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Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscosa* (Sapindaceae)

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Quercetin and isorhamnetin are found in adequately large concentrations in the plant *Dodonea viscosa* (Sapindaceae). Plants that contain flavonoids are effective in the topical treatment of skin or mucous membrane inflammation. In this study, the release profiles of quercetin and isorhamnetin from hydrophilic, amphiphilic and lipophilic creams of the crude extract of *Dodonea viscosa* were determined using a multilayer membrane system. The results revealed that the hydrophilic cream provided the highest rate of release of both flavonoids while there was practically no release from the lipophilic cream. The hydrophilic cream may, therefore, serve most in delivering flavonoids to a diseased skin.

1. Introduction

Dodonea viscosa (Sapindaceae) is an ever green shrub widely distributed almost throughout the world and is known by several vernacular names such as Kitkita, Aliar, Bandaraaku, Cacho venado, Chamana, De Monte, Cuerno De Cabra, Gandin, Hautiwa, Hopbush, Luhahi, Mkapwani, Muendu, Mwajio, etc. It is used for the treatment of boils and other skin infections, sore throat, dermatitis, hemorrhoids, rheumatism, diarrhea, stomach-aches, pains of hepatic or splenic origin, uterine colic and other disorders involving smooth muscles in several countries [1–4]. It is also used to speed up postpartum recovery, to treat menorrhagia, infertility and hemorrhage between menstrual periods, and to prevent miscarriage in Mexico [5].

Previous reports indicated that the methanol extract of the leaves of the Mexican species show weak activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* [6]. The methanol/water extract (1 : 1) of the flowers and leaves of the Nigerian species also demonstrated activity against *B. subtilis*, *E. coli*, *Proteus species*, *P. aeruginosa* and *S. aureus* [7]. A study on the 80% methanol extract of the leaves of *D. viscosa* (collected from Awash area, Ethiopia) showed that the plant possessed moderate activity against *S. aureus*, *Streptococcus pyogenes* and *Corynebacterium diphtheriae*, at concentrations of 25, 50 and 100 mg/ml. On the other hand, no activity was observed against *E. coli* and *P. aeruginosa* [8].

Dodonea viscosa is reported to contain triterpenes [9], sesquiterpenes [10], monoterpenes [11], coumarins [12], steroids [12] and diterpenes [13]. Flavonoids such as quercetin, isorhamnetin, narcissin and rutin were observed in the leaves of the plant [14]. Quercetin and isorhamnetin were also detected in the aerial part [15], the stembark [16], the rootbark [17] and the flowering tops [9]. Several endopharmacological reports confirmed that plants that contain flavonoids in sufficiently large concentration are effective in the topical treatment of skin or mucous membrane inflammation [18, 19].

When drugs, be it modern or traditional, are applied topically to the skin, a pharmaceutically active agent must not be released from its carrier (vehicle) before it contacts the epidermal surface and be available for penetration into the stratum corneum and lower layers of the skin. Drug release and skin penetration are of paramount importance in the treatment of skin diseases that invade the lower layers of the skin. In the literature, there are only publications regarding pharmacokinetics following oral administration of phytopharmaceuticals [20]. Even less information is available about the biopharmaceutical characterisation of topical phytomedicines [21–23]. The objectives of this study were, therefore, to quantify the flavonoid content, particularly, quercetin, kaempferol and isorhamnetin of the leaves of *D. viscosa*, and to evaluate the release profile of the flavonoids that are found in concentrations sufficient for detection from three different creams of the crude extract.

2. Investigations, results and discussion

HPLC/UV analysis (Fig. 1) of the crude extract of *D. viscosa* revealed the presence of quercetin, kaempferol and isorhamnetin in concentrations of 2.65 ± 0.06, 1.59 ± 0.10 and 15.33 ± 0.39 mg/g, respectively (3 repetitions for 3 extracts, $n = 9$). The quantitative detection limits for quercetin and isorhamnetin were calculated using the method previously described by Funk et al. [29] and were found to be 0.440, 0.650 and 0.655 μg/ml, respectively.

The success of dermal therapy depends strongly on the penetration profile of the active therapeutic agents in the diseased skin layer. The concentration of the drugs in these skin layers is important. The measure of drug release as a measure of bioavailability and bioequivalence has been a subject of debate for many years [30, 31]. Few years ago, however, consensus has been reached that an *in vitro* release test can serve as a valuable tool for initial screening of experimental formulations in the product development area and can serve to signal possible bioequivalence. A number of simple and reproducible methods, generally applicable to all topical dermatological dosage forms, has been developed and used to measure *in vitro* release of drugs from their vehicles using synthetic membranes [24, 32–37]. One of those methods (Fig. 2) was employed in this work to study the release of quercetin and isorhamnetin from topical formulations containing crude extract of *D. viscosa*. Plots of percentage of each flavonoid released from hydrophilic and amphiphilic creams are depicted in Fig. 3. From the plot, it is apparent that quercetin and isorhamnetin are better released from the hydrophilic cream than from the amphiphilic cream. The statistical analysis (student t-test) shows that in steady state the differences of quercetin and isorhamnetin between the hydrophilic and amphiphilic cream were significant with a probability of 95%. There was practically no

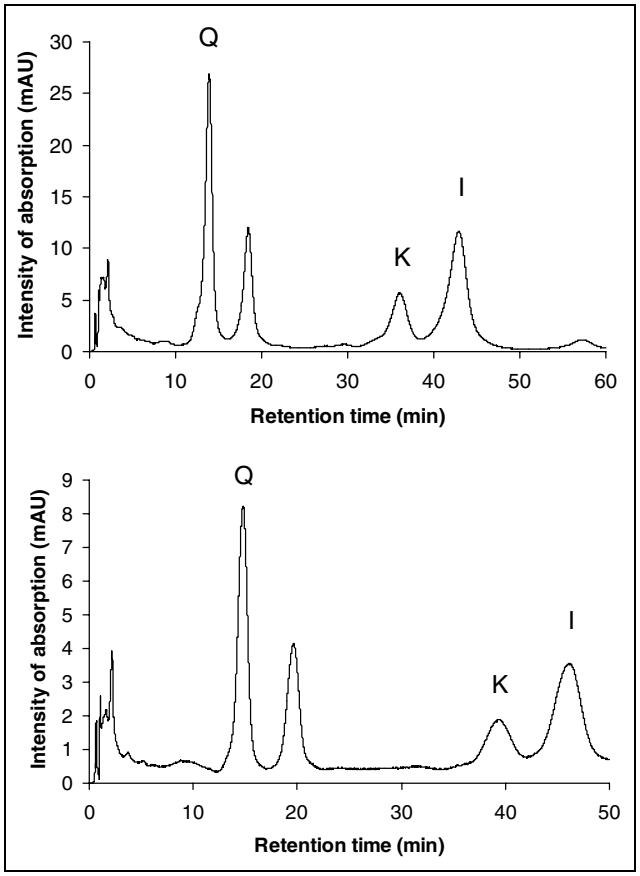

Fig. 1: HPLC/UV Chromatograms of *Dodonea viscosa* extract after liberation from hydrophilic (a) and amphiphilic (b) creams (second peak – unknown compound)

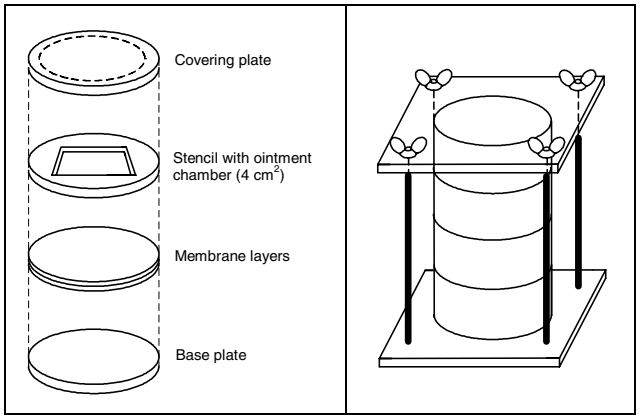

Fig. 2: Schematic representation of the multilayer membrane apparatus used for the study of flavonoid release from the creams

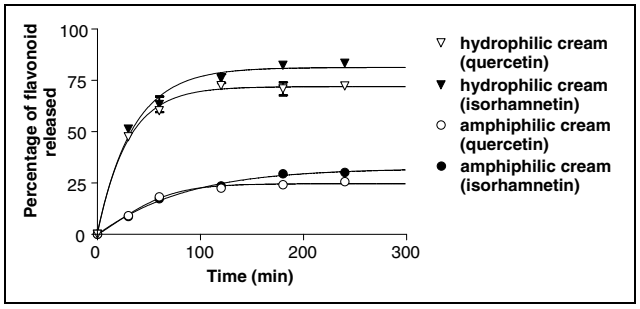

Fig. 3: Percentages of quercetin and isorhamnetin released from the hydrophilic and amphiphilic creams of the extract of *Dodonea viscosa* with time (n = 3)

release of either of these substances from the lipophilic cream during the experimental period. In conclusion, of the three creams, the hydrophilic cream may function best in delivering flavonoids to a diseased skin.

3. Experimental

3.1. Materials

Methanol (HPLC-grade), HPLC-grades of quercetin, kaempferol and isorhamnetin, macrogol-1000, glycerolmonostearate 60 and glycerol were purchased from Carl Roth GmbH & Co, Karlsruhe; petroleum ether, phosphoric acid and hydrochloric acid all of analytical grade were from Riedel-deHaën AG and Gruessing GmbH, respectively. Collodion solution, 4% w/w in ether/ethanol was from Caelo, Hilden. Propylene glycol and medium-chain triglyceride were from Woelm Pharma GmbH & Co, Eschwege while wool alcohol, cetostearyl alcohol, liquid paraffin and white soft paraffin were from Beiersdorf AG, Hamburg. All the companies mentioned above are in located Germany.

3.2. Collection of plant material

The leaves of *D. viscosa* were collected from Awash area, Eastern Shoa, Ethiopia. They were then dried in open air and powdered. Taxonomic identification was made by and sample specimens are deposited in the Natural Herbarium, Department of Biology, Faculty of Science, Addis Ababa University.

3.3. Preparation of crude extract

Powdered plant material (200 g) was macerated with 1 l of petroleum ether for 24 h and the extract was kept aside. The marc then remained was dried in an oven at 40 °C and exhaustively extracted with 80% methanol (3 times, 600 ml each) by percolation. The methanol was removed from the percolate by evaporation using rota vapour (LABO-ROTA C-311, Reseno Technics, Switzerland) at 40 °C and 150 mBar leaving an aqueous residue. The aqueous residue was first deep frozen and then lyophilized by a freeze dryer (BETA 1–8 K, Martin Christ, Osterode am Harz, Germany) at −30 °C and 0.016 mBar for 48 h. The resulting extract was weighed and filled into an amber coloured bottle. The bottle was then stored in a desiccator for future use. The extraction was repeated three times.

3.4. Detection and quantification of flavonoids in the crude extract

The presence of the flavonoids quercetin, kaempferol and isorhamnetin in the crude extract was detected and their amount quantified by HPLC/UV (HP 1100 HPLC System, Agilent, Waldbronn, Germany) equipped with a binary gradient pump, UV diode array detector, autosampler and solvent degasser. For the same purpose, HPLC-grade quercetin, kaempferol and isorhamnetin were used as reference substances.

A solution containing quercetin, kaempferol and isorhamnetin in a ratio of 2 : 2 : 1, respectively, was made in 80% methanol and used as an internal standard both for the solutions of the references and the plant extracts. Separate solutions of each reference substance were made at concentrations of 5, 10, 20, 30 and 40 µg/ml in the same solvent. Equal volumes of the solution of internal standard and each of the reference solutions were mixed and analysed by HPLC/UV to determine the calibration curve.

Solutions of 20 mg/ml of each plant extract in 80% methanol were made in an ultrasonic bath at 40 °C for 2 h, cooled and filtered. In order to facilitate hydrolysis, to each 2 ml of the solutions, 0.5 ml of 5.5% of hydrochloric acid in methanol was added and heated in boiling water for 30 min. The solutions were then allowed to cool and were analysed by HPLC/UV.

The flavonoid contents of the various extracts and solutions of the reference substances were determined using HPLC/UV under the following conditions: Stationary phase: Reverse phase (RP) column (Eurosphere-100 C_{18}, 100 × 4 mm, 5 µm particle size, Dr. Ing. H. Knauer GmbH, Berlin, Germany); mobile phase: 50 : 50 : 3 – methanol : water : phosphoric acid; wave length: 370 nm; flow: 1.5 ml/min; injection volume: 20 µl and run time: 30 min.

3.5. Preparation of creams of D. viscosa extract

A 10% dispersion of the extract of *D. viscosa* was prepared in each of three different bases (hydrophilic, amphiphilic and lipophilic). The extract was dissolved in 50% methanol and incorporated into Hydrophilic Cream DAB 1999 (Syn: Unguentum emulsificans), Amphiphilic Cream DAC 1999 (Syn. Basiscreme, Amphiphile Creme) and Wool Alcohol Cream DAB 1999 (Syn. Lanae alcoholum unguentum). The methanol was then completely evaporated from the resulting cream at about 60 °C.

Composition of the three different creams:

Hydrophilic cream:	Cetylstearyl alcohol, emulsifying	30 parts
	Liquid paraffin	35 parts
	White soft paraffin	35 parts

Amphiphilic cream:	Glycerol monostearat 60	4.0 parts
	Cetylstearyl alcohol	6.0 parts
	Medium-chain triglyceride	7.5 parts
	White soft paraffin	25.5 parts
	Macrogol-1000 glyceromonostearate	7.0 parts
	Propylene glycol	10.0 parts
	Extract solution in 50% methanol	80.0 parts
Lipophilic cream:	Cetylstearyl alcohol	0.5 parts
	Wool alcohol	6.0 parts
	White soft paraffin	93.5 parts

3.6. Preparation of the membranes

Model membranes prepared by modifying the method previously described [24] were used. Collodion solution (100 g) was added to 100 g of 4% glycerol solution in ether/ethanol (85 : 15, w/w). This mixture was placed on an apparatus designed for membrane preparation [25] that provides membranes of uniform thickness and glycerol content. The sheets of membranes then obtained were cut into discs of uniform diameter (4 cm) by a hollow sharp-edge stainless steel cylinder designed for this purpose.

3.7. Study of the release of flavonoids from the creams

The multilayer membrane system, schematically shown in Fig. 2, has been used for the release study. This model was used as a penetration model by various authors [25–28]. The apparatus consisted of 5 polyacrylate (Piacryl®, Piesteritz, Germany) cells each containing 4 membranes. In each experiment, 5 sets of apparatus were employed. The cream (ca. 100 mg) was then applied to the first membrane (with an exposed area of 4 cm^2) of each cell. The cells were then fitted together vertically and the 5 apparatus were placed in a chamber maintained at 32 ± 0.2 °C. At selected time intervals (30, 60, 120, 180 and 240 min), an apparatus was removed from the thermostat chamber. The five cells were separated and the creams remaining on the first membranes of the three cells were carefully removed and discarded. The membranes were then extracted with 80% methanol and the released flavonoid content was determined using HPLC/UV. The creams of each of the other two cells were extracted together with the membranes, quantified and used as references.
The release of flavonoids from different formulations was considered as significant if the confidence intervals with a probability of 95% did not overlap.

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