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Isolation of Quercetin (3, 3', 4' 5, 7 – Penta Hydroxy Flavone) from Ethyl Acetate Fraction of *Acacia gourmaensis (*Leguminoceae/Mimosaceae)

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ABSTRACT

This study aimed at investigating and isolating some phytochemical constituents present in the methanol and dichloromethane extracts and ethyl acetate fraction, and to validate the antimicrobial, antioxidant, and anti-inflammatory claims of *Acacia gourmaensis*. Dried stem bark powder of *Acacia gourmaensis,* locally known as 'Anaka in Igala in North Central and kaya yawo in Hausa of Northern Nigeria of the family Fabaceae, was subjected to cold successive maceration using dichloromethane (DCM) and methanol (MeOH) to afford dichloromethane extract (DCM-E) and methanol extract (MeOH-E). The MeoH-E was partitioned with ethyl acetate to afford ethyl acetate fraction (EA-F) and residual methanol extract (RMeOH-E). The EA-F fraction was subjected to repeated column chromatography leading to the isolation of a yellow substance (coded EAF10). The substance was identified as Quercetin (3, 3', 4', 5, 7 Pentahydroxy flavone) when subjected to physical, chemical and spectroscopic analysis using IR, ^{1}H-NMR, ^{13}C -NMR data and by comparing the data with reference material.

Keywords: *Acacia gourmaensis,* methanol extract, dichloromethane extract, ethyl acetate fraction, quercetin, IR, NMR.

INTRODUCTION

Traditional herbal medicines are naturally occurring, plant derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices [1]. For centuries, humans have relied on nature to meet their primary health needs, precisely for the treatment of a wide spectrum of diseases. The interest in medicinal plant uses has increased in recent decades, because these plants produce a large range of bioactive molecules, making them a rich source of different types of medicines. This approach is attractive, because it constitutes a potential source of bioactive compounds which are safe and often act on multiple and novel target, thereby reducing the potential for resistance [2]. Traditional herbal medicines are getting significant attention in global health debates, in China and it is playing a prominent role in the strategy to contain and treat several diseases [3]. About half of the world medicinal compounds are derived from plants. Medicinal products from plants are generally more important in developing countries than in the developed and industrialized world as 75 – 90 % of the world's rural populations rely comfortably on herbal medicines according to Hamman O. [4]. The *Acacia* species are found all over the world. Almost 1000 of them are found in a wide range in Australian regions followed by 185 species in North and South America, about 144 in the Africa regions (Madagascar included) and 89

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species in Asia-pacific [5]. Ethnobotanical data revealed that preparations from different parts of *Acacia* species are used traditionally for diarrhea, diabetes, gastrointestinal disorders, skin diseases and inflammatory diseases [6-9]. *Acacia* is a member of tribe *Acacieae* within sub-family Mimosoideae of the Fabaceae family. The Mimosoideae, which is sometimes treated as a distinct family contains about 50 - 60 genera that are distributed throughout tropical, subtropical and warm-temperate regions of the world [10]. Previous phytochemical investigations of the genus *Acacia* led to the isolation of 152 chemical constituents, including flavonoids, phenolic acids, terpenoids and other compounds according Abdou *et al,* [2]. Previous studies demonstrated that extracts and isolated compounds have interesting biological properties such as antibacterial, antifungal, antioxidant, anticancer, antiparasitic, antidiabetic, cytotoxicity and other activities as reported by Abdou *et al* [2]. During the seven past decades, about 152 chemical constituents were isolated from the genus *Acacia*, including flavonoids (44), terpenoids and phytosterols (34), phenolic acids (21), fatty acids (11), hydrocarbons (13) and other compounds (29) [1]. Many flavonol, flavone, chalcone derivatives, flavan-3-ols and flavan-3,4-diols, which constitute many of the secondary metabolites from the genus *Acacia* have been reported [11 – 16]. The most frequently encountered flavonoids are catechin found in six species and quercetin found in five species. This study as stated earlier aimed at investigating and isolating some phytochemical constituents present in the methanol and dichloromethane extracts and ethyl acetate fraction, and to validate the antimicrobial, antioxidant, and anti-inflammatory claims of *Acacia gourmaensis* used in traditional medical practices.

MATERIALS AND METHODS

Collection and identification of plant material

The stem bark of the plant growing in wild was collected from Rijana village near Kontagora in Niger state by Mr. Gallah Shehu Umar of the herbarium unit of the Department of Pharmacognosy and Drug Development Kaduna State University on 20th October 2018 where it was authenticated by comparing it with an existing voucher specimen number KASU/PCG/9882 and confirmed in the herbarium unit of the Department of Botany Faculty of life Sciences Ahmadu Bello University Zaria by comparing with an existing sample with a voucher number 01378 by Namadi Sunusi. The plant was air dried under the shade for 2 weeks and then size reduced to a coarse powder using pestle and mortar and hence referred to as the powdered plant material.

Extraction and Isolation.

The powdered stem bark (1,520g) was cold macerated stepwise exhaustively each time with 10.0 liters each of dichloromethane (DCM) and methanol (MeOH), which were referred to respectively as dichloromethane extract (DCM-E) 39.24g and methanol extract (MeOH-E) 50.00g. The methanol extract was partitioned with 2.5 liters of ethyl acetate to afford ethyl acetate fraction (EA-F) with 21.90g and residual methanol extract (RMeOH-E), the residual methanol extract was air dried and kept for further use.

The TLC profile of the EA-F using 100% DCM and hexane: ethyl acetate (9: 1) reveal some prominent spots that necessitated its subjection to column chromatography for isolation.

The EA-F (10g) was subjected to repeated column chromatography using silica gel (60

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– 120) mesh using various solvent systems starting with 100% hexane, hexane: ethyl acetate 99: 1, 98: 2, 97: 3, 96: 4, 95: 5, 90: 10, 80: 20, 70: 30, 50: 50, 30: 70, and 0: 100 (Table 1). A total of 168 collections were made from 12 different solvent systems with about 1,720 ml of solvents Table 1.

Table 1: fractions from the EAF column chromatography

S/No	Solvent system	Fractions collected	Volume (ml)
1	Hexane: ethyl acetate 100: 0	1	50
2	Hexane: ethyl acetate 99: 1	2 – 8	50
3	Hexane: ethyl acetate 98: 2	9 - 15	50
4	Hexane: ethyl acetate 97: 3	16 - 22	50
5	Hexane: ethyl acetate 96: 4	23 - 25	50
6	Hexane: ethyl acetate 95: 5	26 - 32	50
7	Hexane: ethyl acetate 90: 10	33 - 64	250
8	Hexane: ethyl acetate 80: 20	65 - 82	150
9	Hexane: ethyl acetate 70: 30	83 - 116	300
10	Hexane: ethyl acetate 50: 50	117 - 141	220
11	Hexane: ethyl acetate 30: 70	142 - 167	100
12	Hexane: ethyl acetate 0: 100	168	100

Table 2: Pooling and coding of the EAF column chromatography.

S/No	Pooling	Code	No of spot(s)	Remark
1	1 - 9	A12	0	No spot discarded
2	10 - 23	A13	5	Five spots, oily & scanty, discarded
3	24 - 30	A14	0	No spot discarded
4	**31 - 49**	**A15**	**5**	**Five spots, looking separable**
5	50 - 65	A16	3	Three spots heavy at top & below
6	66 - 80	A17	2	Two tailing spots
7	81 - 89	A18	2	Two complex spots
8	90 - 105	A19	1	One bulky spot
9	106 - 120	A20	9	Nine spots
10	121 - 128	A21	7	Seven spots
11	129 - 135	A22	5	Five spots
12	**136 - 150**	**A23**	**4**	**4 spots, looking separable**
13	151 – 164	A24	4	4 faints, tailing spots
14	165 - 167	A25	0	No spot - discarded
15	168	A26	1	One dark/heavy spot

Gel filtration of A23 from ethyl acetate column led to the isolation of compound EAF10 (12mg). Compound EAF10 was isolated as a yellow powder, it gave a single spot-on TLC using DCM: ethyl acetate 1:1, 4: 1 and 9: 1 with R_f values of 0.30, 0.68 and 0.85 respectively (Table 3).

Table 3: Rf values of the TLCs profile of compound EAF10

Solvent system	Ration	R_f value
Dichloromethane: Ethyl acetate	1: 1	0.30
Dichloromethane: Ethyl acetate	4: 1	0.68
Dichloromethane: Ethyl acetate	9: 1	0.85

EAF10 was completely soluble in methanol, and it was found to melt between 236 – 237 ^{O}C. It produces yellow colouration when subjected to Shinoda test [17]. The IR spectrum (Plate I) of compound EAF10 showed absorptions bands at 3205.5cm^{1} for (OH) vibrations of phenol, 3067.6 cm^{1}, 2922.2 cm^{1}, 2855.1 cm^{1}, 2091.0 cm^{1}, 1725.8, cm^{1}, 1289.7 cm^{1} (-C=O for Aryl ether), 1170.4 (- C-CO-C stretch and bending in ketone), 1043.7 cm^{1}, 865.7 cm^{1} & 685.8 cm^{1} (C-H bending of aromatic hydrogen). The proton NMR (Plate II) for compound EAF10 showed signals/peaks at δ 2.510, δ 2.514 & δ 2.517 (for alcohol OH), δ 6.100, δ 6.103, δ 6.410, δ 7.064, δ 7.500 & δ 7.510 (for benzene CH), signals at 6.19 (d,1H), 6.41 (d, 1H), 6.88 (d, 1H), 7.54 (q, 1H) and 7.66 (d, 1H) are for aromatic hydrogens while the signals at 9.36(s,2H), 9.65 (s,1H), 10.87 (s,1H) and 12.48 (s,1H) are for aromatic alcohols (OHs). The ^{13}C – NMR spectrum (Plate III) of compound EAf10 showed signals at δ 93, δ 98, δ 102, δ 114, δ 116, δ 162, δ 176, δ 180.

The NMR data thus showed signals typical of flavonoids which are comparable to those NMR data reported in the literature according to Bharathi, *et al* [18].

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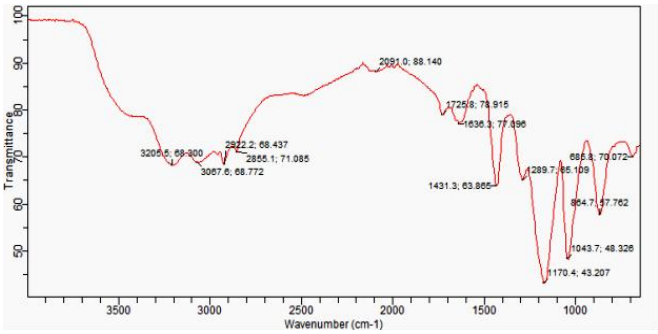

Plate I: IR spectrum of compound EAF10

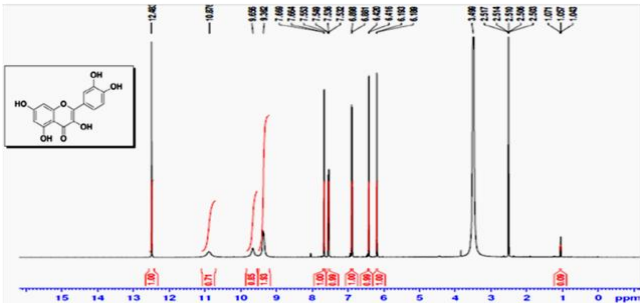

Plate II: The H-NMR spectrum of compound EAF10.

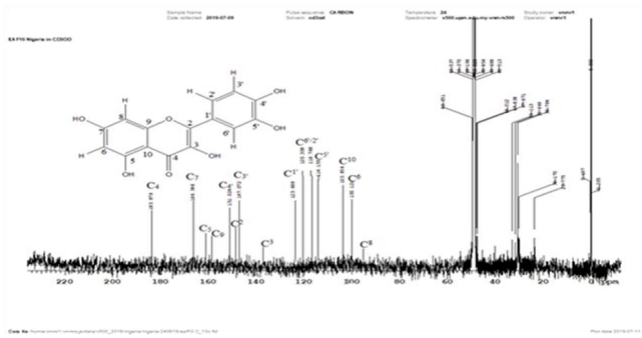

Plate III: ^{13}C – NMR spectrum of compound EAF10.

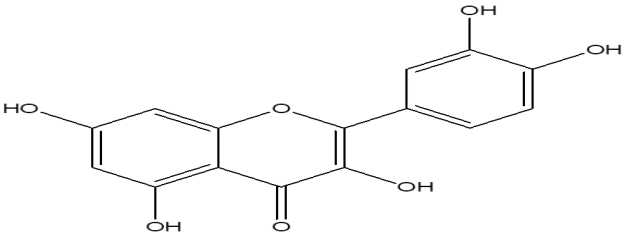

Figure 1: Quercetin ($C_{15}H_{10}O_7$) 3, 3' 4' 5,7 pentahydroxy flavone)

CONCLUSION

The EA-F of *A. gourmaensis* contain some phytochemicals or compounds one of which has been isolated in this study and identified as a flavonoid (quercetin [3, 3', 4', 5, 7 pentahydroxy flavone) when its spectral data were compared to the literature, this can be one of the phytochemicals or compounds responsible for the use of *Acacia gourmaensis* in ethno-medicine for the treatment of convulsion, arthritis, jaundice, cancer, malaria, cough, rheumatism, lumbago, muscular/body pains, as well as in treatments of eye, ear, oral and pulmonary infections, management of snake bites and food poisoning in West African countries particularly Nigeria, thereby validating the use of the plant in traditional herbal practices. We recommend that more study be carried out on other extracts and fractions of the stem bark and other parts of *A. gourmaensis* to isolate more of these phytochemicals which can serve as drug leads.

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