Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 1707-1710
Received: 24-07-2018
Accepted: 26-08-2018

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Identification of antioxidant compound from *Phoenix pusilla* fruits using NMR techniques

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Abstract
The present investigation has been carried out to find the Antioxidant compound present in *Phoenix pusilla* fruits extract. Antioxidant compound was isolated by column chromatography technique. The collected flavonoid fractions was purified by thin layer chromatography. NMR studies was carried out to find the structure of Antioxidant compound Quercetin. ^{1}H- NMR and ^{13}C – NMR that reveals the structure of flavonoids. The compound was identified as 3, 3', 4', 5, 7 – pentahydroxyflavanone by ^{1}H-NMR and ^{13}C – NMR. All these data obtained in the present investigation supported the antioxidant compound present in *Phoenix pusilla* fruits extract and thereby traditional claim associated with *Phoenix pusilla* fruits.

Keywords: *Phoenix pusilla* fruits, antioxidant and NMR

Introduction
A nuclear magnetic resonance spectrum gives the largest amount of information about the structure of a compound. In NMR Spectroscopic method, a substance is placed in a strong magnetic field that affects the spin of the atomic nuclei. A radio wave passes through the substance, and reorients these nuclei. When the wave is turned off, the nuclei release a pulse of energy that provides data on the molecular structure of the substance and that can be transformed into an image by computer techniques Moore and Dalrymple (1997) [18]. The role of plants present in the environment has significant importance to health. The NMR phytochemical analysis of this medicinal and edible plant leaves is essential in line with WHO requirements (2012) [27]. Though Gori and Campbell (1998) [9] emphasized that some herbs only have mild or placebo effects, it is necessary to elucidate the phytochemical basis of their safety and effectiveness. To obtain scientific evidence required to ensure the use of safe, effective and quality products and practices, and to facilitate the understanding of its biological activities, the current study aimed at identifying the major and minor compounds in the antioxidant compound present in *Phoenix pusilla* fruits extract using nuclear magnetic resonance (NMR).

Materials and Methods
Plant materials
The fully mature *Phoenix pusilla* fruits were collected from Kathattipatti (Palaiyapatti North), Sengipatti, Thanjavur District, Tamil Nadu, India. The fruits were identified, authenticated and voucher specimen (A001) has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamilnadu, India.

Preparation of alcoholic extract
The collected *Phoenix pusilla* fruits were washed several times with distilled water to remove the traces of impurities present in the fruits. The fruits were dried at room temperature, seeds were removed and coarsely powdered. The powder was extracted (Maceration technique) with different extracts (chloroform, ethanol and water) for 24 hours and a semi solid mass was obtained after complete removal of solvent under reduced pressure. The *Phoenix pusilla* fruits extract (PPFX) was stored in refrigerator and used for further experiments.

Column Chromatography
Separation of flavonoid compound using Column Chromatography by the method of Javed Intekhab and Mohammad Aslam (2009) [16].

Thin Layer Chromatography

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces followed by the method of Harborne (1984, 1973) [14,13].

NMR Spectroscopy

After the separation of plant extract to fractions using Column chromatography, Thin Layer chromatography was used for further purification of collected fractions. The NMR experiment was carried out in BRUKER-AMX400 MHz instrument with 5mg of purified compound in $DMSOd_6$. Tetra Methyl Silane is used as the internal standard and chemical shifts are expressed in ppm.

Results and Discussion

Quercetin Identified by NMR Studies

^{1}H-NMR spectrum

In ^{1}H-NMR spectrum (500MHz, MeOD) the A ring protons at C-6 and C-8 appear as δ 6.28 and δ 6.43ppm respectively. A two proton singlet at δ 7.67 assigned to H-2′ and H-6 ′Mizuno *et al.* (1992) [17]. The proton at C-5′ appears as δ 6.95ppm as doublet.

^{13}C -NMR spectrum

Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C-NMR data and a complete assignment is given in Table. It was characterized as 2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one (quercetin).

Table 1: ^{13}C-NMR data and their assignment of quercetin obtained by the references (Guvenalp and Omur [11]; Harborne and Williams [12]; Guvenalp and Nurcan [10]).

	Chemical shift δ ppm (Quercetin Nucleus)		
Carbon	**Plant extract**	**Literature Eleni Kyriakou *et al.* [8]**	**Reference**
2	146.10	147.90	147.65
3	137.18	137.20	135.68
4	177.24	177.30	175.79
5	162.03	162.50	160.67
6	99.26	99.30	98.12
7	165.98	165.70	163.83
8	94.30	94.40	93.29
9	157.11	158.20	156.08
10	103.02	104.40	102.96
1'	121.80	124.10	121.90
2'	116.11	116.00	115.55
3'	146.10	146.20	146.75
4'	148.50	148.70	147.65
5'	116.11	116.20	115.02
6'	121.80	121.60	121.90

Based on this the data has been characterized as Quercetin (Molecular Formula: $C_{15}H_{10}O_7$)

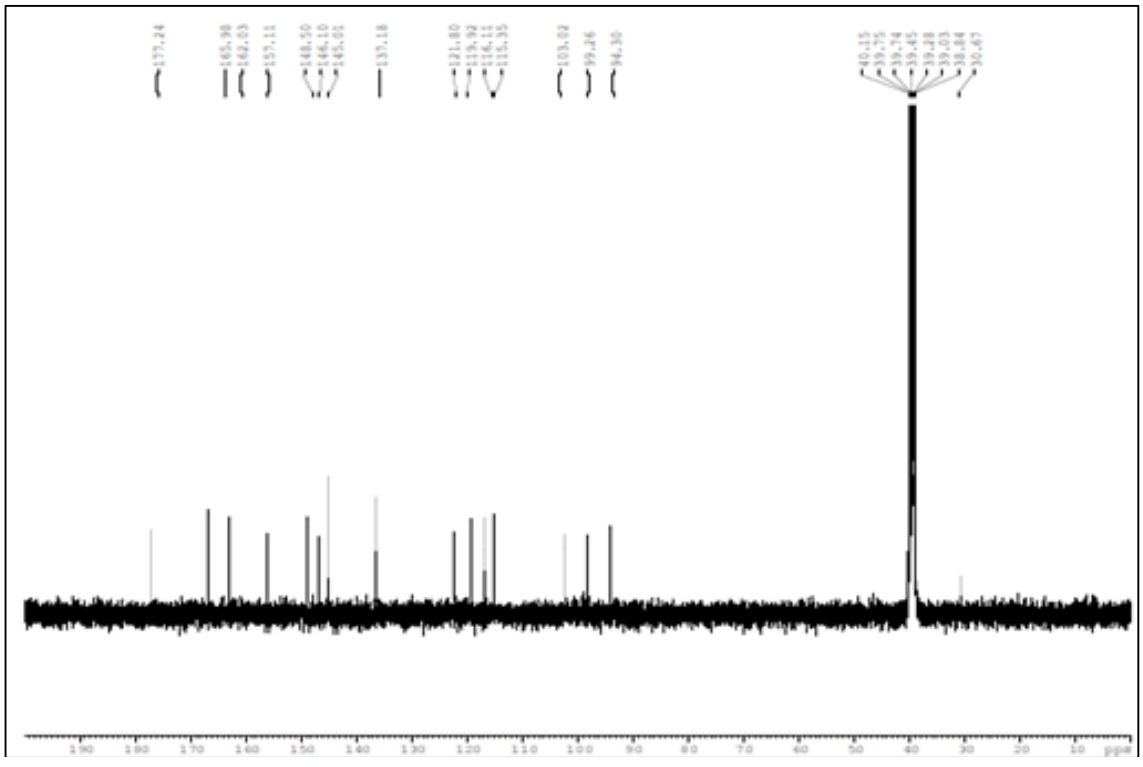

Fig 1: [13] C -NMR spectrum of Quercetin

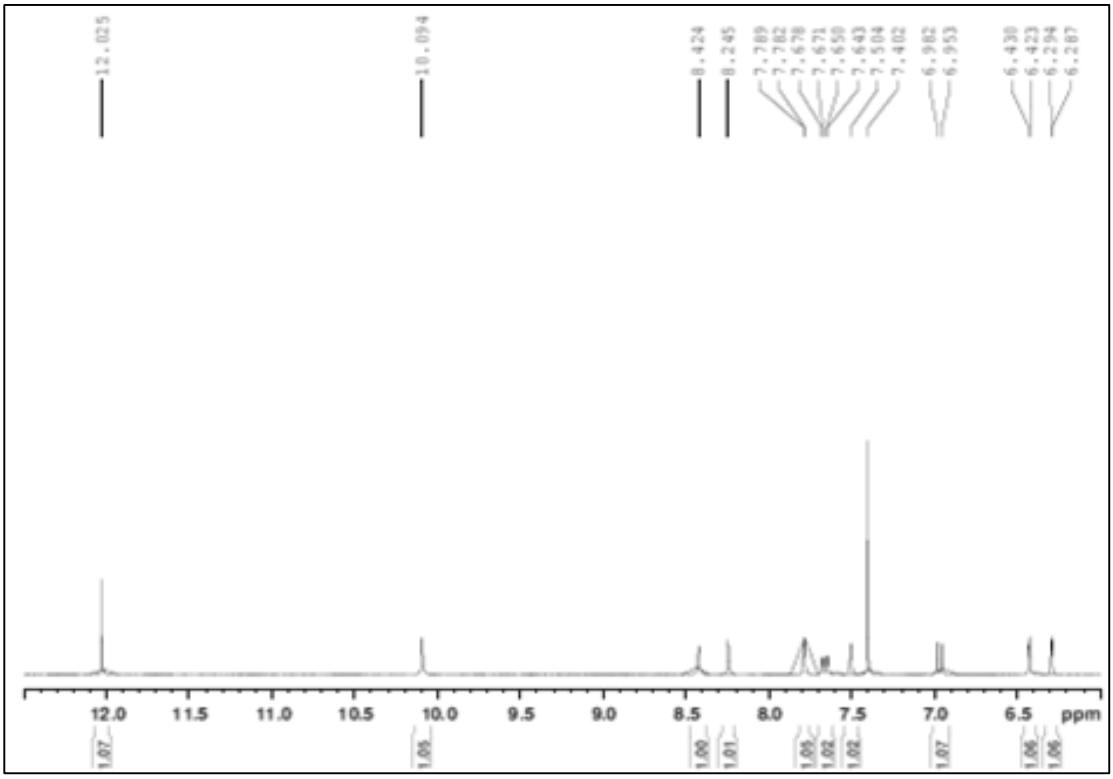

Fig 2: [1] H -NMR spectrum of Quercetin

The effects of these natural antioxidants in scavenging the free radicals are well discussed and reported in the earlier studies. The factors that encourage the use of natural antioxidants are its low cost, compatibility with diet and less harmful effect in the human body. The strong H-donating capacities of various phytochemicals make them as a effective natural antioxidants. Phenols present in plant extracts acts as a potential antioxidant by inhibiting the free radical formation and also prevent auto oxidation Anbudhasan *et al*. (2014) [3].

In the present study Quercetin was isolated and identified from the leaves of *Lanata camera* using NMR. Present finding was agreement with earlier studies Sikorska and Maltlawska (2000); Pratima Tatke *et al*. (2014); Arturo Sánchez-Muñoz *et al*. (2012); Iwona Wawer and Agnieszka Zielinska (2001); Dahham *et al*. (2015) [23, 19, 4, 15, 7].

Quercetin is one of natural flavonoid group that is most common as a secondary metabolite in plants. Production of synthetic flavonoids has not been practiced yet. Hence, plants are the only sources for quercetin Abdelmoaty *et al*. (2010) [1]. Major vegetables and fruits that are commonly consumed comprise different classes of flavonoids in varied amount. It has been found that onion has the highest amount of quercetin (about 300 mg/kg) among tested nutrition Beecher (1999) [5]. The antioxidant character of quercetin is associated to chemical structure, especially the presence and location of the hydroxyl (–OH) substitutions and the catechol-type Bring Rice-Evans *et al.* (1996); Wang *et al.* (2006) [20, 26]. The structural properties of a potent antioxidant capacity is due to the presence of (i) an ortho-dihydroxy or catechol group in the B-ring, (ii) a 2,3-double bond, and (iii) hydroxyl substitution at positions 3 and 5 Bors *et al.* (1990) [6]. Growing evidence has demonstrated that quercetin, which is featured by a hydroxylation form of 3, 5, 7, 30, and 40 and a catechol B-ring, contains all the structural properties of an antioxidant agent Silva *et al*, (2002); Rietjens *et al*. (2005) [24, 21].

Sumit Arora and Prakash Itankar (2018) [25] showed the extraction, isolation and identification of flavonoid from *Chenopodium album* aerial parts. The flavonoids contained in *C. album* aerial parts were extracted, identified and characterized. Sequential soxhlet extraction was subjected to preliminary phytochemical screening and flavonoid quantification. The results showed that maximum yield of the flavonoid (7.335 mg/g) were obtained from acetoneextr act. This acetone extract was subjected to flash chromatography for isolation of flavonoid. Characterization of isolated flavonoid was done by UV, IR, ^{1}H & ^{13}C NMR and MS. On the basis of chemical and spectral analysis structure was elucidated as 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one, a flavonoid.

Selvaraj *et al*. (2013) [22] designed for isolation of bioactive polyphenolic compounds from methanol extract of *Azolla micr*o phylla and their subsequent characterization. The flavonoid compounds were isolated and characterized by using thin layer chromatography (TLC), purified by preparative thin layer chromatography (PTLC) and were identified using High performance chromatography (HPLC). Their structures and chemical bonds were analyzed using Ultraviolet-Visible spectrophotomery (UV spec), Fourier Transform-Infra Red spectroscopy (FTIR) and Nuclear magnetic resonance NMR (^{13}Cand ^{1}H) techniques. Results: Two flavonoids were identified as rutin and quercetin. The isolated compounds showed a potent antioxidant radical scavenging activity, as assessed by non-physiological assays like DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS and FRAP (Ferric reducing antioxidant power). For the first time rutin and quercetin have been isolated successfully from the macrophyte aquatic fern *Azolla microphylla* under the present study.

Ahmadu *et al*. (2009) [2] examined flavonoid glycosides from *Byrsocarpus coccineus* leaves. Schum and thonn (Connaraceae). The bioactive ethyl acetate and N-butanol soluble parts of an ethanolic extract of *Byrsocarpus coccineus* leaves was subjected to column chromatography over silica gel G (60 - 120µ) and repeated purification of the flavonoid

rich fraction over sephadex LH-20 eluted with methanol led to the isolation of three flavonoid glycosides identified as quercetin 3-O-α-arabinoside (I), quercetin (II) and quercetin 3-β-D-glucoside.

Conclusion

In this study concluded that based on ^{1}H- NMR and ^{13}C – NMR study reveals that the structure of Quercetin (3, 3', 4', 5, 7 – pentahydroxyflavanone) was identified in *Phoenix pusilla* fruits. Quercetin, a member of the flavonoids family, is one of the most prominent dietary antioxidants. Especially the ability of quercetin to scavenge highly reactive species such as peroxynitrite and the hydroxyl radical is suggested to be involved in these possible beneficial health effects. All these data obtained in the present investigation supported by traditional claim associated with *Phoenix pusilla* fruits.

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