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Isolation and characterization of kaempferol 3-O-(2''-α-methyl p-coumaryl)-β-d-glucoside from Tabebuia rosea (Flowers)

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ABSTRACT

The aim of the present study was to isolate a new compound from the flowers of *Tabebuia rosea*. The structure of the isolated compound was elucidated through their physical and chemical methods. The isolated compound was characterized using various spectroscopic data such as UV, ^{1}H NMR, ^{13}C NMR, MS.

Keywords: Tabebuia rosea, UV, NMR (^{1}H, ^{13}C) and MS, Kaempferol 3-O-(2''-α-methyl p-coumaryl)-β-d-glucoside

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INTRODUCTION

India is endowed with rich wealth of medicinal plants. India recognizes more than 2500 plant species which have medicinal values [1]. Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available, economical and has less side effects [2]. In plants, flavonoid aglycones (i.e., flavonoids without attached sugar) occur in a variety of structural forms. All contain fifteen carbon atoms in their basic nucleus two six-membered rings linked with a three carbon unit which may or may not be a part of a third ring [3].

The *Tabebuia* genus includes approximately 100 species and is the largest genus in the *Bignoniaceae* family. This plant family is distributed from the southwestern United States to the northern regions of Argentina and Chile [4]. where almost one-half of its genus and species are located [5]. *Tabebuia rosea* is one of the medicinally important plants belonging to the family Bignoniaceae. The herbal products obtained from the bark of Tabebuia trees are called “taheebo”, “lapacho”, and “ipe roxo”. Tabebuia rosea (Bertol.) DC. commonly known as “Pink Trumpet Tree” can grow up to 15 meter and well known for its beautiful flowers. Tea made from the leaves and bark is known to have a fever-reducing effect [6]. Tabebuia is reported to be an astringent, anti-inflammatory, antibacterial, antifungal, diuretic, and laxative [7-10].

MATERIALS AND METHOD

Collection of Flowers

Fresh flowers of *Tabebuia rosea* were collected from Jail Corner, Trichirappalli, Tamil Nadu, India, during the month of May and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS002 dated: 06/11/2015). St.Joseph’s College (Campus), Trichirappalli, Tamil Nadu, India.

Extraction and fractionation

Fresh flowers (3 kg) of *Tabebuia rosea* were extracted with 90% ethanol (8x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (4x250ml), Peroxide free diethyl ether (3x250ml) and ethyl acetate (8x250ml). Ethyl acetate fraction was taken for further study.

Ethyl acetate fraction

(Flavonol glycoside kaempferol 3-O-(2’’-α-methyl p-coumaryl)-β-d-glucosideThe ethyl acetate fraction was concentrated in vacuo. The residue obtained was taken up in acetone and left in an ice chest for 2 hours. A yellow solid (m.p 178-180^{0}C) separated was recrystallized from methanol.

It gave an olive green colour with alc.Fe^{3+}, deep pink colour with Mg-HCl and yellow colour with NH_3 and NaOH. It responded to Wilson's boric acid test, Gibb's test and Molisch's test [11]. But did not respond to Horhammer-Hansel test [12].

It had R_f values as depicted in table I.1

It had λ_{max} MeOH 264, 301sh, 352; + NaOMe 273, 324, 399; + $AlCl_3$ 262, 303sh, 350, 426;+ $AlCl_3$ – HCl 260, 303sh, 350, 426; + NaOAc 276, 301, 352 and + NaOAc - H_3BO_3 276, 301, 320, 352 nm.

(The ^1H, ^{13}C and mass spectra of the glycoside are appended in figure 1,2 and 3)

Table 1: R_f X 100 values of the glycoside and aglycone from the flowers of *Tabebuia rosea* (Whatman No.1, Ascending, 30 ± 2^0C)

Compound	Developing solvents								
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
Glycoside	14	41	43	69	77	70	68	81	82
Aglycone (Complete hydrolysis)	02	01	06	19	51	92	69	62	84

*** 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 = HOAc : Conc. HCl : H_2O = 30:3:10

i = t BuOH : HOAc : H_2O = 3:1:1

Hydrolysis of the glycoside

Acid hydrolysis

The glycoside (50 mg) was dissolved in MeOH (5 ml, 50%). An equal volume of dilute H_2SO_4 (7%) was added to it. The reaction mixture was refluxed at 100^0C for about 2 hours. The excess of alcohol was distilled off in vacuum and the resulting aqueous solution was extracted with Et_2O. The residue obtained was studied [13].

Identification of the aglycone (Kaempferol)

The yellow solid obtained (m.p 276-278^0C) from Et_2O fraction was soluble in organic solvents and insoluble in water. It gave yellow colour with NH_3 and NaOH. It developed olive green colour with alc.Fe^{3+} and orange-red colour with Mg-HCl. It answered Wilson's boric acid test, Gibb's

test and Horhammer-hansel test [12]. But did not respond to Molisch's test [11]. It had R_f values as depicted in table 1

It had λ_{max}MeOH 265, 294sh, 367; + NaOMe 279, 322sh, 417; + $AlCl_3$ 260, 303sh, 350, 424; + $AlCl_3$ – HCl 255, 303sh, 348, 424; + NaOAc 273, 303, 368 and + NaOAc - H_3BO_3 257, 297sh, 320sh, 365 nm. It was identified as kaempferol.

Identification of sugar (glucose)

The aqueous solution from the above hydrolysate was neutralized with $BaCO_3$ and the concentrated filtrate indicated the presence of glucose on paper chromatography. It had R_f values as depicted in table 2. These values are identified with those of glucose. The identity was confirmed by comparison with an authentic sample of glucose [13].

Table 2: R_f x 100 values of the sugars from the glycoside (G_1) from the flowers of *Tabebuia rosea*(Whatman No.1, Ascending, 30±2^{0}C)

Compound	Developing solvent			
	f	g	h	j
sugar from the glycoside	18	38	36	24
Glucose (from literature)	17	38	37	24

j = n BuOH : Benzene **:** Pyridine: H_2O = 5:1:3:3

Spray reagent: Aniline hydrogen phthalate

RESULTS AND DISCUSSION

The fresh flowers of *Tabebuia rosea* have been found to contain *Kaempferol 3-O-(2''-α-methyl p-coumaryl)-β-d-glucoside*

The UV spectrum of the glycoside showed two major peaks at 352 nm (band I) and 264 nm (band II) indicating the presence of flavonol skeleton. The aglycone showed peaks at 367 nm (band I) and 265 nm (band II), revealing the presence of glycosylation at C-3[13]. The presence of 4'- OH is evident from the bathochromic shift of 47 nm seen in the glycoside and 50 nm seen in the aglycone, in their respective NaOMe spectrum [14]. A bathochromic shift of 76 nm in the glycoside (band I) and 57 nm in the aglycone (band I) are noticed in the $AlCl_3$-HCl spectra. This is confirming the presence of free 5-OH in both [15]. This is also supported by the fact that both the glycoside and the aglycone answered Wilson's boric acid test. The NaOAc spectra of both glycoside and aglycone indicated the presence of free –OH at C-7. They showed bathochromic shift of 12 nm (band II) and 8 nm (band II) respectively. No additive bathochromic shift was noticed in the $AlCl_3$ spectra of the glycoside and also of the aglycone as compared with their respective MeOH spectra, ruling out the presence of O-dihydroxy grouping in B-ring. No change

was noticed in band (I) of the glycoside and its aglycone on the addition of NaOAc-H_3BO_3 thereby revealing the absence of O-diyhydroxy grouping in the B-ring [16].

In the ^{1}H-NMR spectrum (DMSO-D_6, TMS) (FigI.1) of the glycoside, the signal appearing at δ 12.654 ppm corresponds to the –OH at C-5 and the signal appearing at δ 10.232 ppm corresponds to the –OH at C-7. C-8 proton, due to meta coupling with C-6 proton, appears as a doublet at δ 6.44 ppm. C-6 proton due to meta coupling with C-8 proton, appears as a doublet at δ 6.21 ppm. The two pairs of ortho coupled doublets of C-2', C-6' and C-3', C-5' protons appear respectively at δ 8.03 ppm and at δ 6.87 ppm. The doublet due to C-3' and C-5'protons occur upfield from that of C-2' and C-6' protons, due to the deshielding influence of C-ring on C-2' and C-6' protons. H-1' of the glucose resonates at δ 5.48 ppm. The remaining sugar protons appear in the range of δ 3.2 to 3.5 ppm. –CH_3 protons of α-methyl p-coumaryl group resonate at δ 1.52 ppm. C-2''' and C-6''' protons and the proton at β-carbon atom of the above group appear at δ 7.57 ppm. C-3''' and C-5''' protons appear at δ 6.79 ppm[13,16].

Supporting evidence is given by ^{13}C-NMR (DMSO-D_6, TMS) spectral data (FigI.2). The signal positions and their complete assignments to different carbons are given in Table I.3. As a result of glycosylation the signal at C-3 is shifted upfield and appear at δ 133.1 ppm. The signals of ortho carbon atoms C-2 and C-4 are shifted downfield. C-1'' of the glucose appears at δ 100.8 ppm. As a result of α-methyl p-coumaryl substitution the signal at C-2'' shifted downfield and appear at δ 76.3 ppm. Upfield shift of C-1''and C-3''signals confirm the acylation at C-2". The rest of the sugar carbons appear in the range of δ 60.4 – 77.4 ppm. Methyl carbon of α-methyl p-coumaryl group appears at δ 28.75 ppm and –CO carbon of the above group appears at δ 167.8 ppm[13,16].

The structure of the glycoside is further evidenced by mass spectrum (Figure 3) of the glycoside, which had a peak at m/z 607 for M^+ ion. The pattern showing RDA and other common fragmentation pattern are shown in fig I.4 and they are supporting the structure of the glycoside. Presence of α-methyl p-coumaryl group is evidenced by the peaks found at m/z 513 and at m/z 472. Peaks seen at m/z 355, m/z 287 and at m/z 149 are also in favour of the structure of the compound [13,16].

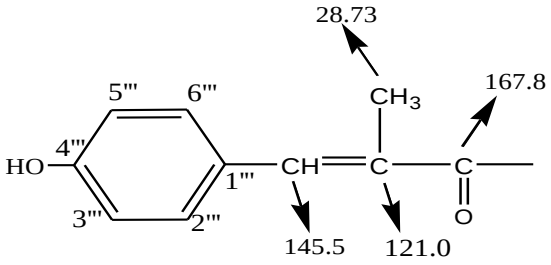
Based on the above evidences, the glycoside has been characterized as Kaempferol 3-O-(2''-α-methyl p-coumaryl)-β-d-glucoside [13,16].

Table 3: ^{13}C NMR data and their assignments for the glycoside from the flowers of *Tabebuia rosea*

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Kaempferol from literature(δ ppm)	146.8	135.6	175.9	160.7	98.2	163.9	93.5	156.2	103.1
Glycoside(δ ppm)	156.34	133.1	177.4	161.18	98.65	164.07	93.6	156.34	103.97
Kaempferol 3-O-glucoside from literature(δ ppm)	156.3	133.0	177.4	161.1	98.7	164.1	93.6	156.3	104.1

Compound	C-1’	C-2’	C-3’	C-4’	C-5’	C-6’
Kaempferol from literature(δ ppm)	121.7	129.5	115.4	159.2	115.4	129.5
Glycoside(δ ppm)	120.8	130.8	115.0	159.9	115.0	130.8
Kaempferol 3-O-glucoside from literature(δ ppm)	121.0	130.7	115.0	159.8	115.0	130.7

Compound	C-1’’	C-2’’	C-3’’	C-4’’	C-5’’	C-6’’
Glucoside from literature(δ ppm)	101.4	74.3	76.8	70.3	77.5	61.3
Glycoside(δ ppm)	100.8	76.3	74.1	69.85	77.4	60.7

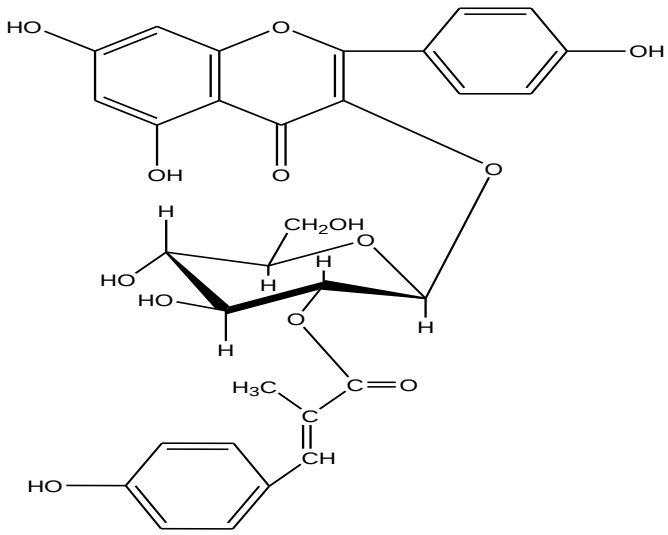


C-1''' → δ 125.6 ppm

C-2''' & C-6''' → δ 130.8ppm

C-3''' & C-5''' → δ 115.8 ppm

C-4''' → δ 161.1 ppm



Kaempferol-3-O-(2''- α – methyl p-coumaryl-) β-d-glucoside

Mass fragmentation pattern

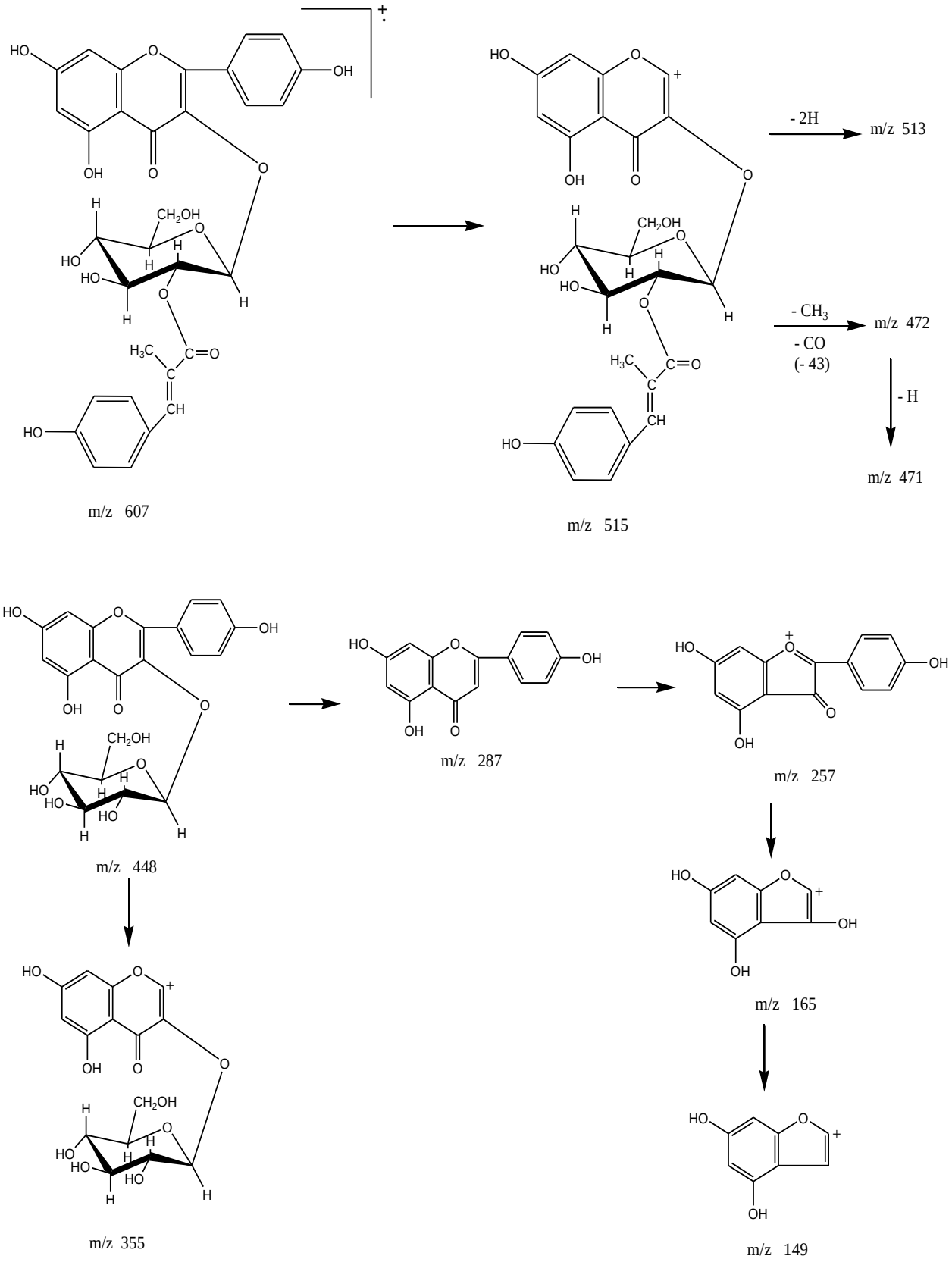


Fig I.4

CONCLUSION

In recent years, ethno-botanical and traditional used of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. It could be concluded that the compound *Kaempferol 3-O-(2''-α-methyl p-coumaryl)-β-d-glucoside* isolated from the ethyl acetate fraction of flowers of *Tabebuia rosea* is of phytopharmaceutical importance. However, isolation of compound subjecting it to biological testing will definitely give fruitful results.

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