The characteristic pair of sp² carbons comprising the double bond of lupeol was observed as shifts at $\delta = 150.6$ and 109.8 ppm. Oxygenated carbon shifts for C-3 was observed at $\delta = 79.2$. In all, the spectra revealed a compound with six methyl groups, thirty carbon atoms (which is equivalent to the total number of carbon atoms in

triterpenoid). Based on these spectral data the compound was identified as betulinic acid (1). The NMR spectral data for the betulinic acid is tabulated in Table 1.

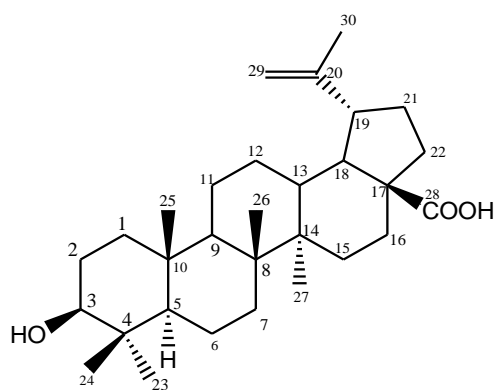
Interpretation of NMR spectral data for other compounds SF2, SF4 and SF5 led to a conclusion that they were fatty acids. In addition, NMR data of compound SF3 showed it as a benzoylated triterpenoid. However its structure is yet to be fully elucidated.

Purification of crude roots extracts of *Dovyalis abyssinica* resulted in three compounds; DF1, DF2, and DF16. Compound DF2 was isolated as a white crystal. The ^1H NMR spectrum of this compound showed two triplets at $\delta = 7.47$ and $\delta = 7.63$ integrating for two protons and one proton respectively. A doublet was also observed at $\delta = 8.13$ integrating for two protons. A broad downfield signal is evident at $\delta = 11.2$ indicating the presence of carboxylic acid proton. The ^{13}C NMR spectrum of compound DF2 also exhibited signals at $\delta = 128.7$, $\delta = 129.5$, $\delta = 130.4$, $\delta = 134$ and $\delta = 172.1$. It is evident from DEPT NMR spectrum of this compound that the three signals at $\delta = 128.7$, $\delta = 130.4$ and $\delta = 134$ were those of aromatic methine carbons. From these spectroscopic data the structure of compound DF2 was proposed to be that of

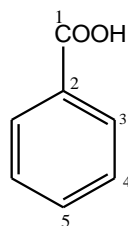
benzoic acid (2). The NMR spectral data for Benzoic acid (6) is summarized in the table 2;

The structure of compound DF16 (3) was proposed based on its NMR Data and comparison with the literature values. The ^1H NMR spectrum of compound DF16 shows a pattern characteristic of a phenolic ester glycoside. This was evident from cluster of peaks at $\delta = 3.80$ - 5.34 ppm which are characteristic of phenolic ester glycoside. Furthermore presence of aromatic protons is evident from peaks clustered at around $\delta = 7.03$ - 8.13 which suggests the presence of substituted Benzene. The ^{13}C NMR spectrum of this compound indicates presence of O-linked carbons of the sugar moiety and carbinol carbon of the phenolic aglycone. The anomeric carbon and the carbonyl carbon of an ester resonated at $\delta = 100.8$ ppm while the two carbonyl carbons of an ester resonated at $\delta = 167.2$ and $\delta = 171.2$ ppm respectively. The aromatic carbons were observed in the region $\delta = 116.7$ - 156.5 ppm. From the spectral data summarized in table 3, the structure of compound DF16 was found to be that of tremulacin (3).

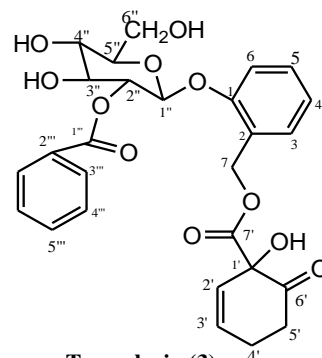
The proposed chemical structures of the isolated compounds are shown below.



Betulinic acid (1)



Benzoic acid (2)



Tremulacin (3)

Table 1: NMR spectral data for Betulinic acid (1).

position	¹³ C Isolated compound (δ)	¹³ C Literature value (δ) (Sharma <i>et al.</i> , 2010)	DEPT Isolated compound
1	40.2	39	CH ₂
2	28.19	27.6	CH ₂
3	79.8	78.2	CH
4	39.98	39.1	C
5	57.63	55.5	CH
6	19.6	18.4	CH ₂
7	35.7	34.5	CH ₂
8	43.7	40.8	C
9	50.6	50.7	CH
10	42.1	37.3	C
11	22.23	21	CH ₂
12	27.03	25.7	CH ₂
13	39.8	38.1	CH
14	40.2	42.5	C
15	30.9	30.2	CH ₂
16	31.85	32.9	CH ₂
17	49.7	47.1	C
18	49.6	48.1	CH
19	49.7	49.2	CH
20	152.1	150.1	C
21	31.8	30.6	CH ₂
22	38.2	37	CH ₂
23	28.1	27.9	CH ₃
24	16.25	15.5	CH ₃
25	16.7	16.4	CH ₃
26	16.87	16.7	CH ₃
27	15.25	15	CH ₃
28	180.2	180.3	C=O
29	110.3	108.9	CH ₂
30	19.59	19.6	CH ₃

Table 2: NMR spectral data for Benzoic acid (2)

Position	¹³ C	¹ H	DEPT
1	172.1	-	-
2	129.5	-	-
3	130.4	8.13	-CH-
4	128.7	7.47	-CH-
5	134	7.63	-CH-

Table 3: NMR spectral data for tremulacin (**3**).

Position	¹³ C Isolated compound (δ)	¹³ C Literature value (δ) (Rasmussen <i>et al.</i> , 2006)	¹ H	DEPT	HSQC-DEPT
1	156.5	156	-	-	-
2	129.2	127.5	-	-	-
3	131	129.5	7.14	-CH-	7.21
4	123.8	123.1	6.97	-CH-	7.03
5	131.2	130.1	7.23	-CH-	7.30
6	116.7	115.6	7.02	-CH-	7.30
7	64.02	63.6	4.95	-CH ₂	4.97(d)
1'	78.4	78.3	-	-CH-	3.64 (d)
2'	129.6	127.5	5.68	-CH-	5.76
3'	133.3	132.1	6.01	-CH-	6.17
4'	27.2	26.6	2.41	-CH ₂	2.53(m)
5'	36.8	35.3	2.52	-CH ₂	2.57(m)
6'	207.4	207.5	-	-	-
7'	171.2	170	-	-	-
1''	100.8	99.5	5.15	-CH-	5.33
2''	75.9	74.0	5.30	-CH-	5.36
3''	75.5	74.8	3.86	-CH-	5.34
4''	71.6	69.9	3.86	-CH-	3.88
5''	76	76.1	3.01	-	-
6''	62.5	61.3	3.86	-CH ₂	4.01
1'''	167.2	166.0	-	-	-
2'''	130.2	129.5	-	-	-
3'''	130.9	129.9	7.99	-CH-	8.13
4'''	129.7	128.4	7.34	-CH-	8.12
5'''	134.4	133.3	7.49	-CH-	7.62

DISCUSSION

The structure of betulinic acid was confirmed by comparison with literature values (Sharma et al., 2010) and it has been reported to originate from lupane (Chudzik et al., 2015). This is a group of pentacyclic triterpenes, characterized by cytotoxic properties, which may be isolated from plants (e.g., *Spirostachys africana*) (Mathabe et al., 2008) or synthesized. Their activity has been proved for cell lines of lung cancer (A549), colorectal carcinoma (DLD-1), breast cancer (MCF-7) and prostate cancer (PC-3), at no activity toward cutaneous fibroblasts (WS1-1) (Chudzik et al., 2015). The pentacyclic triterpene is commonly found in plants (Njue et al., 2017) and not only are capable of inhibiting life of neoplastic cell lines, but also induce apoptosis of cancer cells, to cause their "suicidal" death, with no threat to normal cells of the body. Such properties, in particular the selectivity of triterpenes' activity, present them as alternatives in cancer treatment and prevention.

Benzoic acids are C₆-C₁ aromatic carboxylic acids that serve as precursors for a wide variety of essential compounds and natural products playing crucial roles in plant fitness. Plant benzoic acids (BAs) are the building blocks and acts as important structural elements for numerous primary and specialized metabolites including plant hormones, cofactors, defense compounds, attractants for pollinators and seed dispersers (Widhalm and Dudarera, 2015).

The curative properties of plants are derived from a number of different compounds, among which phenolics, as secondary metabolites, are the most numerous (Djurđević et al., 2013). Compound **3** is a phenolic glycoside known as tremulacin and such compounds are characterized by a sugar portion or moiety attached by a special bond to one or non-sugar portions (Kabera et al., 2014). Many plants store chemicals in the form of inactive glycosides, which can be activated by enzyme hydrolysis. For this reason, most glycosides can be classified as prodrugs since they remain inactive until they

are hydrolyzed leading to the release of the aglycone, the right active constituent. It has been reported that glycosides have anticancer, expectorant, sedative and digestive properties (Zhou et al., 2013). Phenolic glycosides are some of the most abundant secondary metabolites known in plant tissues, and play an important role as anti-herbivore defenses in the Salicaceae (Boeckler et al., 2011).

Tremulacin may also be considered benzoic acid derivative and such derivatives have been reported to have varied biological properties. For instance, they have been reported to exhibit anti-sickling properties *in vitro* (Pierre et al., 2015).

Other biological activities of *D. abyssinica* have been reported in literature. For instance the leaf aqueous extract was tested for its cytotoxicity and recorded a CC₅₀ value which was greater than 90 µg/ml and this confirmed it as non-toxic (Kimutai, 2017). Furthermore it has been tested for its *in vivo* antitrypanosomal activity and proved to be active (Tadesse et al., 2015).

Conclusion

The chemical structures of the three compounds; betulinic acid, benzoic acid derivative and tremulacin, isolated from this study have been proposed. It was evident from literature survey that these compounds have been isolated previously and their anticancer properties have also been reported extensively. The two medicinal plants under study therefore have some basis to justify their ethnobotanical use.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

DKC was the principal investigator; PKC JOO and MKL contributed fully to the work. MKL did the spectroscopic analysis of the compounds isolated in the study. All authors proposed the chemical structures of the compounds, read and approved the final manuscript.

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