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ISOLATION AND CHARACTERIZATION OF THE FLAVONOID KAEMPFEROL-3-O-(2''-O-(3-HYDROXY BUTANOYL)-A-L ARABINOSIDE FROM DATURA INNOXIA FLOWERS

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ABSTRACT:
The present study was isolating the new compound Kaempferol-3-O-(2''-O-(3hydroxy butanoyl)-α-L arabinoside from the flowers of Datura innoxia. The extraction and fractionation was carried out using the solvents of ethanol, benzene, petroleum ether, diethyl ether and ethyl acetate. The structure of the isolated compound was investigated by physical and chemical methods. The new compound was characterized by various spectroscopic techniques viz UV, ^{1}H NMR, ^{13}C NMR and MS.
Key words: Datura innoxia, ^{1}H NMR, ^{13}C NMR, MS, Kaempferol-3-O-(2''-O-(3-hydroxy butanoyl)-α-L arabinoside.

INTRODUCTION:

In current and traditional medicines, medicinal plants and its parts constitute an effective source of medicinal compounds so plants are act as a synthetic laboratory of its biochemical compounds like alkaloids, terpenoids, phenolics, and glycosides. The medicinal plants and its products have played a vital role in treatment and prevention of various human diseases [1]. *Datura* is an essential medicinal plant and well known resource of different phytochemicals [2].
The genus *Datura* belongs to Solanaceae family commonly known as Thorn Apple, Jimson weed is disseminated throughout the earth and is comprised of fourteen species, of which ten species are spread out in India. Among them *D. innoxia, D. stramonium, D. metel* are important medicinal species [3]. The World Health Organization suggested that, medicinal plants were the greatest resource to achieve a variety of drugs for human diseases [4].
The proximate and elemental analysis shows that *Datura innoxia* seeds contains, high level of Nitrogen, Nickel and Phosphorus. Potassium, Calcium and Sodium were found to be present in considerable quantities. The elements Lead and Cadmium are considered harmful and carcinogenic which could be why the plant is poisonous and reasonable concentrations for variety of essential and non essential amino acids also present [5]. The seeds and leaves of *D. innoxia* are extensively used in herbal medicine as antispasmodic, anesthetic and bronchodilator. The antibacterial studies of the *D. innoxia* leaves, seeds and roots methanol extracts of 50% concentration was active against most of the bacteria and *Aspergillus* niger [6]. Entire parts of the medicinal plants are psychoactive however the flowers, leaves and seeds contain the maximum content of alkaloids [7]. The medicinal plant ingredients are extensively used in traditional medicine in all parts of the world [8]
Mostly alkaloids and tannins were present in methanolic and ethyl acetate extracts of leaves of the medicinal plant were highly active against the micro organisms K. pneumonia, E. coli, A. niger and P. vulgaris [9]. *Datura* Species has utmost antibacterial action against Enterobacter and the antifungal action against S. cerevicae. So the *datura* Species are the important source of effective pharmacological significance and prospective resource of new-fangled drugs [10]. In the latest

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periods, inflectional diseases are increased to a quantity of great extent and antibiotics battle effects grow to be rising therapeutic crisis [11]. In Africa and Sudan, Datura innoxia seeds are widely used in traditional medicinal parts of various diseases. The leaves of the plant are generally used as a medicine in antispasmodic, bronchodilator, anesthetic, and as hallucinogenic [12 and 13]. The phytochemical screening of the *D. innoxia* exposed the presence of significant pharmacological bioactive chemicals as well as therapeutic and nutritional potentials in the root, seed, leaf, stem, and pod [14].

The present study was isolated the flavonoid *Kaempferol-3-O-(2''-O-(3-hydroxy butanoyl)-α-L arabinoside* from the flowers of the Datura innoxia using the different solvents in the order of polarity.

MATERIALS AND METHODS:

Flower collection:

Exactly three kilogram fresh flowers of *Datura innoxia* were collected from V. Pudhur village, Sivaganga District, Tamil Nadu State, India in the January session and documented by Dr. S. John Britto, Director of the Rapinat Herbarium and Centre for Molecular Systematics. St.Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Extraction and fractionation:

The extraction was carried out by the collected fresh flowers of *Datura innoxia* were soaked in ethanol (5x500ml) for seven days. The collective ethanolic extract was concentrated by the distillation process and the aqueous extract was sequentially fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether and diethyl ether parts did not yield any isolable material. Ethyl acetate fraction dried and yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

Ethyl acetate fraction:

(Kaempferol-3-O-(2''-O-(3-hydroxy butanoyl)-α-L arabinoside)

The ethyl acetate fraction was concentrated further in vacuo. The solid obtained was dissolved in acetone and kept in ice cold temperature for three hours. The mass obtained was recrystallised from methyl alcohol. The recrystallised yellow slid has the melting point 184-185^o C. it gave intense yellow colour with NaOH and NH_3, dark pink colour with Mg-HCl and green colour with alc. $FeCl_3$. It answered Gibbs test, Wilson's boric acid test and Molisch's test.

It had R_f values as given in table 1.1.1

Table 1

R_f X 100 Values of Glycoside (G1) and aglycone (A1) from the flowers of *Datura innoxia* (Whatman No.1, Ascending, 30±2^{0}C)

Compound	Developing solvents								
	a	**b**	**c**	**D**	**E**	**f**	**G**	**h**	**i**
Glycoside (G1)	11	36	41	66	78	71	72	51	70
Aglycone (A1)	-	-	4	14	48	90	64	86	64

*** 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

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It had the λmax MeOH 262, 300sh,354; + NaOMe 270,320,401; $AlCl_3$ 260, 302sh, 351, 429; + $AlCl_3$-HCl 259, 302sh, 349, 428; + NaOAc 275, 302, 353 and NaOAc- H_3BO_3 275,303,352nm.
The H^1, C^{13} NMR and mass spectra of the compound are given in fig 1.1.1, 1.1.2 and 1.1.3 respectively.

Hydrolysis of flavonol glycoside:

50 mg of the glycoside was dissolved in methanol (5ml, 50%) and an equal volume of 7% H_2SO_4 was added to it. This mixture was refluxed for 2 hours at 100° C. The excess of alcohol was distilled off in vacuo and the resulting solution was extracted with diethyl ether. On evaporation a residue was obtained from this ether fraction.

Identification of the above residue (*Flavonol- Kaempferol*):

The above solid has the melting point 277-278° C. It was soluble in organic solvents and insoluble water. It developed Orange –red colour with Mg-HCl, green colour with alc. Fe^{3+} and yellow colour with NaOH and NH_3. It responded to Horhmmer - Hansal test, Wilson's boric acid test and Gibbs test. It did not answer Molisch's test.
It had λmax MeOH 262,300sh,369; + NaOMe 278,320sh, 420; + $AlCl_3$ 259, 302sh, 350, 428; + $AlCl_3$-HCl 256,302sh,350,427; + NaOAc 274,302,360 and + NaOAc- H_3BO_3 256, 297, 321sh, 368 nm. From these evidences, the compound obtained from the ether fraction was identified as Kaempferol.

Identification of sugar (*Arabinose*):

The aqueous solution obtained from the above hydrolysis process was neutralized with $BaCO_3$ and concentrated. It had R_f values as given in table 1.1.2. These values are similar to that of arabinose. The identity was confirmed by comparison with an authentic sample of arabinose.

Table 2
R_f X 100 Values of Glycoside (G1) and aglycone (A1) from the flowers of *Datura innoxia* (Whatman No.1, Ascending, 30±2°C)

Compound	Developing solvents			
	f	G	H	j
Sugar obtained from the glycoside	19	43	-	28
Arabinose (authentic) (from literature)	18	43	-	29

j = n BuOH : Benzene **:** Pyridine: H_2O = 5:1:3:3 Spray reagent **:** Aniline hydrogen phthalate

Table 3
^{13}C-NMR spectral data and their assignments for the glycoside (G1) from the flowers of *Datura innoxia*

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 G1 (δ ppm)	156.0	133.1	176.99	161.0	98.5	164.2	93.6	156.6	103.8
Kaempferol-3-O-α-L arabinoside	153.3	133.2	177.5	161.0	98.5	164.5	93.6	156.7	103.9

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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 G1 (δ ppm)	120.1	129.4	115.6	158.7	115.6	129.4
Kaempferol-3-O-α-L arabinoside	120.6	130.7	115.3	159.7	115.3	130.7

Compound	C-1’’	C-2’’	C-3’’	C-4’’	C-5’’
Arabinose from literature δ ppm (authentic)	107.9	81.9	76.8	86.2	60.7
Glycoside G1 δ ppm	105.0	84.0	73.0	86.4	60.67

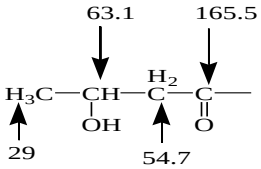
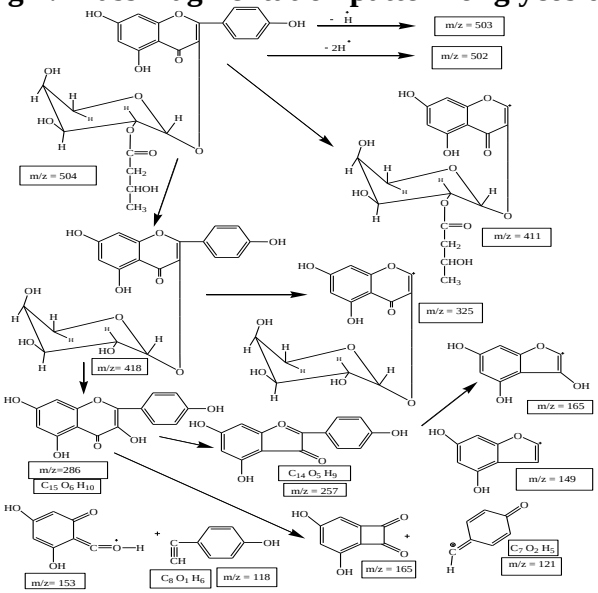


Fig 1: 3-Hydroxy butanoyl group

Fig 2: Mass fragmentation pattern of glycoside



RESULTS AND DISCUSSION:

Kaempferol-3-O-(2”-(3-hydroxy butanoyl)- α-L-arabinoside has been found in the flowers of Datura innoxia

The UV spectrum of the glycoside have been major peaks at 354 nm (band I) and at 262 nm (band II) confirming the presence of flavonol skeleton. The aglycone obtained after hydrolysisshowed peaks at 369 nm (band I) and at 262 nm (band II), showing the presence of hydroxylate ion at C-3. Bathochromic shift of 47 nm in the glycoside on 51 nm in the aglycone seen in their respective NaOMe spectrum (band I), are indicating the presence of 4’-OH. The presence of free 5-OH is evident from the bathochromic shift of 74 nm seen the glycoside (band I) and 58nm seen in the

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aglycone (band I) in their respective $AlCl_3$-HCl Spectrum. This is also supported by the positive response of the glycoside and aglycone to Wilson's boric acid test. The NaOAc spectrum of the glycoside and the aglycone showed bathochromic shift of 13 nm and 12 nm (band II) respectively, indicating the presence of free 7- OH. No additive bathochromic shift was found in $AlCl_3$ spectra of the glycoside and aglycone, when compared with their respective $AlCl_3$ - HCl spectrum. This indicates the absence of O-dihydroxy grouping in B- ring both in glycoside and aglycone. This also supported by NaOAc – H_3BO_3 spectrum in which no change was noticed in band I of the glycoside and its aglycone as compared with their respective MeOH spectrum.

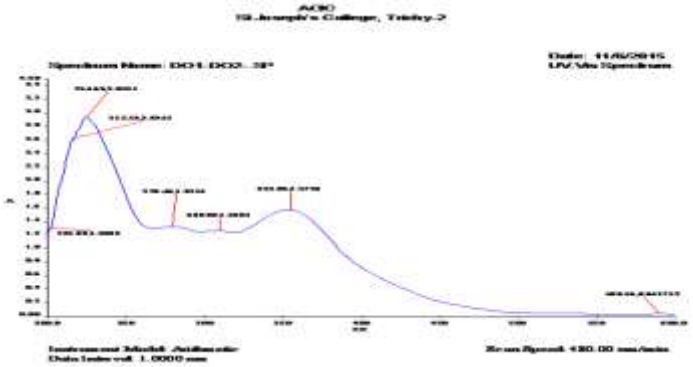

Fig 3: UV spectrum

In the H^1 NMR spectrum (DMSO-d6, TMS) of the glycoside, presence of 5-OH is evidenced by the presence of a peak at δ 12.5 ppm. C-6 proton due to meta coupling with C-8 proton, appears as a doublet at δ 6.24 ppm C-8 proton due to meta coupling with C-6 proton appears as a doublet at δ 6.43 ppm. The two pairs of ortho coupled doublets of C-2', C- 6' and C- 3'- C-5' protons appear at δ 7.99ppm and at δ 6.86 ppm.

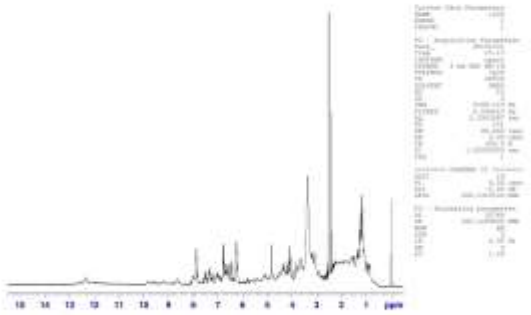

Fig 4: H^1 NMR spectrum

Supporting evidence is given by C^{13} –NMR data (DMSO-d6, TMS) in table 1.1.3. due to glycosylation C-3 signal shifted upfield and appears at δ 133.1ppm. the signals of ortho carbon atoms C-2 and C-4 are shifted downfield and appears at δ 156.0 ppm and at δ 176. 99 ppm respectively. C-1" of arabinose appears at δ 105.0 ppm. Due to substitution C-2" signal shifted downfield and appears at δ 84.0 ppm. The two ortho carbons C-1" and C-3"show upfield shift, evidencing the acylation at C-2". Methyl carbon of 3- hydroxy butanoyl group appears at δ 29 ppm and =C=O carbon appears at δ 165.5 ppm.

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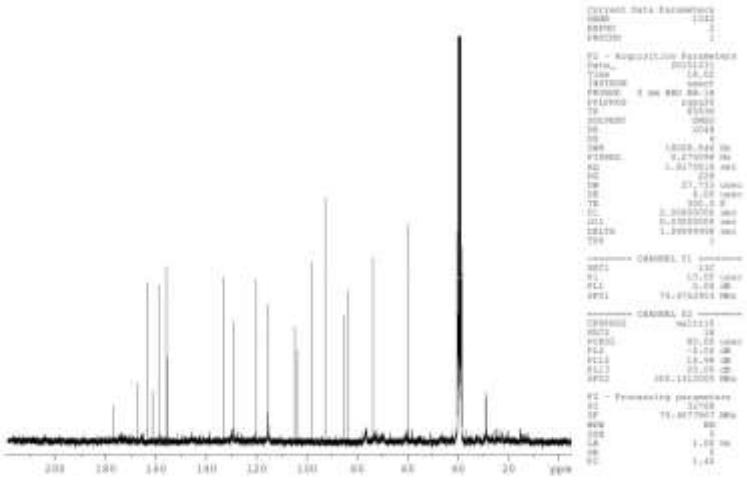

Fig 5: C^{13} –NMR spectrum

Structure of the isolated flavonol glycoside is further evidenced by mass spectrum of the glycoside. It had a peak at m/z 5.4 for M^+ ion. The fragmentation pattern following RDA and other common patterns are shown in fig 1.1.4 and they are strongly supporting the structure of the glycoside. Presence of 3- hydroxy butanoyl group is evidenced by the peak seen at m/z 411. Peaks at m/z 418, m/z 325, m/z 286, m/z 257, m/z 165, m/z 149, m/z 153 and at m/z 118 are also following the structure of the compound.

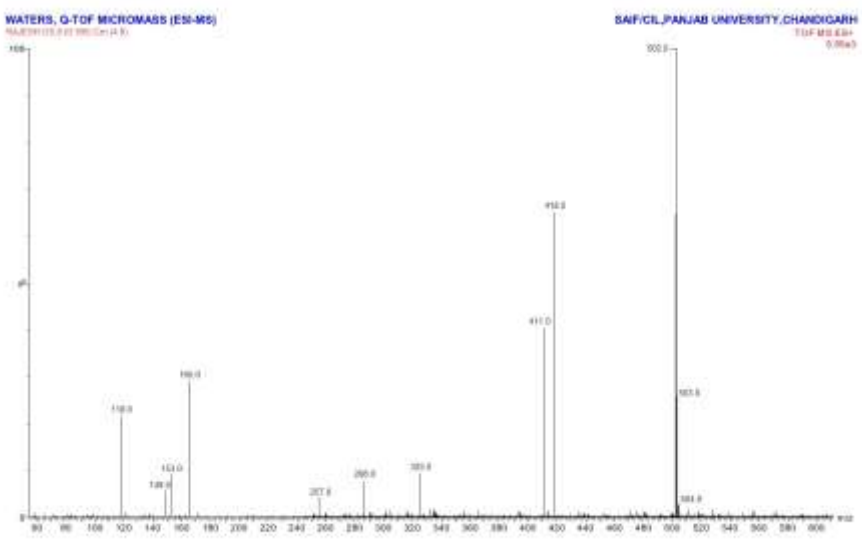

Fig 6: Mass spectrum

Based on the above evidences, the structure of the compound has been identified as **KAEMPFEROL -3-O-(2”-(3-HYDROXY BUTANOYL)- A-L-ARABINOSIDE** :

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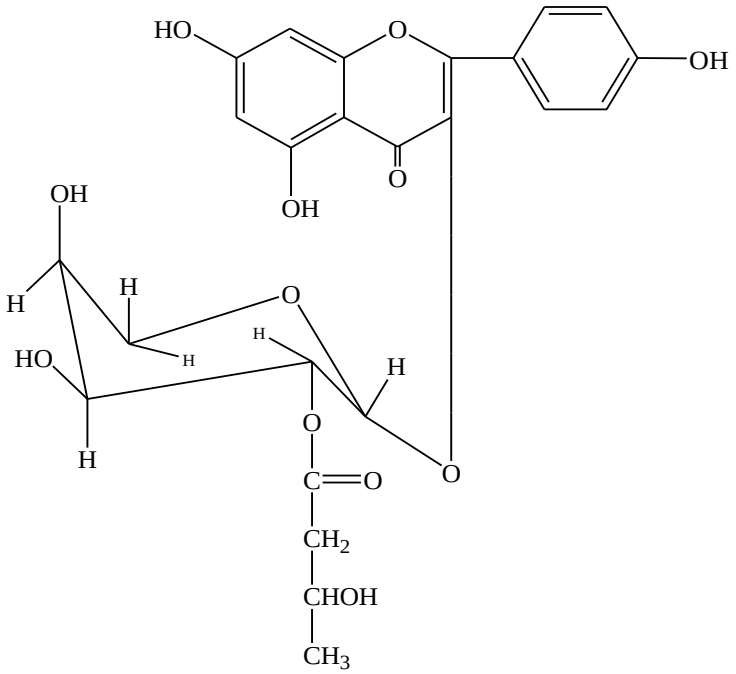

Fig 7: Kaempferol -3-O-(2”-(3-hydroxy butanoyl)- α-L-arabinoside.

CONCLUSION:

Natural medicinal plants contains lot of phytochemicals such as alkaloids, terpenoids, tannins, etc,. the present study reported that the flavonoid Kaempferol -3-O-(2”-(3-hydroxy butanoyl)- α-L-arabinoside was isolated from the flower part of *Datura innoxia* medicinal plant and characterized by UV, H^1 NMR, C^{13} NMR, and Mass spectral techniques. We hope the above flavonoid have better activities.

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