World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

ISOLATION AND PARTIAL STRUCTURE OF A FLAVONOL FROM SUDANESE *PSIDIUM GUAJAVA* L.

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Article Received on 12/03/2017 **Article Revised on 01/04/2017** **Article Accepted on 22/04/2017**

ABSTRACT

Fruits, like guava, contain bioactive components and regular consumption of fruits has many health promoting properties. This study was carried out to investigate the flavonoids of the medicinally important species *Psidium guajava* - guava. The flavonoids were extracted by ethanol and the crude extract was fractionated by TLC chromatography to afford a chromatographically pure flavonol. The structure of this isolate was partially elucidated by sensitive analytical tools (UV and ^{1}HNMR).

KEYWORDS: *Psidium guajava*, Isolation, Flavonol, Partial Structure.

INTRODUCTION

Psidium guajava L. is native to South America. It is a small tree with two common varieties: red guava (*Psidium guajava* var. *pomifera*) and white guava (*Psidium guajava* var. *pyrifera*).[1,2] Fruits,like guava, contain bioactive components and regular consumption of fruits has many health promoting properties.[3-6]

Guava leaves are used in Sudanese traditional medicine against cough, while fruit pulp is a treatment for diarrhea. Seed are reported to possess antimicrobial, antiallergic and anticancer activities.[7-11]
The plant contains: vitamins, flavonoids, tannins, terpenes and essential oils.[2] The fruit pulp is rich in carotenoids, polyphenols and vitamins.[12-14] It has been demonstrated that the fruit has antioxidant properties.[15-17]

Clinical studies testified that consumption of guava for 12 weeks reduced blood cholesterol, blood pressure and level of triglycerides.[18,19] *In vivo* studies showed that feeding model animals with guava pulp resulted in significant decrease in weight and increased levels of $HDL_{-c.}$ Fruit pulp also showed significant hypoglycemic effect in diabetic models.[10]

MATERIALS AND METHODS

Materials

Analytical grade reagent were used. The UV spectra were recorded on a Perkin-Elmer UV-1800 Spectrophotometer. ^{1}HNMR was recorded on a Jeol ECA 500 NMR Spectrophotometer.

Plant material

Psidium guajava leaves were collected from the Khartoum state, Sudan and athenticated by the Aromatic and Medicinal Plants Research Institute –Khartoum - Sudan.

Methods

Extraction of flavonoids

Powderd shade-dried leaves of *Psidium guajava* (1Kg)were macerated exhaustively at room temperature with 95% ethanol. The solvent was evaporated under reduced pressure to give a solid mass.

Phytochemical screening

The crude ethanolic extract was screened for major secondary metabolites according to the method described by Harborne.[20]

Isolation of flavonoids

Thin layer chromatography was used for fractionation the crude ethanolic extract. Silica gel for TLC- from LOBA (India) was used. The composition of the mobile phase (60% acetic acid) was determined by TLC analysis. After the usual workup compound I (R_f 0.65) was isolated in chromatographically pure form.

UV shift reagents

Sodium methoxide spectrum

Three drops of sodium methoxide solution were added to a solution of compound I in methanol (2ml) and the UV spectrum was immediately recorded.

Aluminium chloride spectrum
Six drops of a methanolic solution of aluminium chloride were added to a solution of compound I in methanol (2ml) and the UV spectrum was recorded immediately.

Sodium acetate spectrum
Excess coarsely - powdered anhydrous sodium acetate was added with shaking to a cell containing (2ml)of the solution of compound I in methanol and the UV spectrum was recorded after two minutes.

Boric acid /sodium acetate spectrum
Sufficient powdered anhydrous H_3BO_3 was added with shaking to a cuvette containing the solution used for the sodium acetate spectrum. The UV spectrum was recorded after two minutes.

RESULTS AND DISCUSSION

Phytochemical screening
Phytochemical screening of leaves revealed the presence of tannins, saponins, terpenes, flavonoids, steroids, alkaloid and glycosides.

Identification of compound I
Compound I was isolated as pale yellow powder from the leaves of *Psidium guajava*.The structure was partially elucidated via spectral techniques (UV and ^{1}HNMR).

The UV spectrum of compound I (Fig. I) showed λ_{max} (MeOH) 247, 363 nm. Such absorption is characteristic of flavonols.[21]

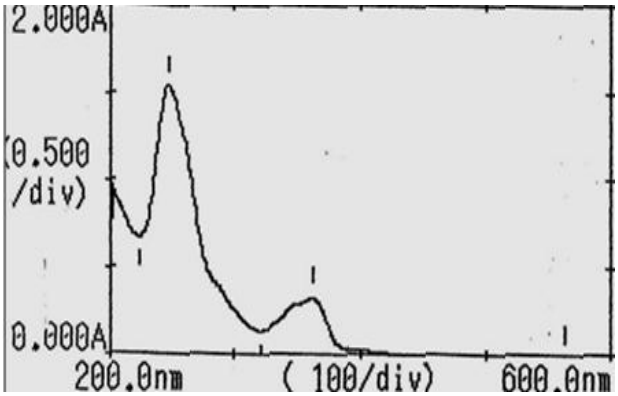


Fig.1: UV spectrum of compound I.

Next the hydroxylation pattern of the isolated flavonol was studied via the UV shift reagents: sodium methoxide, sodium acetate, aluminium chloride and boric acid. The strong base –sodium methoxide is diagnostic of 3- and 4`-OH functions, where a bathochromic shift is detected in both cases, but with decrease in intensity in case of a 3-OH function. Aluminium chloride gives bathochromic shifts in presence of 3- or 5-OH functions or catechol moieties. The presence of such groups is evidenced by bathochromic shifts. The boric acid spectrum is useful diagnostic agent for catechol systems.[21]

Addition of sodium methoxide to a methanolic solution of compound 1 (Fig.2) gave a bathochromic shift (35nm) in band I without decrease in intensity Indicating a 4`-OH group, while the presence of a 3-OH function is evidenced by a small bathochromic shift in band II with decrease in intensity.

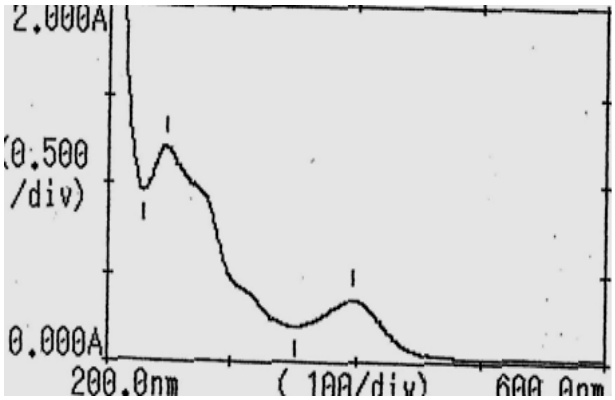


Fig. 2: Sodium methoxide spectrum of compound I.

Sodium acetate is a weaker base than sodium methoxide and as such ionizes only the more acidic hydroxyl group in flavonoids.[21] When this shift reagent was added to a methanolic solution of compound 1 a bathochromic shift(36 nm) was observed(Fig. 3). This indicates the presence of a 7-OH function.

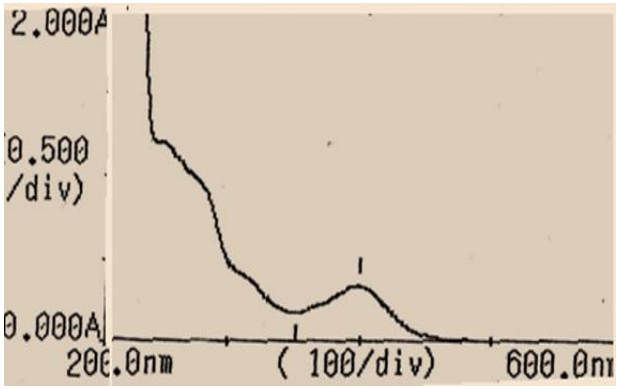
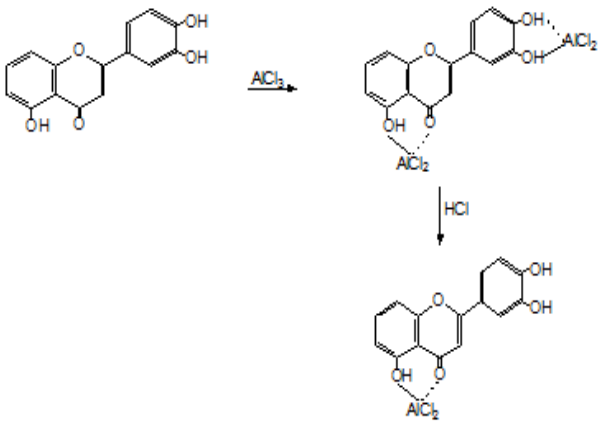


Fig. 3: The sodium acetate spectrum of compound I.

Alumium chloride chelate with functional groups such as the 5 – hydroxyl – 4- keto -; 3- hydroxyl -4- keto systems as well as catechol moieties giving bathochromic shifts[21]:However, only the complexes formed with the 3 – OH (5-OH) and 4 –keto function are acid –stable as shown below:



When the shift reagent aluminium chloride was added to a methanolic solution of compound 1 (Fig.4) no detectable bathochromic shift was observed. This indicates absence of 5- hydroxylation as well as catechol systems. The absence of catechol is further confirmed by the boric acid spectrum (Fig.5).

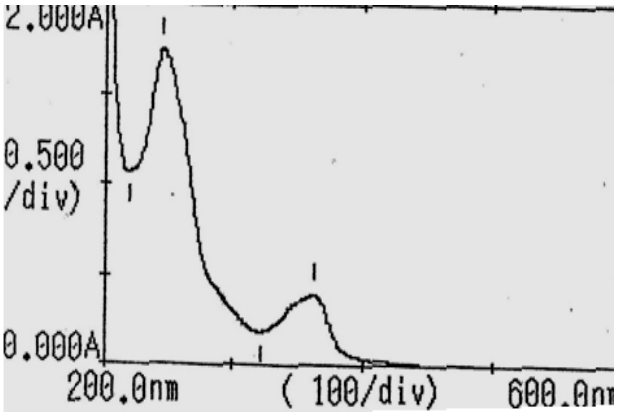


Fig. 4: Aluminium chloride spectrum of compound I.

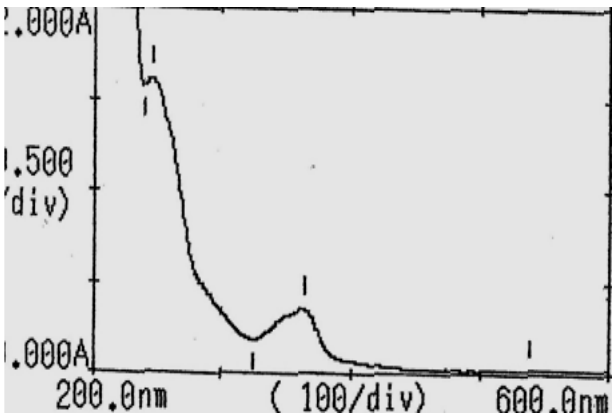


Fig. 5: Boric acid spectrum of compound I.

The ^{1}H NMR spectrum (Fig.6) showed a signal at δ 0.95ppm assigned for a methyl function. The singlet at δ 4.00ppm accounts for a methoxyl group, while the resonances at δ7.20 and δ 8.15 account for the aromatic protons.

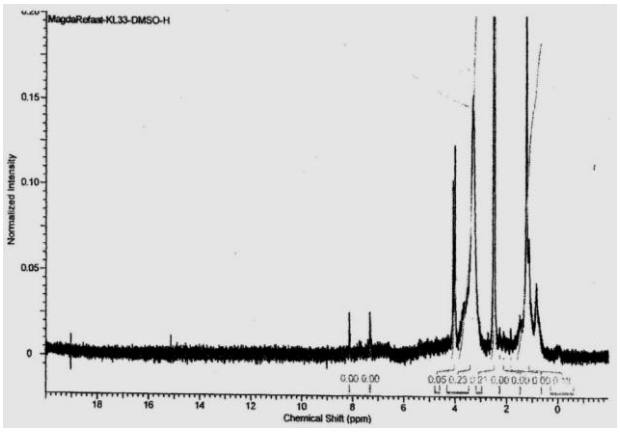
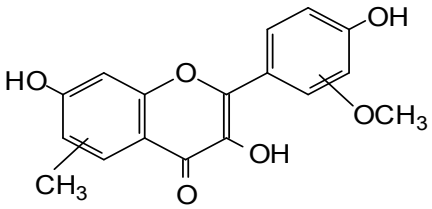


Fig 6: H^{1}NMR spectrum of compound 1.

Comparison of the above spectral data with literature data gave the following partial structure for the isolated flavonol:



Compound I

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