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Identification of Flavonoids in Methanolic Extract of *Caryota urens* (Fish Tail Palm): A Phytochemical Screening Involving Structure Analysis by FTIR Spectroscopy

Amit Kumar Srivastav, Raghvendra Singh, S. Manimegalai and V. Devi Rajeswari
School of Biosciences and Technology, VIT University, Vellore, 14, India

Corresponding Author: V. Devi Rajeswari, School of Biosciences and Technology, VIT University, Vellore, 14, India

ABSTRACT

This study is to delineate the isolation of active compound from the plant fish tail palm (*Caryota urens*). A methanol extract of the dry pulverized fruit pulp of *Caryota urens* was obtained by the Soxhlet cold extraction method. The extract was subjected to a preliminary phytochemical screening by standard methods. Thin layer chromatography was performed to confirm the number of compounds present in the methanol extract by using three different solvent systems. High performance liquid chromatography was performed with the respective standards. To separate the compound by using silica gel column chromatography with methanol and phosphoric acid as mobile phase. The structure of compound was elucidated by NMR and FTIR spectrum. The different frequencies of the peaks in FTIR spectrum is found to fall in the same range of frequencies of functional groups which is present in rutin.

Key words: *Caryota urens*, active compound, rutin, plant pigment

INTRODUCTION

Palms are the most beneficial plants to people in the Tropics. The botanical name of Fishtail palm is *Caryota urens* which name gets because of the present of stinging chemicals known as 'Urens' in the fruit (Vaishnavi and Suneetha, 2013). The *Caryota urens* is a member of the family Arecaceae. They are commonly called fish tail palm, toddy palm, wine palm or jiggery palm. The sap of fishtail palm is sweet in nature. So, it is used to produce sugar which is known as jaggery and also to make palm wine (Toddy) (Namoff *et al*., 2011). These species are commonly found in Asia especially from India to Myanmar and some islands country like Sri Lanka. *Caryota urens* flower is used to treat gastric ulcer, migraine headaches, snake bite poisoning as well as rheumatic swellings (Kumar *et al*., 2013).

Caryota urens species are well known for their medicinal role and are used as ancient medicine to treat hemicranias and rheumatic swelling. Ancient medicine technologies recommend these flowers of the trees are used as a home remedy and improve the hair growth. The roots of the trees are used as the tooth ailments. The fruit skin and immature fruit of *Caryota urens* is known to have antibacterial activity against the tested pathogens (*Escherichia coli*, *Vibrio cholerae*, *Salmonella typhii*, *Staphylococcus aureus* and *Shigella flexneri*). Its leaf extract is also well known for exhibiting antioxidant activity. Root bark and the terminal bud is employed for treating rheumatic swellings and snake bite. The qualitative and quantitative studies of bioactive compounds from plant materials mostly rely on the selection of proper extraction method. Extraction is the first step of any medicinal plant study, plays a significant and crucial role on the final result and outcome

(Jeyadevi *et al*., 2013). This project objective was to determine the active species in the fruit sample of *Caryota urens* both qualitatively and quantitatively. The main challenge was to analyse the active phytochemical compound in the fruit sample of *Caryota urens*.

MATERIALS AND METHODS

Sample collection: The fruits of fish tail palm plant were collected from VIT University, Vellore campus during the month of February. The brown colour fruit was oval in shape and contains significant moisture in its pulp.

Preparation of plant extract: The dried fruit pulp (250 g) of *Caryota urens* was extracted with methanol (500 mL) using Soxhlet apparatus. The liquid was then filtered and kept in hot air oven at 65°C for 8 h to get more solid extract. Then the methanol extract was concentrated and weighed (10 g). The dry extract was stored at 4°C until used.

Phytochemical analysis: Phytochemical screening of methanol extract of *Caryota urens* was subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as reducing sugar, proteins, alkaloids, Phenols, tannins, saponins, glycosides, flavonoids, terpenes and steroids etc., using standard procedures (Hossain *et al*., 2013).

Thin layer chromatography: Thin layer chromatography (Revathi *et al*., 2010) was performed to know the number of active compound is present in sample. Thin-layer chromatography is performed on aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. Three solvents methanol and phosphoric acid (pH 3), water and phosphoric acid (pH 3) and methanol were used as mobile phase to run the extract.

High performance liquid chromatography: High performance liquid chromatography (Shaik *et al*., 2012), was performed to separate the components in the crude extract. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. The extract was mixed with the solvent consist of methanol and phosphoric acid (pH 3) and HPLC grade methanol was used as a mobile phase to perform HPLC. Oxalic acid and maleic acid are used as standards. The UV detector was set at 214 nm and the flow rate of mobile phase was 0.7 mL min^{-1}.

Column chromatography: Column chromatography method (Sivakumar *et al*., 2012) used to purify individual compounds from mixtures of compounds. Sample was prepared by mixing the plant extract with silica and then keeping it in water bath at 60°C for 1 h. The mixture was then dried to make it in powder form so that it could be used in column chromatography. Column is first washed with solvent (methanol) and then one fourth of column is filled with solvent (methanol) followed by stationary phase silica is added in column carefully so that there is no gap in between the silica layers. Column was continuously tapped so that silica loading could be done properly without any gap in layers. After silica loading, all the sample was poured very slowly and carefully

in column and then mobile phase consisted of methanol plus phosphoric acid was added in column to run the column. After addition of mobile phase, the loaded sample was allowed to run by opening the tap on the bottom. The different components in sample interacted with stationary phase differently and ran in the stationary phase at different velocity. The different components of sample was collected in 20 different test tubes. All the 20 fraction collected was ran on TLC plate with solvent methanol plus phosphoric acid (pH 3) to identify if any active compound is present in the any of the fractions.

Nuclear magnetic resonance: The NMR analysis was done to elucidate the structure of compound obtained in fraction after column chromatography. The solubility of the fraction was checked in water. When it was found soluble, the extract was mixed in deuterium water and given for NMR testing (Goodman *et al*., 1992).

Fourier transform infrared spectroscopy: The FTIR analysis of the extract was done to confirm the functional group of present in the compound (Chua, 2013).

RESULTS

Phytochemical screening of the leaf extracts of *Caryota urens* confirmed the presence of organic acids, phenols, flavonoids and reducing sugars (Table 1). Thin layer chromatography A clear spot was observed on TLC plate which confirms the presence of at least one active compound in the extract (Fig. 1). In high performance liquid chromatography, the retention time of standard oxalic acid and standard malic acid was obtained 2.90 and 2.99, respectively (Fig. 2-3). The retention time of extract (sample) was obtained 2.599 (Fig. 4) which does not match with any of the standard

Table 1: Phytochemical screening of the fruit pulp extracts of *Caryota urens*

Phytochemical tests	Qualitative analysis	Results
Organic acid	Oxalic acid test	+
Carbohydrates	Benedict's test	+
Proteins and amino acid	Lead acetate test	-
Glycoside	Borntrager's test	-
Saponins	Foam test	-
Steroids and sterols	Salkowski test	++
Phenols	Lead acetate test	+
Flavonoids	Sodium hydroxide test	++

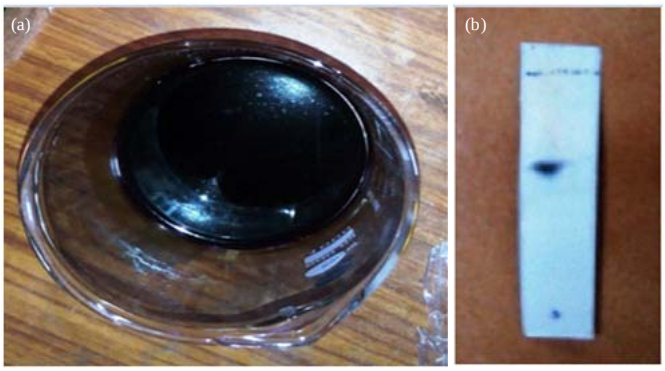
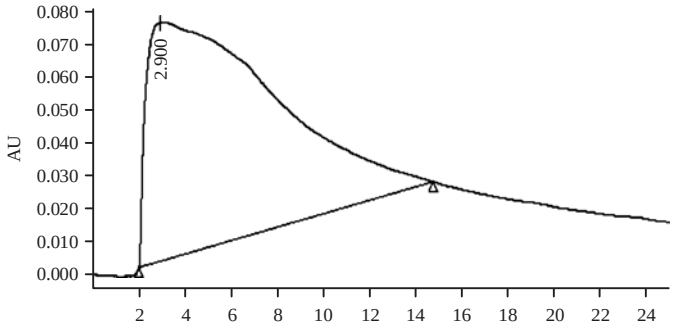


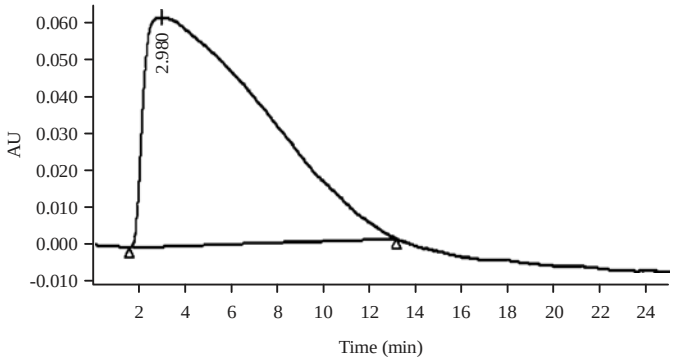
Fig. 1(a-b): Fractionation of crude methanol extract of *Caryota urens* using silica coated TLC using (a) Methanol and (b) Phosphoric acid as mobile phase



Peak results

	Name	RT	Area	Height	Amount	Units
1		2.900	27657306	72358		

Fig. 2: HPLC chromatogram of malic acid standard



Peak results

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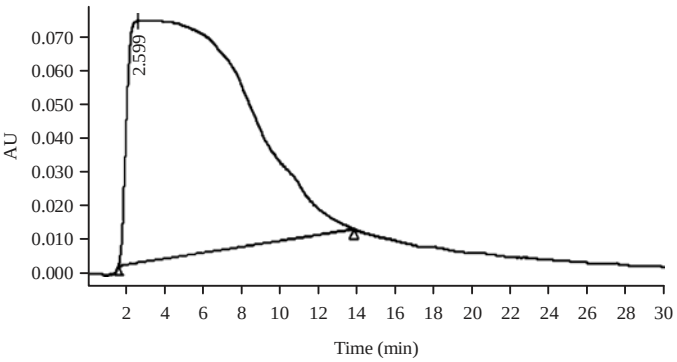
1		2.980	22041939	61497		

Fig. 3: HPLC chromatogram of Oxalic acid standard



Peak results

	Name	RT	Area	Height	Amount	Units
1		2.599	30103765	71735		

Fig. 4: HPLC chromatogram of *Caryota urens* fruit pulp extract

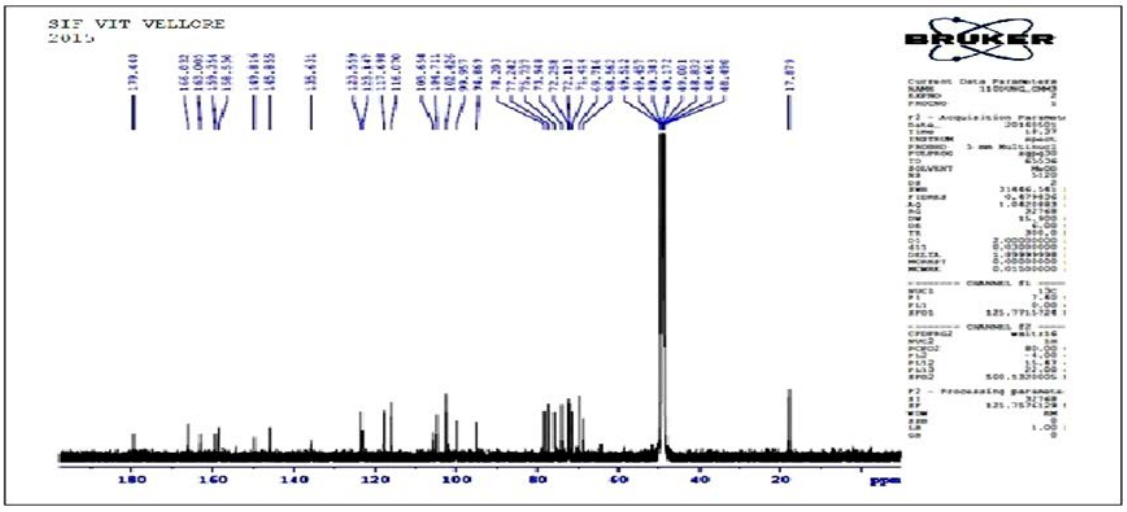


Fig. 5: NMR-C13 spectra (600 MHz) of *Caryota urens* fruit extracts from four different geographical origins

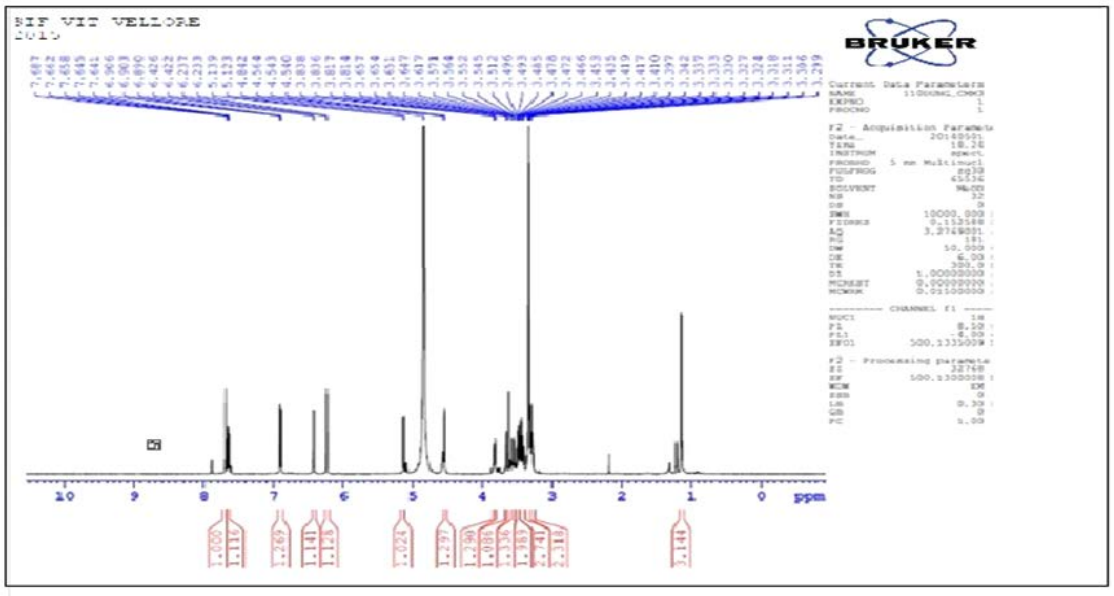


Fig. 6: NMR H1 Spectrum of *Caryota urens* fruit extracts

which confirms that already reported compound in the extract is neither oxalic acid nor malic acid. Column chromatography after column run 20 different fractions was collected in different test tubes which were expected to have different compound in each fractions. To confirm any active compound TLC was performed for all the fraction. Only one fraction gives a clear band which confirms the presence of active compound in that fraction. The NMR analysis was done to elucidate the structure of compound obtained in fraction after column chromatography (Fig. 5-10). The 1H NMR spectrum (500 MHz) shows two ortho-coupling aromatic protons at δH 7.65 ppm (1H) and

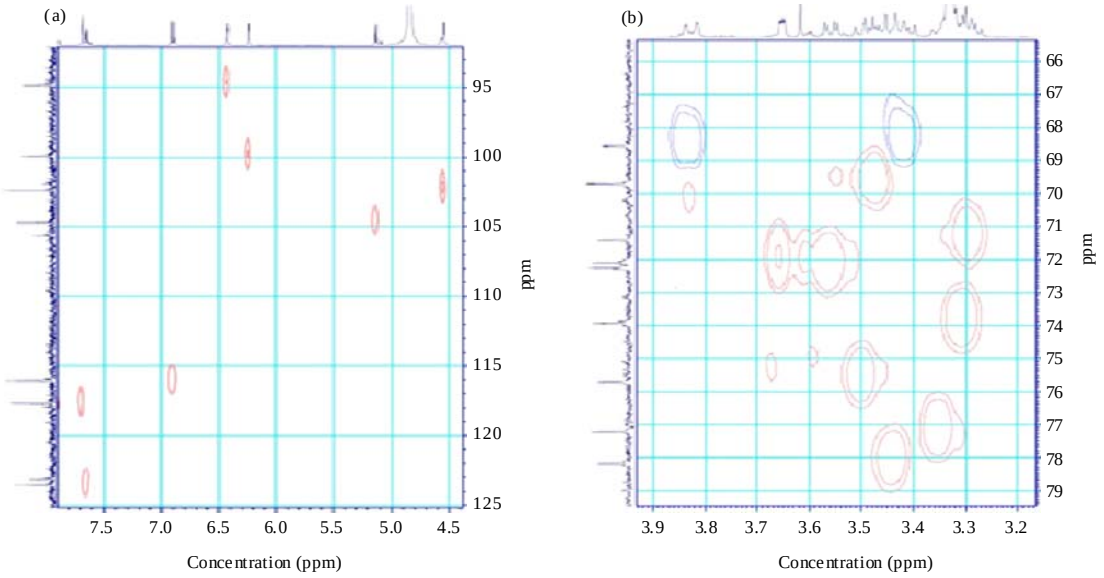


Fig. 7(a-b): NMR HSQC spectrum of *Caryota urens* fruit extracts

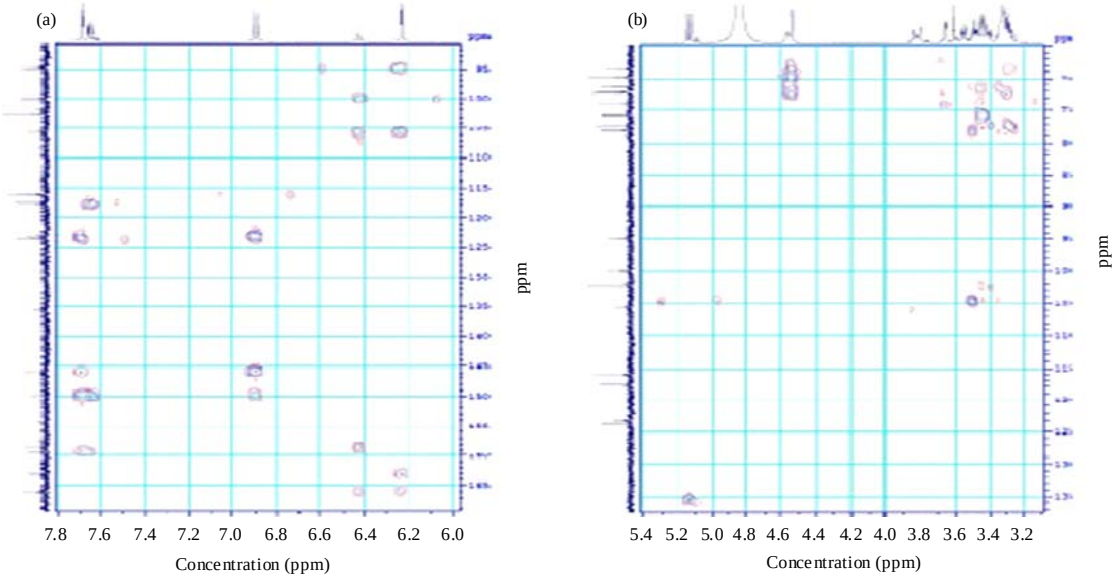


Fig. 8(a-b): NMR HMBC spectrum of *Caryota urens* fruit extracts

δH 6.90 ppm (1H), two meta-coupling aromatic protons at δH 6.23 ppm (1H) and δH 6.42 ppm (1H) and a singlet aromatic proton at δH 7.69 ppm (1H). The 1HNMR spectrum also supported the presence of two sugar moieties with the anomeric proton signals at δH 5.13 ppm (1H, d) and δH 4.54 ppm (1H). The coupling constants (J = 8 Hz and J = 6.3 Hz) of two anomeric protons certified the α-linkage of rhamnose. A doublet of methyl group of rhamnose was observed at high field δH 1.13 ppm (3H). The rest of protons in the sugar moiety resonated between 3.27 and 3.51 ppm (Table 2).

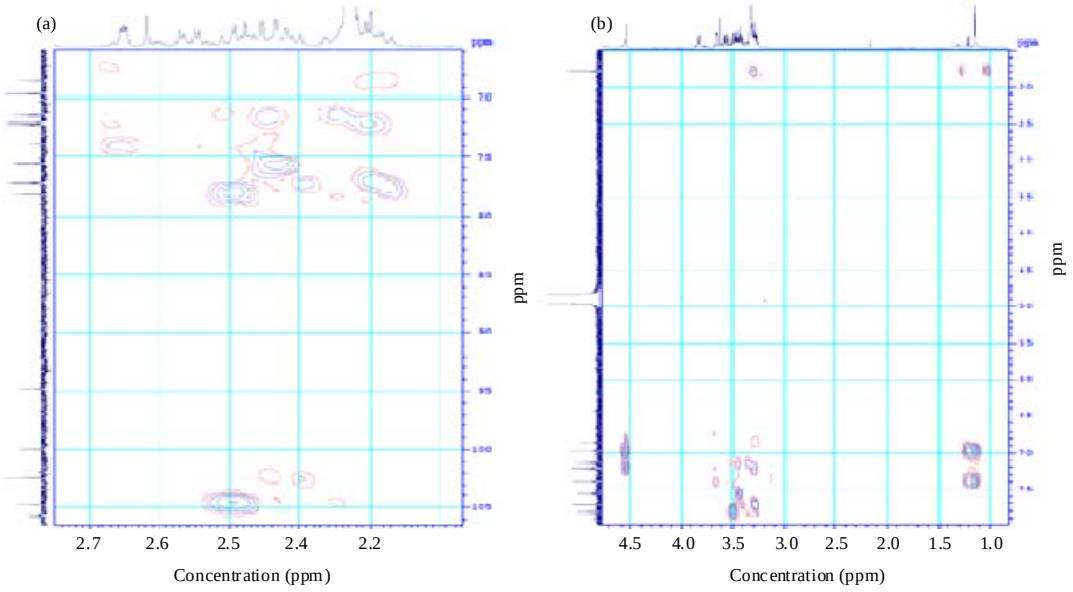


Fig. 9(a-b): NMR-HMBC spectrum of *Caryota urens* fruit extracts

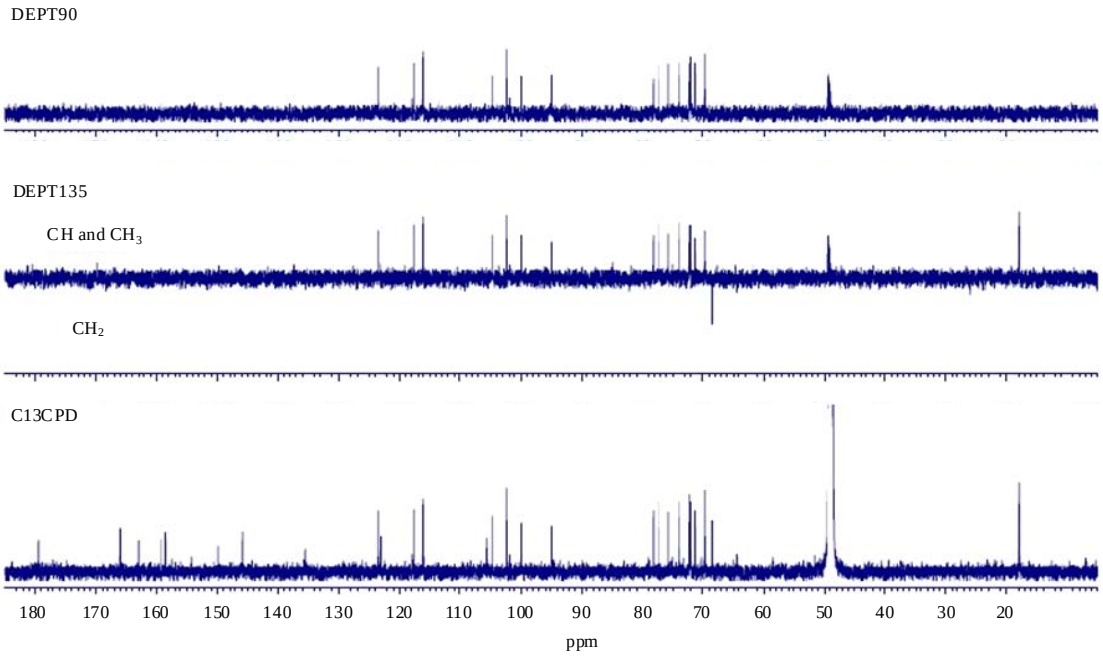


Fig. 10: DEPT spectrum of *Caryota urens* fruit extracts

The 13C NMR spectrum showed 27 carbon signals. The DEPT spectrum of the isolated compound, revealed the presence of one methyl carbon (δC = 17.9 ppm, C-6''') of rhamnose, one methylene carbon (δC = 68.6 ppm, C-6''), 15 methine carbons and 10 quaternary carbons. In FTIR spectrum, different peaks were obtained at different frequencies, The peaks in spectrum shows the presence of different functional groups of the found compound (Fig. 11).

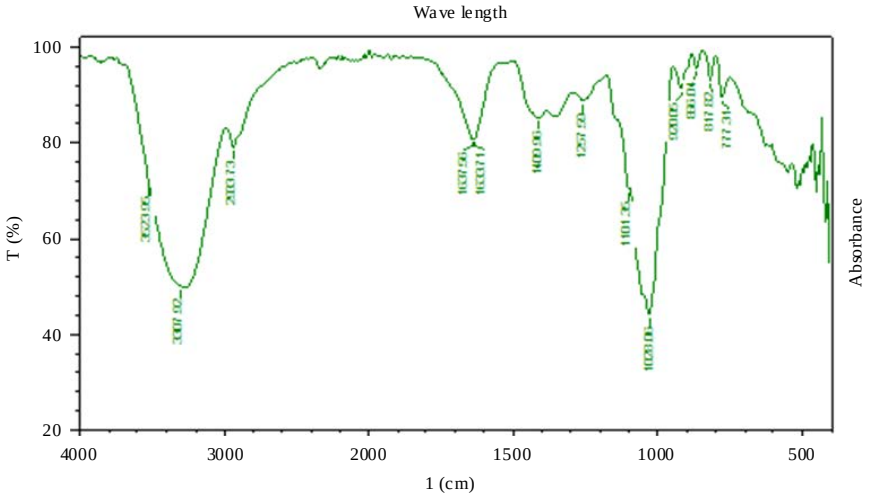


Fig. 11: FTIR spectrum

Table 2: Comparison of ^{1}H-and ^{13}C-NMR data of compound obtained and Rutin

		Active compound		Rutin	
Position	DEPT	δH ppm 500 Mhz	δC ppm	δH ppm	δC ppm
Aglycone					
6	= CH^{-}	6.24	99.8	6.23	99.9
6	= CH^{-}	7.63	123.5	7.65	123.6
8	= CH^{-}	6.42	94.9	6.41	94.9
2'	= CH^{-}	7.71	117.6	7.69	117.7
5-OH					
Glc					
1"	>CH^{-}	5,13	104.6	5.13	104.7
Rha	= CH^{-}				
3"'	>CH^{-}	3,56	72.2	3.55	72.2
6"'	-CH_3^{-}	1.10	17.9	1.13	17.9

DISCUSSION

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in vegetables, fruit, grains, roots, bark, stems, flowers, wine and tea. These natural products were known for their favourable effects on human health and long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been isolated and characterized. Many of which are responsible for the attractive colours of flowers, fruit and leaves. The association between flavonoid intake and the long term effects on mortality was studied subsequently (Sulaiman and Balachandran, 2012).

Isolation of compound from the plant fish tail palm (*Caryota urens*) was accomplished by preparing its extract, getting a fraction containing compound by silica gel chromatography, confirmation of compound by TLC result and structure of compound by NMR and FTIR analysis. The structure of compound was elucidated by NMR and FTIR spectrum. The different peaks and graphs obtained in NMR spectrum and HSQC, HSBC and DEPT reports of NMR was studied by NMR library which shows that the characteristic of the obtained compound is in close similarity with the compound Rutin. The different frequencies of the peaks in FTIR spectrum is found to fall in same range of frequencies of functional groups which is present in Rutin. The plant pigment

(flavonoid) that is found in certain fruits and vegetables. Rutin is used to make medicine. Rutin is a common dietary flavonoid that is widely consumed from plant derived beverages and foods as traditional and folkloric medicine worldwide. Rutin is believed to exhibit significant pharmacological activities, including anti-oxidation, anti-inflammation, anti-diabetic, anti-adipogenic, neuroprotective and hormone therapy (Selloum *et al*., 2003). Oligorutin has also elicited antimutagenic potential (Rhouma *et al*., 2012). Rutin is known to illustrate positive effect on spatial memory as well as the concentration of brain neurotransmitters in aged rats (Xua *et al*., 2014). Aging is also affected during diabetes by treatment of rutin (Binic *et al*., 2013). The current work was focused to isolate flavonoid, rutin. The results of the studies are encouraging but more systematic studies are required to confirm these effects.

REFERENCES

Binic, I., V. Lazarevic, M. Ljubenovic, J. Mojsa and D. Sokolovic, 2013. Skin ageing: Natural weapons and strategies. Evidence-Based Complement. Altern. Med. 10.1155/2013/827248

Chua, L.S., 2013. A review on plant-based rutin extraction methods and its pharmacological activities. J. Ethnopharmacol., 150: 805-817.

Goodman, B.A., B. Williamson and J.A. Chudek, 1992. Nuclear Magnetic Resonance (NMR) microimaging of raspberry fruit: Further studies on the origin of the image. New Phytol., 122: 529-535.

Hossain, M.A., K.A.S. AL-Raqmi, Z.H. AL-Mijizy, A.M. Weli and Q. Al-Riyami, 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pac. J. Trop. Biomed., 3: 705-710.

Jeyadevi, R., T. Sivasudha, A. Ilavarasi and N. Thajuddin, 2013. Chemical constituents and antimicrobial activity of Indian green leafy vegetable *Cardiospermum halicacabum*. Indian J. Microbiol., 53: 208-213.

Kumar, K.P.P., V.S.S. Shankar, R. Deepak, V. Suneetha and B. Mishra, 2013. A report on rubber degrading bacterial Sps. from vellore soil contaminated with tyre waste. Res. J. Pharmaceut. Biol. Chem. Sci., 4: 45-51.

Namoff, S., A. Veloz, F. Jimenez, R.A. Rodriguez-Pena and B. Peguero *et al*., 2011. Sweet drinks are made of this: Conservation genetics of an endemic palm species from the Dominican Republic. J. Heredity, 102: 1-10.

Revathi, R., R. Ravi, V.S. Saravanan, T. Ethira and T. Sudhamani, 2010. Isolation and characterisation of (-) hydroxy citric acid from *Garcinia cambogia* fruit extract. Int. J. Pharmacol. Res. Dev., 2: 1-6.

Rhouma, G.B., L. Chebil, M. Krifa, M. Ghoul and L. Chekir-Ghedira, 2012. Evaluation of mutagenic and antimutagenic activities of oligorutin and oligoesculin. Food Chem., 135: 1700-1707.

Selloum, L., H. Bouriche, C. Tigrine and C. Boudoukha, 2003. Anti-inflammatory effect of rutin on rat paw oedema and on neutrophils chemotaxis and degranulation. Exp. Toxicol. Pathol., 54: 313-318.

Shaik, A.H., S.N. Rasool, A.V.K. Reddy, M.A. Kareem, G.S. Krushna and K.L. Devi, 2012. Cardioprotective effect of HPLC standardized ethanolic extract of *Terminalia pallida* fruits against isoproterenol-induced myocardial infarction in albino rats. J. Ethnopharmacol., 141: 33-40.

Sivakumar, P., C. Nethradevi and S. Renganathan, 2012. Synthesis of silver nanoparticles using *Lantana camara* fruit extract and its effect on pathogens. Asian J. Pharma. Clin. Res., 5: 97-101.

Sulaiman, C.T. and I. Balachandran, 2012. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J. Pharm. Sci., 74: 258-260.

Vaishnavi, R. and V. Suneetha, 2013. Phytochemical analysis on *Caryota urens* (fishtail palm) fruit from VIT university campus for pharmaceutical use. Der Pharm Lett., 5: 71-75.

Xu, P.X., S.W. Wang, X.L. Yu, Y.J. Su and T. Wang *et al*., 2014. Rutin improves spatial memory in Alzheimer's disease transgenic mice by reducing Aβ oligomer level and attenuating oxidative stress and neuroinflammation. Behav. Brain Res., 264: 173-180.