IGBINEDION UNIVERSITY OKADA
JOURNAL OF PHARMACEUTICAL SCIENCES

https://pharmacy-journal.iuokada.edu.ng/ Volume 3, no. 1, pp. 037-042 (2024)

ISSN (online): 2992-5746 **ISSN (print): 2992-5339**

SHORT COMMUNICATION

ISOLATION AND CHARACTERISATION OF SOME FLAVONOIDS FROM METHANOL LEAF EXTRACT OF *FICUS SYCOMORUS* L. (MORACEAE)

Atiku I[1,*], Pateh UU[1], Sule MI[1], Isyaka MS[2], Isiyaku S[3]

[1]*Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria.*
[2]*Department of Chemical Sciences, Pen Resource University, Gombe, Nigeria*
[3]*Department Chemistry, Ahmadu Bello University, Zaria, Nigeria.*

***Corresponding author's email**: iatiku@abu.edu.ng. **Telephone:** +234(0)7039605662

ABSTRACT

Background and aim: *Ficus sycomorus* L. (Moraceae) is a plant used in African traditional medicine to treat mental illness, dysentery, cough, diarrhoea, tuberculosis and cancer. The aim of this study was to isolate and characterise some flavonoids from the leaf extract of the plant.

Methods: Dried pulverised leaf was macerated sequentially using dichloromethane and methanol with occasional shaking for three days followed by filtration. The concentrated extract was subjected to flash column chromatography using the mobile phase, which progressed from 100% n-hexane to dichloromethane and ethylacetate. Silica gel (60-120 mesh size) was used as the stationary phase.

Results: Further purification of the flash column fractions led to isolation of three known flavonoid compounds for the first time viz: two aglycones (epicatechin and kaempferol) and one glycosides (kaempferol-3-*O*-α-L-rhamnoside). The fourth isolated glycoside quercetin-3-*O*-β-*D*-glucopyranoside is previously reported in the plant. The structures of these compounds were established by analysis of their spectral (IR, GC-MS ^{1}H, ^{13}C and 2D NMR) data and comparing them with literature data. Kaempferol and (+)-catechin are being isolated from *F. sycomorus* leaf for the first time.

Conclusion: Four flavonoids consisting of two aglycones and two glycosides with reported biological activities were isolated and characterised from the methanol leaf extract of *F. sycomorus*.

Keywords: *Ficus sycomorus*, leaf, Moraceae, flavonoids, flash chromatography, dichloromethane extract, ethylacetate extract.

INTRODUCTION

Medicinal plants are associated with the presence of polyphenols, polysaccharides and hydrolysable tannins. Various extracts from the plants contain diverse array of secondary metabolites such as flavonoids, triterpenes and tannins that possess antioxidant properties [1].

Flavonoids are a large group of C-15 (C6–C3–C6) secondary metabolites widespread in higher plants and are also detected in some lower plants such as algae. An important number has been reported from natural and synthetic sources due to their several applications in the pharmaceutical and diet industries. Flavonoids occur in natural products especially blooming plant species, and the colours of flowers could be indicative of the class of compounds [2]. Several methods have been used for extracting flavonoids in plant materials [3]. Polar solvents are used to obtain flavonoid glycosides, whereas non-polar solvents extract mostly their aglycones. Most of the investigations conducted in the extraction of flavonoids in plant materials have been done by maceration and infusion [4].

Ficus sycomorus L. (Moraceae) is a large, semi-deciduous spreading savannah tree, up to 21 m. It is found growing in Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia, and Cote d'lvoire. In Nigeria, the plant is mostly found in semi-arid regions [5]. The plant is referred to by several local names: it is commonly known as Baure (Hausa), Tarmu (Kanuri), and Kamda (Babur/Bura) among others [6]**.** Different parts of the plant are traditionally used to treat various ailments such as tumors and diseases associated with inflammation. Examples are fruits in different stages of ripening, fresh or dry, stem bark, leaves, twigs and young shoots, and also latex from the bark, fruit and young branches [7]. Oghenesuvwe *et al.*, [8] evaluated the ethno-botany, phytochemistry, pharmacological properties and toxicological effects of *F. sycomorus* using electronic data bases. Its ethnomedicinal claims with validated pharmacological properties include; anti-diabetic, anti-microbial, anti-oxidant, hepatoprotective, neuroprotective, antidiarrheal and hypotensive activities. El-Sayed *et al.* [9] have previously reported the isolation of quercetin, gallic acid, quercetin 3-*O*-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (rutin), quercetin 3-*O*-β-D glucopyranoside (isoquercitrin), quercetin 3,7-*O*-α-L-dirhamnoside, quercetin 3-*O*-β-D-galactopyranosyl(1→6)-glucopyranoside and β-sitosterol-3-β-glucopyranoside from *F. sycomorus* leaf. Similarly, non-flavonoid compounds including 2-acetyl-3-methylaminocyclopentenone, 4-(3,4-dimethoxyphenyl)-5-methyl-2-thiazolamine and cyclobarbital were also reported to be isolated from this plant [10].

MATERIALS AND METHODS

***Collection and preparation of plant materials*:** Leaves were collected from Turunku village, Igabi Local Government Area, Kaduna State, Nigeria, in July 2013. Identification was done by Namadi Sanusi of Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, (voucher number: 1466). Leaves were air-dried and powdered using a cleaned pestle and mortar and bagged.

Extraction: Powdered leaf (2.5 kg) was subjected to cold maceration with dichloromethane (DCM) and occasional shaking for three days. The extract was drained and filtered using Whatman no. 1

filter paper and concentrated to dryness using rotary evaporator at 45^{o}C to yield a dark green residue (73.5 g) referred to as dichloromethane extract (DCM-E). The marc was further extracted by the same method with absolute methanol to yield a dark brown residue (70.2 g) referred to as Methanol Extract (ME). The ME was used for this study and DCM-E kept for future study.

***Flash column chromatography*:** The ME was subjected to flash column chromatography (FCC). Silica gel (Sigma-Aldrich) was added to the leaf extract slurry, dried and ground into fine powder. The powder was loaded into a sample cartridge and fitted to the FCC instrument, along with a 150 g sample-mass- cartridge packed with silica gel (Sigma-Aldrich). Gradient elution was used starting from 100% n-hexane to DCM:EtOAc (1:1). A total of 150 fractions (45 ml each) was collected, fractions with similar R_f values upon TLC analysis (DCM:EtOAc, 3:7; 10% H_2SO_4 at 115^{o}C) were pooled for either immediate NMR analysis or further purification. A combination of column chromatography over both silica gel and Sephadex® **(**a size exclusive cross-linked dextran gel) and preparative TLC (prep-TLC) were used for purification of the pooled fractions.

***Isolation of compound A1*:** (+)-Catechin was obtained from FCC fractions 86-87 of ME, subjected to silica gel column chromatography and eluted with EtOAc (100%) to give 15 fractions (2 ml each). Fraction 5 yielded a single homogenous spot on the analytical TLC.

***Isolation of compound A2*:** Kaempferol was obtained from FCC fractions 79-84 of the ME, subjected to silica gel column chromatography and eluted with DCM:MeOH (25:1) to give 22 fractions (2 ml each). Fractions 17 and 18 yielded a single homogenous spot on analytical TLC

***Isolation of compound A3*:** Kaempferol-3-*O*-rhamnoside was obtained from FCC fractions 88-91 of the ME, subjected to gel filtration using Sephadex LH20 and eluted with methanol (100%) to give 21 fractions (2 ml each). Fractions 14 and 15 showed a single spot on analytical TLC.

***Isolation of compound A4*:** Quercetin-3-*O*-β-*D*-glucopyranoside was obtained from FCC fractions 95-101 of the ME, subjected to silica gel column chromatography and eluted with EtOAc:MeOH (8:2) to give 31 fractions (2 ml each). Fractions 17 and 19 yielded a single homogenous spot on analytical TLC.

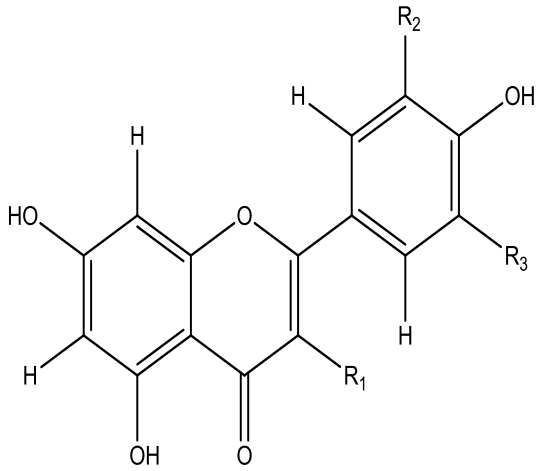
RESULTS

***Compound A1*:** Isolated as white crystals (R_f = 0.41, DCM:EtOAc 7:3) has a specific rotation of +55^{o} (c= 0.04, CH_2Cl_2) and a m.p. 237-238^{o}C, was identified as (+)-epicatechin. The ^{13}C-NMR spectrum showed 15 signals indicating 15 carbon atoms which viz: seven quaternary carbons, seven tertiary carbons and one secondary carbon at δ_C 29.4 (C-4). The spectral data are similar to those reported by in the literature [11,12].

***Compound A2*:** Isolated as a reddish-brown paste and had a melting point of 227-229^{o}C, was identified as kaempferol. ^{13}C-NMR/DEPT-135 spectra showed a total of twenty-one carbon signals. These included six methines, four oxygenated methines, nine quaternary carbons and one methyl carbon signals. The spectrum also an α,β unsaturated ring system with resonances at δc 157.2, 134.0 and 179.0 which were ascribed to C-2, C-3 and C-4 of ring C of flavonoid nucleus. These spectral data were in agreement with that of kaempferol reported in the literature [13,14]. To the best of our knowledge this is the first time this compound is isolated from the leaf of *F. sycomorus*.

***Compound A3*:** Obtained as a yellow powder (R_f = 0.48, DMC:MeOH 9:1), m.p. 182-184ºC, $[\alpha]^{21}_D$ = +0.33(c = 0.063 CH_2Cl_2) and was identified as kaempferol-3-*O*-α-L-rhamnoside. The ^{1}H NMR and ^{13}C NMR (CD_3OD 500 MHz) spectrum of A13 showed that the compound had similar structural skeleton as that of kaempferol. Moreover, its ^{13}C-NMR spectrum showed a total of twenty-one carbon signals which included six methines, four oxygenated methines, nine quaternary carbons and one methyl carbon signals. An anomeric carbon signal was observed at δc 103.7 which showed a correlation in the HSQC-DEPT spectrum with the anomeric proton signal observed at δ_H 5.39. This indicated the presence of a sugar moiety. The HMBC correlation observed between the anomeric proton H-1" (δ_H 5.39) and the carbon signal at δc 136.4 (C-3) confirming that the sugar was attached at C- 3. The sugar was determined to be rhamnose by the presence of the typical rhamnose C-6 methyl carbon signal at δc 17.8 (C-6"). The coupling constant value for the anomeric proton, (*J* = 1.5 Hz) suggested that the sugar moiety is α-L-rhamnoside [15]. The spectral data reported here for compound A13 agrees with the NMR data of kaempferol-3-*O*-rhamnoside previously reported [16].

***Compound A4*:** Obtained as a pink amorphous powder, m. p. 193-195ºC, and $[\alpha]^{21}_D$ +55º (c= 0.04, CH_2Cl_2) was identified as quercetin-3-*O*-β-*D*-glucopyranoside. The ^{13}C NMR spectrum showed a total of twenty-one carbon signals. This, according to the DEPT-135 spectrum, included 10 quaternary carbons, ten methine carbons and one methylene carbon. The anomeric carbon signal was seen to appear at δc 104.4 which corresponded to the anomeric proton at δ_H 5.25. This showed that the compound is a flavonoid glycoside. The sugar unit was identified as glucose due to the typical glucose –CH_2OH group observed at δc 62.7 (C-6"). The coupling constant of the anomeric proton (*J* =7.6 Hz) defined the stereochemistry of glyosidic linkage as β [17]. The HMBC correlation observed between the anomeric proton and the carbon signal at 135.8 (C-3) confirmed that the glucose moiety was attached at C-3. Its structure was confirmed by comparing its spectral data by those previously reported [18,19].



A1: R_1= OH, R_2= H, R_3= OH
A2: R_1= OH, R_2= H, R_3= H
A3: R_1 = Glucosyl, R_2 = H, R_3= H
A4: R_1 = Rhamnosyl, R_2 = OH, R_3= H

Figure 1: Chemical structures of compounds A1 ((+)-catechin), A2 (kaempferol), A3 (kaempferol-3-*O*-rhamnoside) and A4 (quercetin-3-*O*-β-*D*-glucopyranoside)

CONCLUSION

The aforementioned studies revealed that *F. sycomorus* leaf contained important flavonoid aglycones, epicatechin and kaempferol and flavonoid glycosides, kaempferol-3-*O*-α-L-rhamnoside and quercetin-3-*O*-β-*D*-glucopyranoside with reported biological activities.

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