DOI: 10.15415/jce.2016.22008

Flavonoids of *Calophyllum inophyllum Linnaeus.* C. von

K. SHAKILA[1*]

[1]Assistant professor of Chemistry, Bharathiyar College of Engg & Tech., Karaikal. 609-609

***Email: shakila.manickaraj@gmail.com**

Received: November 16, 2015| Revised: November 25, 2015| Accepted: December 28, 2015

Published online: March 14, 2016

Abstract : Calphyllum inophyllum *Linnaeus C.von (Syn.)* Calophyllum bintagar *Roxb of Guttifereae (Clusiaceae)* is commonly known as ' Indian laurel' or 'Alexandhian laurel' a broad leaved evergreen tree occurring as a littoral species along the beach crests, sometimes seen inland . It is widely distributed and cultivated throughout tropics from West Africa to Pacific islands (as far) Eastas Tahiti. It blossoms during March to October. Fresh flowers of calophyllum inophyllum have been treated for their flavonoids contents. They are rich flavonol quercetin and flavonol glycosides quercitrin. They have been characterized with the help of modern physical methods like UV, H-1 NMR , C-13 NMR, chemical reactions, chromatographic techniques and hydrolytic studies.

Keywords: Clusiaceae; Quercetin; Quercitrin.

1. INTRODUCTION

The antibacterial / antineoplastic, anti-inflammatory, antiplatelet, antipsychotic, antiviral and photoprotection have been observed in C. Inophyllum[1]. The bark of the plant has astringent activities and its decoction is used to cure the diarrhoea and dysentery. However, the juice of the bark and the fruits are also found to be purgative. The latex of the palnt is also an emetic [10-11]. Investigations on cancer chemopreventive acivity of 4 – phenylcoumarins from C. inophyllum establishes the property of the species[2] . Examinations on healing of ocular burns shows considerable activity of C. Inophyllum[3]. The cytoprotective effects of C. inophyllum against UV induced damage show the efficacy of the species[4]. The toxicities have also been studied in detail. Myricetin 7 - glucoside has been isolated from C. Inophyllum[12]. With a view to locating additional flavonoids, the flowers of C. Inophyllum have been investigated and the results are presented hereunder.

Journal of Chemistry, Environmental Sciences and its Applications
Vol - 2, No - 2
March 2016
pp. 135–144
CHITKARA UNIVERSITY

2. EXPERIMENTAL

2.1. Extraction and fractionation

Fresh flowers (1 kg) of C. inophyllum collected from Thirukalachery, in Nagapattinam District, during September were extracted with 90% MeOH (4 × 500 ml) under reflux. The specimen for the C. inophyllum is kept at Rapinat Herbarium and Centre for Molecular Systematics, St. Josheph's college (Campus), Tiruchirappalli- 620 002, the specimen number being SA 010. The alc. extract was concentrated in vacuo and the aq. concentrate successively fractionated with petrol (b.p. 60 - 80°C) (3 × 250 ml), peroxide free Et_2O (3 × 250 ml) and EtOAc (4 × 250 ml).

The petrol and Et_2O fractions did not yield any isolable material.

2.2. Et_2O fraction: [flavonols - quercetin]

The Et_2O fraction was concentrated *in vacuo* and left in the ice chest for about a week. A yellow solid that separated was filtered and studied. It came out as pale yellow needles m.pt. 316-318°C yield (0.01%) on recrystallisation from MeOH. It was soluble in organic solvents and sparingly in hot water. It gave red colour with Mg-HCl, Olive – green colour with alc. Fe^{3+}, golden yellow colour with NH_3 and NaOH, yellow solution with a pale green flurescence with conc. H_2SO_4 and appeared yellow under UV with or without ammonia. It reduced ammonical $AgNO_3$ (in hot) and Fehling's solution. It answered the Horhammer – Hansel[5] , Wilson's boric acid[6] and Gibb's tests[7-8]. It did not answered Molisch's tests. It had nm 255, 269sh, 301sh, 370; +NaOMe 247sh, 321(dec.); +$AlCl_3$ 272, 304sh, 333, 458; + ($AlCl_3$ – HCl) 265, 301sh,

Table: 1 hRf [Rf × 100) Values of the constituents of the flowers of *C. Inophyllum*

(Whatman No 1, Ascending, 30 ± 2°C)

Compound	Mobile Phases								
	a	b	c	d	e	f	g	h	i
Aglycone the Et_2O fraction	–	01	04	17	38	85	39	72	48
Quercetin (authentic)	–	01	04	17	38	85	39	72	48

Solvent Key : a – H_2O ; b – 5% aq. HOAc ; c – 15% aq. HOAc ; d – 30% aq. HOAc; e – 60% aq. HOAc; f-n- BuOH: HOAc: H_2O = 4:1:5(upper Phase); g- phenol saturated with water; h –t- BuOH: HOAc: H_2O = 3:1:1; i – Forestal = HOAc: Conc. HCl: H_2O = 30: 3: 10 j - Formic = EtOAc: HCOOH: H_2O = 10: 2: 3

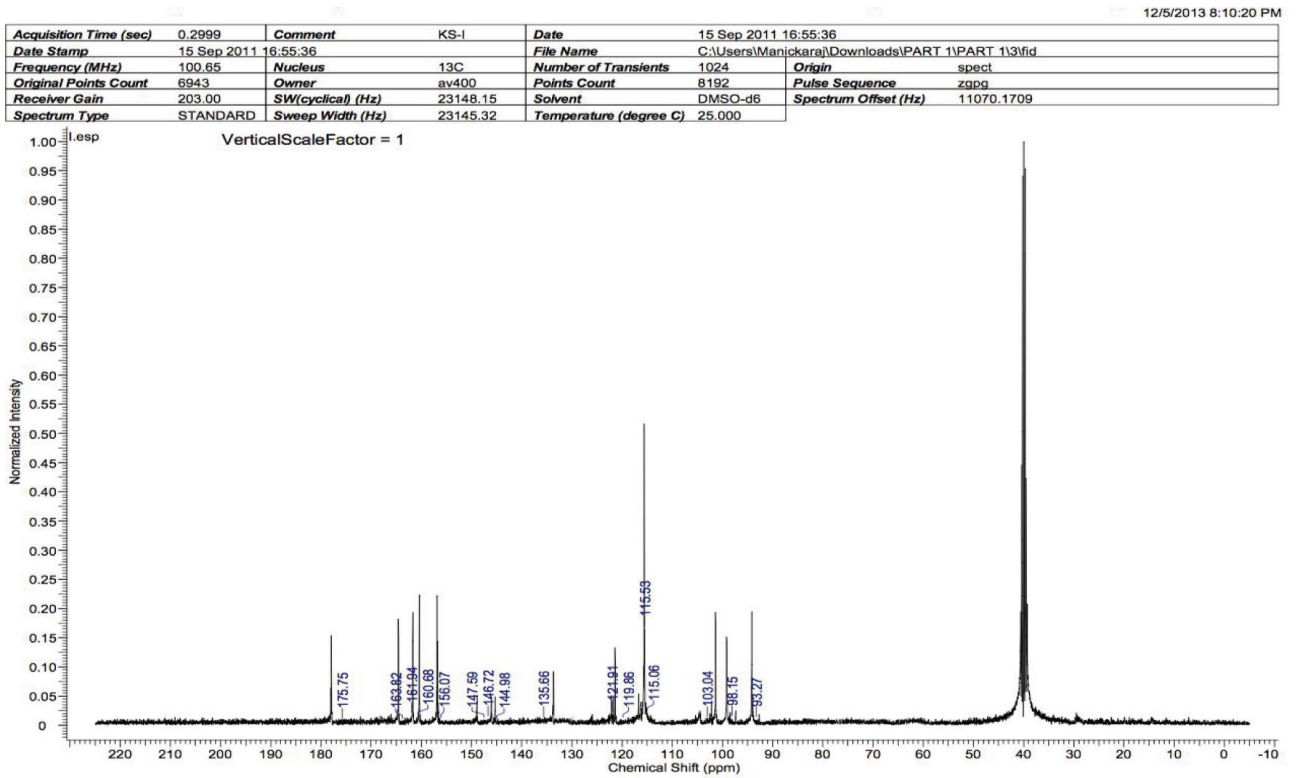

Fig. 1: This report was created by ACD/NMR Processor Academic Edition. For more informaition go to www.acdlabs.com/nmrproc/

359, 428; + NaOAc 257sh, 274, 329, 390(dec) and + (NaOAc – H_3BO_3) 261, 303 sh, 388 and had R_f values as depicted in Table.1. ^{13}C NMR of the flavonol is appended (Fig.1). It was identified as quercetin and the same was confirmed by co – and mixed – PC and m.m.p with an authentic sample of quercetin from *Physalis minima*[9].

2.3. Preparation of acetyl derivative:

The glycoside (0.05g) was dissolved in freshly distilled acetic anhydride (2 ml). It was treated with fused anhydrous sodium acetate (2 g) and the mixture gently refluxed at 140°C for about 2 hr. The reaction mixture was then poured in a thin stream with constant stirring into excess of ice water containing crushed ice. The crude acetate that separated was filtered and recrystallised from EtOAc. It gave a pentaacetate, m.pt. 200 - 201°C.

2.4. Preparation of benzoyl derivative

The pigment (0.05 g) was dissolved in AR benzoylchloride (1 ml) and treated with aq. NaOH (1 ml) in a conical flask. After the addition of benzoyl chloride, the conical flask was shaken vigorously for about 15 – 20 m till there were no smells of benzoylchloride. The separation of a brown solid indicated the completion of the reaction. When the reaction was completed, the crude benzoate that separated out was filtered and recrystallised from EtOAc. It gave a pentabenzoate derivative, m.p 200 – 01°C.

2.5. EtOAc fraction: (quercitrin)

The EtOAc fraction was concentrated *in vacuo* and left in an ice – chest for a few days. A yellow solid that separated was filtered and studied.

Table: 2 hRf [Rf × 100) values of the constituents of the flowers of C. Inophyllum

(Whatman No: 1, Ascending, 30 ± 2° C)

Compound	**Mobile Phases**								
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
Gycoside from EtOAc fraction	42	34	53	74	80	82	65	83	81
Quercetin 3-rhamnoside (authentic)	42	35	53	74	80	83	65	89	81

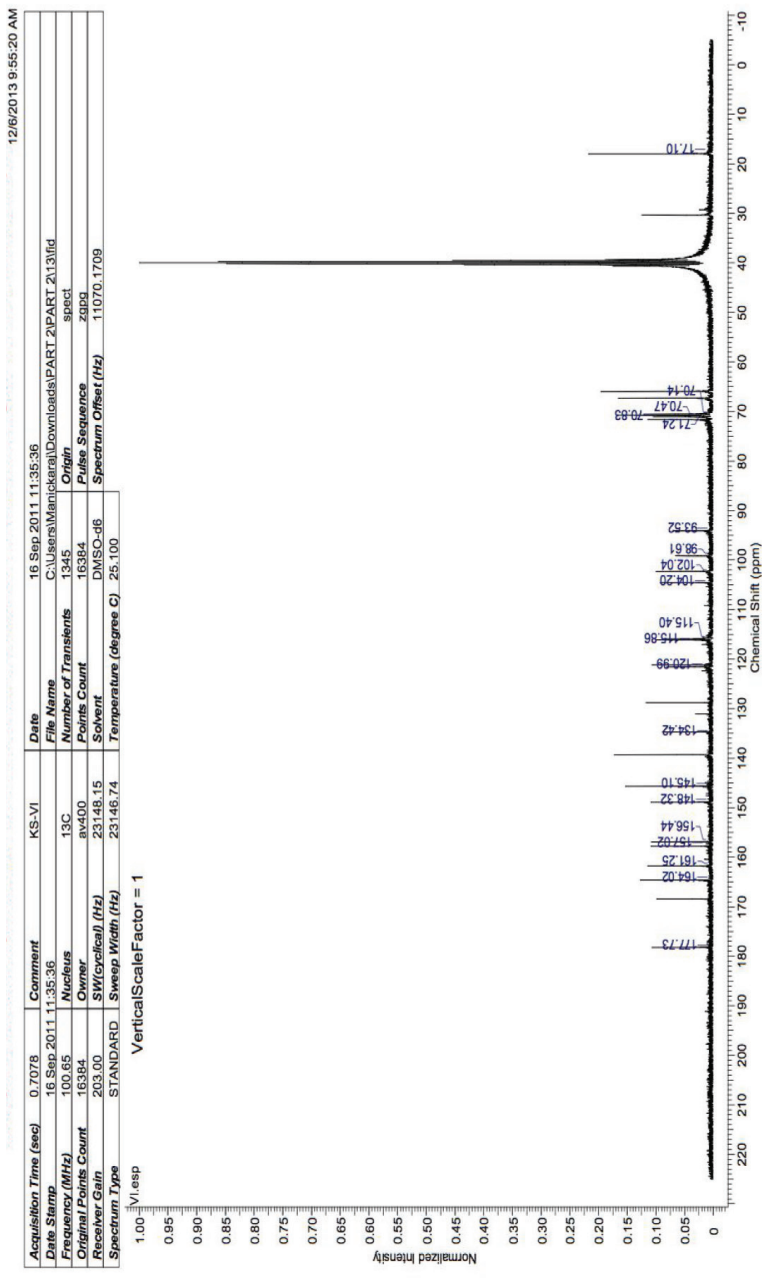

Fig. 2: This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

Table: 3 hRf [x 100) values of the constituents of the flowers of *C. Inophyllum*

(Whatman No: 1, Ascending, 30 ± 2˚ C)

Compound	Mobile Phases			
	e	f	g	i
Sugar from the Gycoside from EtOAc fraction	76	30	60	92
Rhamnose (authentic)	76	30	60	91

It came out as pale yellow leaflets, m.p. 182 - 85° C (yield 0.05%) on recrystallisation from MeOH. It was soluble in EtOH and EtOAc but insoluble in cold water. It developed green colour with Fe^{3+}, pink colour with Mg^{+2}- HCl and a yellow precipitate with aq. (basic) lead acetate. It reduced ammonical silver nitrate solution but not Fehling's solution. It appeared deep, purple under UV that turned yellowish green on exposure to NH_3. It responded to wilson's boric acid, Molisch's and Gibb's tests, but did not answer the Horhammer – Hansel test. It had nm 256, 265sh, 301sh, 350; +NaOMe 270, 326, 393; +($AlCl_3$ – HCl) 272,303sh, 353, 401; + NaOAc 272,322sh, 372, and +(NaOAc – H_3BO_3) 260, 300sh, 367. It had R_f values as depicted in Table.2. The ^{13}C NMR spectra of the glycoside is appended (Fig.2).

2.6. Hydrolysis of the glycoside

The glycoside (0.05g, 0.1 m mole) was dissolved in hot aq. MeOH (2ml, 50%) and also was hydrolysed with H_2SO_4 (5%) at 100°C for about 2 h and the hydrolytic products identified as described below.

2.7. Identification of aglycone: (Flavonol: quercetin)

The aglycone on crystallisation (Me_2CO), gave yellow needles, m.p. 316 - 318°C. The aglycone that resulted was characterised as quercetin, by comparing it with an authentic sample isolated from Physalis minima.

2.8. Identification of Sugar: (rhamnose)

The concentrated filtrate from the neutralized aq. hydrolysate when examined by PC gave R_f values (Table.3) corresponding to those of rhamnose. The identity of the sugar was also confirmed by direct comparison with an authentic sample

Table 4: 13c nmr spectral data their assignment for the aglycone from the flowers of *C. Inophyllum*

Compound	C2	C3	C4	C5	C6	C7	C8	C9	C10	C1'	C2'	C3'	C4'	C5'	C6'
Quercetin (from Literature)	146.77	135.61	175.75	160.65	98.10	163.80	93.25	156.09	103.0	121.91	115.04	144.97	147.61	115.53	119.90
Aglycone from Et_2O fraction (δppm)	146.72	135.66	175.75	160.68	98.15	163.82	93.27	156.07	103.04	121.91	115.06	144.98	147.59	115.53	119.86

Table 5: 13c nmr spectral data and their assignment for the glycoside from the flowers of *C. Inophyllum*

Compound	C2	C3	C4	C5	C6	C7	C8	C9	C10	C1'	C2'	C3'	C4'	C5'	C6'
Quercitrin (from Literature)	156.4	134.4	177.7	161.2	98.6	164.0	93.5	157.0	104.2	121.0	115.4	145.1	148.3	115.8	121.0
Glucoside from EtOAc fraction (δ ppm)	156.4	134.4	177.7	161.2	98.6	164.0	93.5	157.0	104.2	121.0	115.4	145.1	148.3	115.8	120.0

Continue Table 5

Compound	C1''	C6''	C6''	C6''	C6''	C6''
Quercitrin (from Literature)	102.0	70.4	70.0	71.2	70.1	17.1
Glucoside from EtOAc fraction (δ ppm)	101.7	70.4	70.5	71.2	70.1	17.4

of rhamnose. A quantitative hydrolysis revealed the aglycone sugar ratio to be 1:1 confirming the presence of a monoside.

The glycoside was therefore identified as quercetin – 3- rhamnoside and confirmed by co – and mixed – PC with an authentic sample of quercetin – 3-rhamnoside isolated from *Leucaena leucocephala.*

3. Results and Discussion

The fresh flowers of *C. inophyllum* have been found to contain quercetin and its glycoside (quercitrin).

The UV spectrum of the Et_2O soluble exhibited λ_{max} at 370 nm (band I) and 255 nm (band II) indicating a flavonol skeleton. A bathochromic shift of +58 nm on the addition of $AlCl_3$ – HCl indicates the presence of free –OH at C-5 in A ring[13]. A comparison of $AlCl_3$ and $AlCl_3$ – HCl spectrum revealed an additional bathochromic shift of 30nm in the case of $AlCl_3$ spectrum (without acid) which again points to the presence of catechol type of B –ring. It is further substantiated by the bathochromic shift of 18 nm noticed in band I on the addition of H_3BO_3. The presence of a free –OH at C-7 is evident from the bathochromic shift of 19nm in band II on the addition of NaOAc.

In the ^1H –NMR spectrum (400MHz, DMSO – d_6, TMS) (Fig.1) of the aglycone, the hydroxyl proton at C-5 shows up at δ12.49pppm as a distinct singlet. The sharp singlets at δ10.9 and δ9.56ppm correspond to –OH protons at C-7 and C-3. The doublet at δ9.31ppm (J = 9Hz) accounts for the hydroxyl protons at C-3' and C-4'. The C-5' proton appears as a doublet at δ 6.68ppm (J = 9 HZ). The signals due to the protons at C-2' and C-6' overlap at δ7.54ppm. a ring protons at C-6 and C-8 could be located at δ 6.16 (d, J = 2.5Hz) and δ 6.41ppm (d, J = 2.5 Hz) respectively.

The supporting evidence for the structure of the flavonols is provided by the ^{13}C-NMR (100MHz, DMSO –d_6, TMS) Fig.2 spectra data. A complete assignment of the various signals is provided in Table.4.

A comparison of band I absorption of the glycoside and that of the aglycone revealed that there may be 3- glycosylation in the flavonols as mentioned under Et_2O fraction. A bathochromic shift of 43 nm (band I) on the addition of NaOMe confirmed the presence of a free –OH at C-4'. The $AlCl_3$ spectra (with and without HCl) showed four absorption peaks to reveal the presence of a free 5 – OH group. It was confirmed by the bathochromic shift of 51 nm on the addition of ($AlCl_3$ –HCl) in the glycoside. The presence of a free –OH at C-7 was evident from the 16nm (band II) shift on the addition of NaOAc. The band I absorption in $AlCl_3$ spectrum is 29 nm more than that noticed on addition of $AlCl_3$ – HCl. This is indicative of the existence of an O-dihydroxyl group in the B –ring. The O-dihydroxyl grouping in B –ring is once again ascertain from a bathochromic shift of 17nm on the addition of H_3BO_3.

In the ^1H – NMR spectrum (400 MHz, DMSO – d_6, TMS) the A- ring protons at C-6 and C-8 appear as doublets at δ 6.2 and δ 6.4 ppm respectively. The 5 – OH proton resonates at δ12.63ppm. The protons at C-5' appear at δ 6.8ppm as a doublet. The protons at C-2' and C-6' appear at δ7.3 and δ 7.6ppm. The methyl protons of rhamnose moiety can be located as a doublet at δ 0.9ppm. The H-1' of the rhamnoside resonates at δ 5.2ppm. The remaining sugar protons appear in the range δ 3.2 – 3.4ppm[14].

Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C - NMR(100 MHz, DMSO-d_6, TMS) data and a complete assignment is given in Table.5. Due to glycosylation at 3 positions, C-2 and C-4 carbons absorb at δ156.5 and δ177.8ppm respectively. C-1" absorbs at δ101.8ppm. The rest of the carbons of the rhamnosyl unit except C-6" appear between δ70.4 and δ71.2ppm. C-6" appear at δ17.4ppm.

REFERENCE:

[1] Hwee Ling Koh, Chua Tung Kian, Chay Hoo Tan (2009), A guide to Medicinal plants: An illustrated, scienfific and Medicinal Approach. World scientific publishing co.Pte. Ltd. Singapore, 32.

[2] Hoigawa M, Ito C, Tan HT, Kuchide M, Tokuda H, Nishino H, Furukawa H.(2001), Cancer Chemopreventive agents, 4 – phenylcoumarins from *C. inophyllum*, Cancer LeH . **169(1)**, 15.

[3] Said T, Dutot M, Labbe A, Warnet JM, Rat P.(2009) Ocular burn: rising and healing with ionic marine solutions and vegetable oils. Ophthalmolgical, **223(1),** 52.

[4] Said ,T., Dutot ,M., Martin, C., Beaudeux, J.L., Boucher, C., Enee, E., Baudowin, C., Warnet, J.M., Rat, P. (2007), Cytoprotective effect against UV – induced DNA damage and oxidative stree: role of new biological UV filter. Eur J Pharm Sci., **3(4)**, 203.

[5] Horhammer, L., and Hansel, R.,(1995) Arch. Pharm. Berl., 288, 315.

[6] Wilson, C.W. (1939), J.Amer. Chem. Soc., **61**, 2303.

[7] King, F.E., King , T.J., and Manning, L.C. (1957), J.Chem.Soc., 563.

[8] Shinoda .J. (1928) J. Chem. Pharm. Soc., **48**.

[9] Senthuraman,V., and Sulochana,N.(1988), Fitoterapia, **59**, 355.

[10] Khare , C. P.(2007) Indian Medicinal Plants: An illustrated Dictionary Springer – Verlag Berlin, 113.

[11] Khare C.P (2007), Indian Herbal Remedies : Rational western therapy, Ayurvedic, and other Springer – Verlag, Berlin, 118.

[12] Lewis nelson, Richard, D., Shih, Micheal ,J., Balick (2007), Handbook of poisonous and injurious plants, New York, Botanical Garden Springer – Verlag, Berlin, 100.

[13] Markham, K.R. , and Chari, V. M. (1982), on 22 of J. B. Harborne and T. J. Mabry, The Flavonoids – Advances in Research , Chapman and Hall, London, 29.

[14] Geissman, T.A., and Hinreiner, E.(1975), in 'The Flavonoids' Harorne, J.B., Mabry, T.J., and Mabry, H., (Eds.,) Chapman and Hall, London, 65.