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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF FLAVONOLS FROM THE LEAVES OF CHROZOPHORAPLICATA (VAHI) A. JUSS, EX.SPRENG

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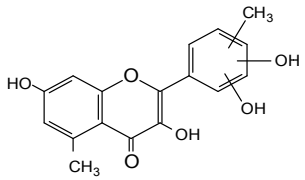
ABSTRACT

In this study the phenolics of two medicinally important plants were investigated. Ethanol. The phytochemical screening of the ethanolic extract of the leaves indicated the presence of flavonoids, tannins, alkaloids and terpenoids. The crude extract was subjected to thin layer chromatography and column chromatography to give compounds I. The structure of compound I was elucidated by a combination of spectral techniques (UV, 1HNMR and MS) and the following tentative structures were proposed:



Compound I

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INTRODUCTION

Phytochemicals are defined as the substances found in edible fruits and vegetables that exhibit a potential for modulating human metabolism in a manner beneficial for the prevention of chronic and degenerative diseases (Claisenand Claparede, 1881). Flavonoids are secondary constituents with a wide array of biological activities including: antibacterial, antifungal, antimalarial and antitumour activities (Elgazali *et al.,* 2016). The study of flavonoid chemistry has emerged, like that of most natural products, from the search for new compounds with useful physiological properties (Harborne and Williams1992). Semi synthetic endeavors of oligoflavonoids are in most instances confined to thosesubstitution patterns exhibited by monomeric natural products that are available inquantities sufficient for preparative purposes (Vonand Rossbach, 1896). In order to alleviate these restrictions, several programs focusing on synthesis of enantiomeric pure flavonoid monomershave been undertaken (Harborne and Williams, 1992). However, synthesis of the desired enantiomer in opticallypure forms remains a daunting objective and is limited to only a few typesof compounds. Chalcone epoxides, α- and β-hydroxydihydrochalcones, dihydroflavonols, flavan-3-ols, flavan-3,4-diols, isoflavans, isoflavanones, and pterocarpansthus far have been synthesized in reasonable yields and purity. Flavonoids are a class of secondary plant phenolics with significant antioxidant and chelating properties. In the human diet, they are mostconcentrated in fruits, vegetables, wines, teas and cocoa.Flavonoids are secondary metabolites characterized by flavan nucleus (Heim *et al., 2002*) and C6-C8-C6 carbon-skeleton (Peterson and Dwyer, 1998; Tsuchiya, 2010).These is group of structurally related compounds with a chromane-type skelton having phenyl substituent in C2-C3 position (Rijke *et al*., 2006). The basic structural feature of flavonoid is 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C) as shown in fig (I) (Cushnie *et al.,*2005).

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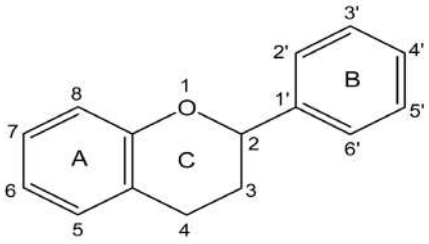
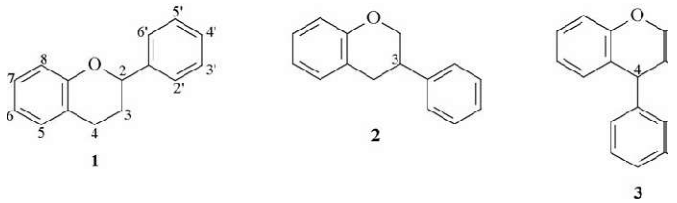


Fig (I): Basic structure of flavonoids: Their cardioprotective effects stem from the ability to inhibit lipid peroxidation, chelate redox-active metals, and attenuate other processes involving reactive oxygen species. Flavonoids occur in foods primarily asglycosides and polymers that are degraded to variable extents in the digestive tract. Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. Widely distributed in the leaves, seeds, bark andflowers of plants, over 4,000 flavonoids have been identifiedto date. In plants, these compounds afford protection againstultraviolet radiation, pathogens, and herbivores (Cushnieand Lamb, 2005). The term "flavonoid" is generally used to describe a broad collection of naturalproducts that include a C6-C3-C6 carbon framework, or more specifically aphenylbenzopyran functionality. Depending on the position of the linkage of thearomatic ring to the benzopyrano (chromano) moiety, this group of natural productsmaybe divided into three classes: the flavonoids (2-phenylbenzopyrans) 1, isoflavonoids (3-benzopyrans) 2, and the neoflavonoids (4-benzopyrans) 3. These groups usually share a common chalcone precursor, and therefore are biogeneticallyand structurally related.



Flavonoids classification

Over 5000 naturally occurring flavonoids havebeen characterized from various plants. They havebeen classified into six subgroups:

- Flavones (luteonin, apigenin, tangeritin).
- Flavonols (quercetin, kaemferol, myricetin, isorhamnetin, pachypodol, rhamnazin).
- Flavanones (hesteretin, naringenin, eriodictyol).
- Flavan-3-ols: (catechins (catechin, gallocatechin, catechin 3-gallate, gallocatechin3-gallate) and epicatechins (epicatechin, epigallocatechin, epicatechin 3-gallate, epigallocatechin 3-gallate)).
- Isoflavones (genistein, daidzein, glycitein) andAnthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin).

Most of them are present in our everyday'slife (Doly 1992).

Forinstance, flavones, such as luteolin and apigeninglycosides, are contents of parsley and celery. Therichest sources of flavonols, like quercetin, arecapers, lovage, apples, tea plant, onions, red grapes, citrus fruits, curly kale, leeks, broccoli, cherries,raspberry, cranberry and blueberry. Flavanonesare abundant in high concentrations in citrus fruit. The flavonoids are known for their anti-inflammatory and antiallergic effects, for antithrombitic and vasoprotective properties, for inhibition of tumour promotion and as a protective for the gastric mucosa. These effects have been attributed to the influence of flavonoids on arachidonic acid metabolism. Many flavonoid containing plants are diuretic or antispasmodic. Some flavonoids have antibacterial and antifungal properties (Manal *et al., 2010*).

Chrozophora genus

Chrozophora genus is a plant of the family Euphorbiaceae and the sole genus comprised in the subtribechrozophorinae. It comprises 8-7 species, which are mostly monoecious herbs under shrubs. This genus is distributed in Pakistan, India, West Africa and Mediterranean regions. Previous phytochemical investigation of the genus Chrozophora resulted in the isolation of several types of chemical constituents including essential oils, terpens, sterols, phenylpropanoid glycosides, xanthones, chromone and flavonoids. It was reported that the plant contained essential oils and flavonoids. A literature search revealed only flavonoid aglycones and an acylatedglucoside of apigenin. In continuation of our studies on phenolic constituents from Egyptian plant, we report herein on the isolation and structure elucidation of a novel brocchlin carboxylic acid and its methyl ester from the aqueous ethanolic extract of Chrozophorabrocchiana, together with eight known phenolic compounds, gallic acid, methylgallate, ethylgallate, ellagic acid, methoxyellagic acid, methylenedioxyellagic acid, apigenin (Burkill 1994). This review paper aims to review Chrozophora genus plants e. g. Chrozophorabrochiana, C. senegalnsis, C. plicata, C. rotteri, C. tinctoria and C. oblongifolia with emphasis on their chemical composition, food, feed, and medicinal uses.(Antonio *et al, 2006*; Audu *et al, 2008*).

Type of Chrozophora genus

1-Chrozophoraborcchiana

Chrozophoraborcchiana

***2. Chrozophorasenegalensis A*. Juss**

Chrozophorasenegalensis

3. Chrozophoraplicata **(Vahl) A. Juss.ex Spreng**

Chrozophoraplicata

MATERIALS AND METHODS

Collection of plant material

For this study, the leaves of chrozophoraplicata were collected from the surroundings of Niala, western Sudan. The plant was kindly authenticated by the Institute of Aromatic and Medicinal Herbes, Khartoum, Sudan. After being authenticated by botanist, sample specimens of leaves of each plant have been deposited atKhartoum UniversityFaculty of Science. Fresh mature leaves were shade - dried at room temperature and powdered.

Extraction of flavonoids

Powdered shade-dried leaves of Chrozophoraplicata were macerated at room temperature with 95% ethanol (5L) for 48hr. The solvent was evaporated under reduced pressure and part of residue was used for the following tests

Phytochemical screening

The leaves of Chrozophoraplicata was screened for steroids, flavonoids, alkaloids, tannins and glycosides.

Test for steroids

Part of the crude plant extract was stirred with petroleum ether to remove most of the coloring matter. The residue was extracted with 20ml chloroform and the solution was dehydrated over anhydrous sodium sulphate. A 5ml Portion of the solution was mixed with 0.5ml acetic anhydride, followed by two drops of concentrated sulphuric acid.

Test for alkaloids

A 5ml of 2N hydrochloric acid were added to the crude plant extract and the solution was heated with stirring in a water bath for 10 minutes. The cooled solution was filtered .To portion 5ml of this solution; few drops of Dragendroffs reagent were added. No precipitate was formed.

Test for flavonoids

Part of the crude plant extract was defatted by extraction with petroleum ether. The defatted residue was dissolved in 30ml 95% ethanol and filtered. The filtrate was used for the following tests:

- To 3ml of filtrate, few drops of 1% methanolicaluminium chloride were added. Formation of yellow color indicated the presence of flavonoids.
- To 3ml of filtrate, few drop of potassium hydroxide solution were added, a dark yellow color indicated the presence of flavonoids.
- To 3ml of filtrate, few drops of ferric chloride solution were added. Development of a blue coloration was taken as a positive test for flavonoids.

Test for glycosides

Part of the powdered air-dried plant was vigorously shaken in a test tube with water. The presence of a froth that persisted for one hour indicated the existence of glycosides.

Isolation of flavonoids from plant material

Thin layer chromatography (TLC)

The TLC was carried out using aluminium sheets precoated with kiesel gel 60 F 254 of 0.2 mm thickness to detect a suitable solvent system for separation of flavonoids and to monitor fractions from column .The spotted thin layer sheets were developed using suitable solvent systems. TLC sheets were then viewed in both short and long UV wavelengths.

Column chromatography

Open wet column (100× 4 cm) was used for fractionation of the ethanolic extracts of Chrozophoraplicata. Silica gel with particle size 120-200 mesh (LOBA) was utilized as stationary phase. The composition of the mobile phase (50% acetic acid) was determined by TLC analysis. The column was packed with slurry of silica gel with 50% acetic acid and then allowed to equilibrate for one hour before use. The ethanolic extract of Chrozophoraplicata (4g) was mixed with 10 g of silica gel and then applied on the top of the column. Fractions of 10 ml were collected. Depending on their TLC pattern fractions F4 – F50 were pooled together, concentrated and subjected to further purification by silica gel TLC using 50% acetic acid as solvent. The spots were visualized under UV lights using both short and long wavelengths with and without exposure to NH_3.The chromatogram with (Rf 0.70) was eluted from silica with absolute ethanol.Removal of solvent under reduced pressure gave compound I. The purity was checked by TLC using silica gel and the solvent systems: (i) ethyl acetate saturated with water (ii) BAW (5:1:6) and finally (iii) methanol: toluene (2:1).

RESULTS AND DISCUSSION

The ethanolic extracts of the medicinally important species: Chrozophoraplicata was fractionated by column chromatography followed by further purification via TLC. In this way compound I were isolated from Chrozophoraplicata. In their UV spectra, flavonoids may exhibit two absorption bands; band I and II. Band I is associated with the absorption of the cinnamoyl system, while band II originates from the benzoyl system. Flavones, flavonols, chalcones and aurones give band I and band II, due to conjugation between the carbonyl function and the aromatic B ring. The UV absorption of some flavonoids, namely, flavones, flavonols, chalcones and aurones .Flavonols which differ from flavones by the presence of a 3-OH function are distinguished from flavones by band I. While flavones absorb in the range: 320350nm, flavonols have band I in the range: 350-390nm. Isoflavones, dihydroflavonols, dihydrochalcones and flavanones exhibit only band II. This is

attributed to loss of conjugation between the carbonyl function and ring B.

Table 1. The UV absorption of flavones, flavonols, chalcones and aurones[1]

Flavonoid class	Band I	Band II
Flavones	330-350	250-270
Flavonols	350-390	250-280
Chalcones	365-390	240-260
Aurones	390-430	240-270

Compound I

Compound I was isolated from the ethanolic extract of Chrozophoraplicata via a combination of column and TLC techniques. The UV spectrum of compound I gave λmax210, 316,365nm (Fig. 1). This absorption is characteristic of flavonols.Thoughchalcones have the same range for band I as flavonols, but they are characterized by a dominant band I
a dominant band I.

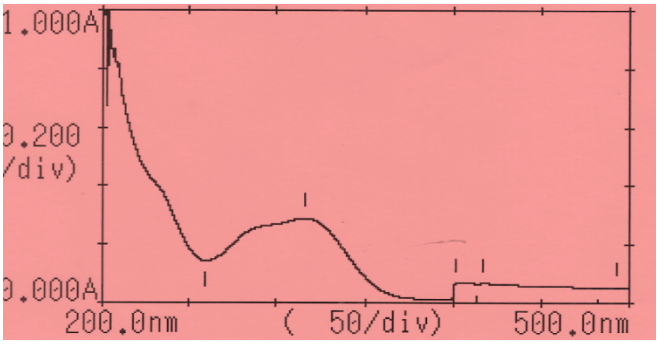


Fig. 2. UV spectrum of compound I

Considerable structural features are gained by using the UV shift reagents: sodium methoxide, sodium acetate, aluminum chloride, hydrochloric acid and boric acid. Use has been made of the effect of NaOMe on the UV spectra of flavonoids for the detection of free 3-and/or 4`-hydroxyl groups.A bathochromic shift is observed in case of 3- or 4`-hydroxylation but with decrease in intensity in case of 4`-hydroxylation. The sodium methoxide spectrum of compound I revealed a bathochromic shift (Fig. 2) with decrease in intensity indicating a 3-OH function.

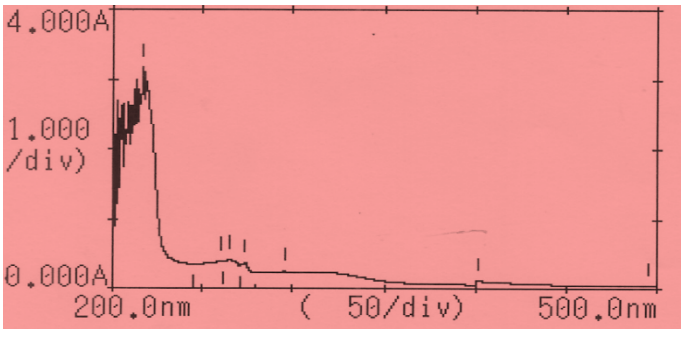


Fig. 2. Sodium methoxide spectrum of compound I

Sodium acetate usually ionizes the more acidic hydroxyl groups. The shift reagent: sodium acetate is particularly useful diagnostic reagent for specific detection of 7-hydroxylation (Middleton and Kandaswami, 1994; Geissman and Crout, 1969). When sodium acetate was added to a methanolic solution of compound I(Fig.3) a bathochromic shift was observed indicating a 7-OH function.

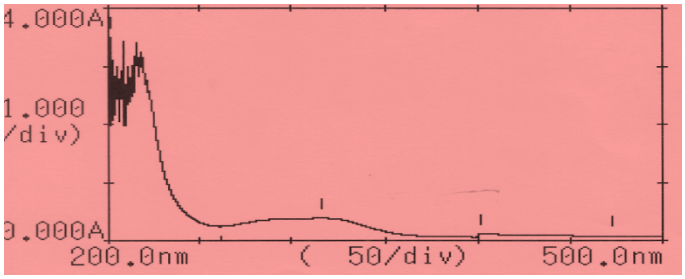


Fig. 3. Sodium acetate spectrum of compound I

Flavonoids which possess a C-3-OH or C-5-OH form acid-stable complexes with aluminium chloride. Also, aluminum chloride forms acid- labile complexes with flavonoids which contain catechol moieties. When $AlCl_3$ was added to a methanolic solution of compound I, a bathochromic shift was observed (Fig.4). The spectrum degenerated in acidic medium (Fig. 5) and this indicates the presence of a B ring catechol system.

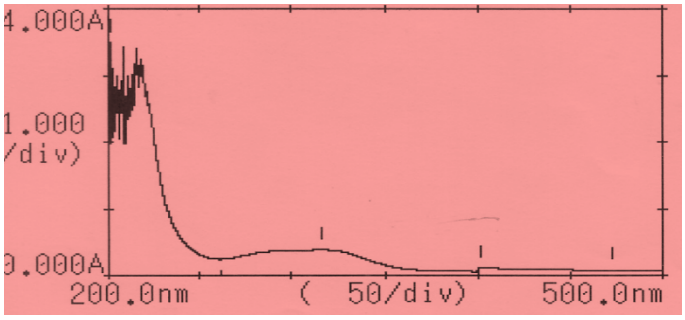


Fig. 4. Aluminium chloride spectrum of compound I

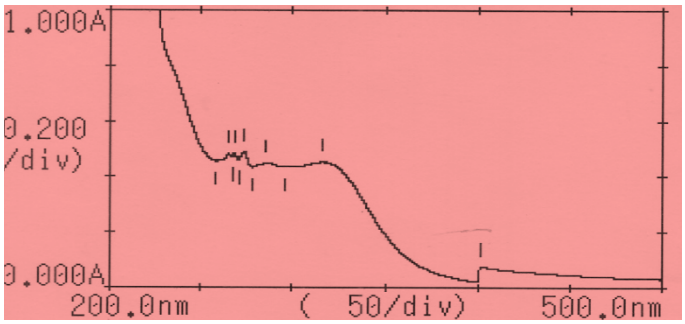


Fig. 5. Aluminium chloride/HCl spectrum of compound I

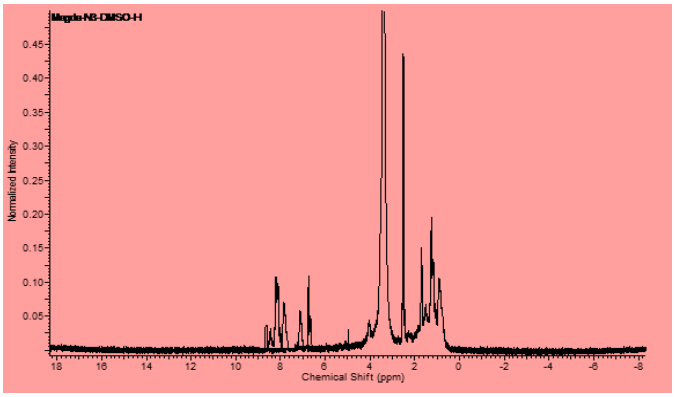
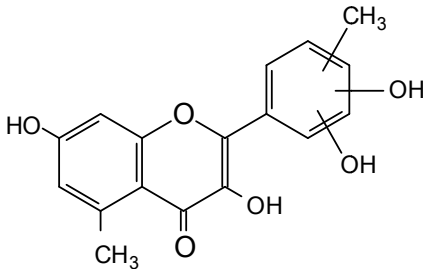


Fig. 6. [1]HNMR spectrum of compound I

The ^{1}HNMR spectrum (Fig.6) showed: δ0.90(s,3H), δ1.20(s,3H) assigned for two methyl groups; δ6.60(d,1H), δ7.25(d,1H) attributed for C_6- and C_8- protons. The former resonates downfield relative to the latter due to the desheliding influence of the 4-keto function; δ6.20-7.0(m,) accounting for C_6- and C_8- protons; δ7.20 (s), δ7.80-8.70 (m, 2H) assigned for B ring protons. The mass spectrum (Fig.7) showed m/z314 for the molecular ion. The retro Diels –Alder fission (Scheme I) revealed peaks at m/z 152 and m/z164 for intact A and B rings. Such cleavage supports the following tentative structure proposed for compound I:



Compound I

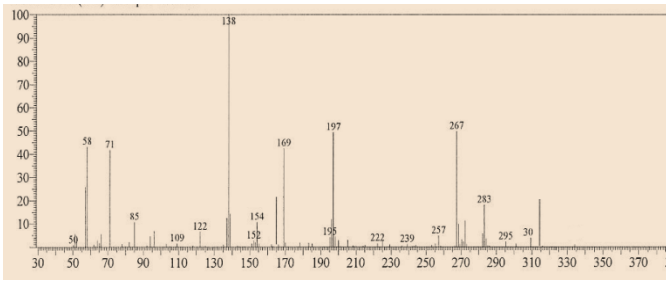
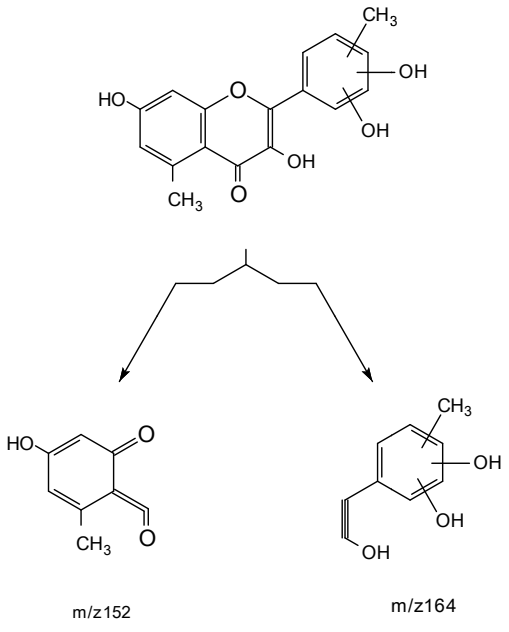


Fig. 7. Mass spectrum of compound I



Scheme I. Retro Diels-Alder fission of compound I

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