Research Article

# Isolation and Characterization of 5,7 di-O-methyl luteolin-6-C-(3''-O-benzoyl)-β-d-xyloside from *Cucumis sativus* Flowers

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**Accepted on:** 25-05-2016; **Finalized on:** 30-06-2016.

**ABSTRACT**

Cucumber (*Cucumis sativus*) is a widely cultivated plant in the gourd family, Cucurbitaceae. It is a creeping vine that bears cylindrical fruits that are used as culinary vegetables. There are three main varieties of cucumber: slicing, pickling, and burpless. Within these varieties, several different cultivars have emerged. The cucumber is originally from Southern Asia, but now grows on most continents. In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases, India has been one of the pioneers in the development and practice of well-documented original systems of medicine, particularly Ayurveda, Siddha and Unani. A new compound has been isolated from the flowers of *Cucumis sativus*. The isolated flavone glycoside was identified as 5,7 di-O-methyl luteolin-6-C-(3''-O-benzoyl)-β-d-xyloside. The chemical structure of this compound was elucidated based on spectroscopic data like UV, NMR ($^{1}H$, $^{13}C$) and MS. This is the first report of isolation of this compound from *Cucumis sativus* flowers.

**Keywords:** *Cucumis sativus*, UV, NMR ($^{1}H$, $^{13}C$) and MS, 5,7 di-O-methyl luteolin-6-C-(3''-O-benzoyl)-β-d-xyloside, etc.

## INTRODUCTION

The "*Cucumis sativus*", an herbaceous vine of the botanical family Cucurbitaceae bears the fruit Cucumber. Cucumbers contain a very large amount of water, about 96% of its content. Thus, it is said that "eating a cucumber is like drinking a glass of water". Cucumbers provide the sulphur needed for healthy skin cells, hair and nails, and also helps in hydrating the skin. Moreover, they cleanse the bloodstream of toxic wastes. Cucumbers are therefore highly recommended for patients suffering from dermatosis, eczema and psoriasis. They are applied locally by rubbing it directly on the skin or on the affected areas. Applying cucumbers on the skin regularly helps make it soft, smooth and fresh.

Cucurbits (Cucurbitaceae family) are composed of 118 genera and 825 species. Members of this family are distributed primarily in tropical and subtropical regions of the world (Wang)[1]. The most economically important cucurbits according to world's total production are watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) (FAO 2006). The Cucurbitaceae includes two subfamilies Zanonioideae and the Cucurbitoideae. Cucumber is thought to have been first domesticated in India, then distributed to Greece and Italy and introduced into China around 100 BC. Cucumber has been cultivated for food for at least 3000 years. It appeared in France in the ninth century, England in the fourteenth century and North America in the middle of the sixteenth century (Plader)[2].

In addition cucumber is cultivated because its extract has soothing, cleansing and softening properties which are important for the cosmetics industry. Cucumber also serves as a pesticide because of its steroid content including cucurbitacins (Wang)[1]. It is an important factor which has a positive effect on yield and constitutes a major component of cucumber improvement programs (Serquan)[3].

Oxygen is absolutely necessary for the life processes, in particular cell respiration. However, the metabolism of oxygen may generate reactive elements called free radicals, in particular the superoxide ion ($O^{2-}$) and the hydroxyl ion ($OH^{-}$). There is a growing body of evidence suggesting that free radicals play an important role in the development of tissue damage and pathological events in living organisms. Free radicals damage DNA, essential cellular proteins and membrane lipids (lipid peroxidation), which may lead to cell death[4]. The use of plant cell and tissue culture methodology as a means of producing medicinal metabolites has a long, history. Cultured plant cells synthesize, accumulate and sometimes exude many classes of metabolites. Medicinal compounds are of particular interest and much effort has been devoted to obtain some of the most active and precious therapeutics. Numerous alkaloids, saponins, cardenolides, anthraquinones, polyphenols and terpenes have been reported from in vitro cultures and reviewed several times[5]. Many health-related properties including anticancer, antiviral, antimicrobial, anti-inflammatory activities, antioxidant properties, effects on capillary fragility, and an ability to inhibit human platelet aggregation have been ascribed to phenolics[6]. The etiology of several degenerative and aging-related diseases has been attributed to oxidative stress, and

numerous studies have been undertaken to search for the most effective antioxidants[7].

In Literature, GC/MS analysis indicated presence of many compounds and details of 26 compounds from ethyl acetate fraction indicated that it has activated macrophages to increase the production of TNF-α as well as secretion of TGF-β which may have a new bioactive material for the treatment of skin lesions in acne vulgaris. Cucumber's ability to eliminate water from the body makes it important for heart and kidney problems. Helps to dissolve uric acid accumulations such as kidney and bladder stones. Cucumber juice helps intestines, lungs, kidneys, and skin. Eaten as a vegetable, it is a good diuretic and can help prevent constipation. It is also applied to inflammations, bed sores, scalds and burns. Cucumber is used for cleansing the skin, bleaching (for freckles and discolored skin); also for sunburn, and rough skin. Cucumber slices or juice is applied to the face and/or hands, and left on 10-15 minutes, then rinsed off. Best for normal or oily skin. Researchers are now investigating an extract of cucumber as a possible "cholesterol buster[8]." The present work has been aimed at isolation and structure elucidation of bio active compound from ethyl acetate soluble fraction of *Cucumis sativus* flowers.

**Extraction and Fractionation**

Fresh flowers (3 kg) of *Cucumber sativus* were collected from O. Koothur village, Ariyalur district, Tamil Nadu, India. The flowers 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°C) (4x250ml), Peroxide free diethyl ether (3x250ml) and ethyl acetate (8x250ml). Ethyl acetate fraction was taken for further study.

**Ethyl Acetate Fraction**

(Flavone glycoside-(5,7 di-O-methyl luteolin-6-C-(3''-O-benzoyl)-β-d-xyloside)

The ethylacetate fraction was concentrated in vacuo. The residue obtained was taken up in acetone and left in an ice chest for 3 hours. A yellow solid (m.p 256-258$^0$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 NaOH. It responded to Gibb's test and Molisch's test (F.E. King)[9]. But not answered Horhammer-Hansel test and Wilson's boric acid test (L. Horhammer)[10], (V. Ragunathan)[11].

It had $R_f$ values as depicted in Table 1.

It had $\lambda_{max}$ MeOH 252, 294sh, 324; + NaOMe 255, 276, 388; + $AlCl_3$ 227, 276, 354; + $AlCl_3$ – HCl 230, 296, 324; + NaOAc 251, 284, 330 and + NaOAc - $H_3BO_3$ 259, 301sh, 360 nm.

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

**Hydrolysis and Demethylation of the Glycoside**

The glycoside was resisted to hydrolysis even on prolonged heating for 24 hours with $H_2SO_4$ and hence the presence of a C-glycosylation was suspected. 20 mg of the glycoside was dissolved in 10 ml of glacial acetic acid. An equal volume of HI (10%) was added and the mixture was refluxed for 7 hours. The resulting aqueous solution was extracted with $Et_2O$. The residue obtained from $Et_2O$ fraction was studied as stated below (K.R. Markham)[12].

**Identification of the Aglycone (Luteolin)**

The yellow solid obtained (m.p 330-332°C) from the above $Et_2O$ fraction was soluble in hot water and organic solvents but insoluble in cold water. It gave yellow colour with $NH_3$ and NaOH. It developed orange red colour with Mg-HCl and green colour with alc $Fe^{3+}$. It answered Wilson's boric acid test and Gibb's test (F.E. King)[9]. But not answered for Horhammer-Hansel test (L. Horhammer)[10] and Molisch's test (F.E. King)[9], (V. Ragunathan)[11]. It had $R_f$ values as depicted in Table 1.

It had $\lambda_{max}$ MeOH 254, 267, 291sh, 349; + NaOMe 266, 327sh, 401; + $AlCl_3$ 273, 300sh, 327, 426; + $AlCl_3$ – HCl 266, 275, 293sh, 355, 386; + NaOAc 269, 327sh, 385 and + NaOAc - $H_3BO_3$ 259, 301sh, 370 nm.

**Identification of Sugar (Xylose)**

The aqueous solution from the above hydrolysate was neutralized with $BaCO_3$ and the concentrated filtrate indicated the presence of xylose on paper chromatography. It had $R_f$ values as depicted in Table 2. These values are identified with those of xylose. The identity was confirmed by comparison with an authentic sample of xylose (K.R. Markham)[12].

## RESULTS AND DISCUSSION

The fresh flowers of *Cucumber sativus* have been found to contain 5,7 di-O-methyl luteolin-6-C-(3''-O-benzoyl)-β-d-xyloside.

The UV spectrum of the glycoside showed two major peaks at 324 nm (band I) and 253 nm (band II), indicating the flavonoids skeleton.

The presence of 4'-OH is evident from the bathochromic shift of 64 nm in the glycoside and 52 nm in the aglycone found in the (band I) NaOMe of spectrum as compared with their respective MeOH spectrum (O. Barbara)[13].

In the $AlCl_3$-HCl spectrum of the aglycone a bathochromic shift of 37 nm (band I) was seen, indicating the presence of free 5-OH. But no such bathochromic shift was noticed in the glycoside, suggesting a substitution at C-5.

This is also supported by the fact that the aglycone answered Wilson's boric acid test but the glycoside did not answered (T.A. Geissman)[14].

Additive bathochromic shifts seen in the $AlCl_3$ spectrum of the glycoside and the aglycone reveal the presence of

O-dihydroxy group in B ring as compared with their respective $AlCl_3$-HCl spectrum.

This is also supported by the bathochromic shift found in the NaOAc-$H_3BO_3$ spectrum (band I) of the glycoside and the aglycone as compared with their respective MeOH spectrum (T.A. Geissman)[14], (K.R. Markham)[15].

The aglycone showed a bathochromic shift of 15 nm (band II) in NaOAc spectrum. But in the glycoside no such shift was seen, indicating the substitution at C-7.

Both the aglycone and the glycoside did not answered Horhammer-Hansel test, proving the absence of 3-OH in both (L. Horhammer)[10].

In the $^1$H-NMR spectrum (DMSO-$d_6$, TMS) (fig 1) of the glycoside, the proton at C-3 appears as a singlet at δ 6.633 ppm. Since C-6 carbon is glycosylated, C-8 proton appears as a singlet at δ 6.5 ppm.

C-2' proton appears as a doublet at δ 7.34 ppm and C-6' proton appears at δ 7.372 ppm (dd). C-5'proton, ortho coupled with C-6', appears as a doublet at δ 6.9 ppm.

–$OCH_3$ protons resonate at 4.0 ppm as a singlet. In the benzoyl group, C-2''' and C-6''' protons appear at δ 7.93 ppm, C-3''' and C-5''' protons appear at δ 7.38 ppm and C-4''' proton appears at δ 7.47 ppm.

The H-1 proton of the xylose appears at δ 5.3 ppm.

Supporting evidence is given by $^{13}$C NMR (DMSO-$d_6$, TMS) (fig 2) spectrum. The signal position and their complete assignments to different carbon are given in Table 3.

As a result of C-glycosylation C-6 signal is shifted downfield and appears at δ 108.5 ppm. Presence of –$OCH_3$ groups are evidenced by the downfield shifts found at C-5 and at C-7. –$OCH_3$ carbon resonates at δ 55 ppm.

In contrast to the O-linked xyloside, the C-1'' carbon atom did not show any significant downfield shift and appears at δ 73.5 ppm.

Presence of benzoyl group at C-3" is evidenced by the downfield shift of 1.5 ppm shown by that carbon.

The two ortho carbon atoms C-2'' and C-4'' are showing upfield shift. The carbonyl carbon of the benzoyl group resonates at δ 167.9 ppm.

The structure of the glycoside is further evidenced by mass spectrum (Fig 3) of the glycoside which had a peak at m/z 566 for $M^+$ ion.

The pattern showing RDA and other common fragmentation pattern are shown in fig 4 and they are supporting the structure of the glycoside.

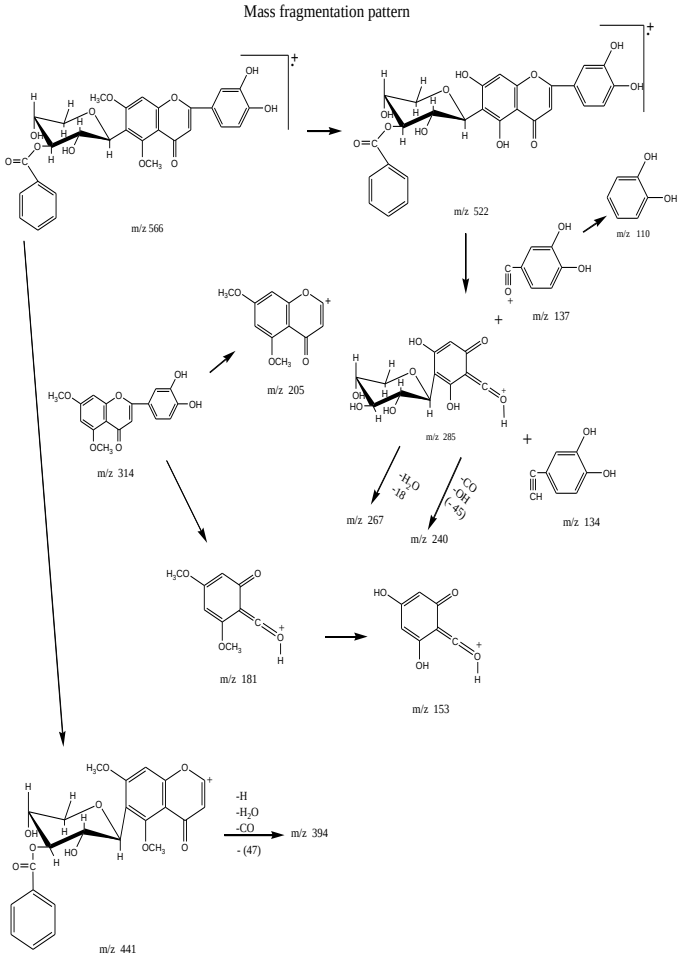
Presence of –$OCH_3$ groups at C-5 and at C-7 is evidenced by the peaks seen at m/z 314 and at m/z 205.

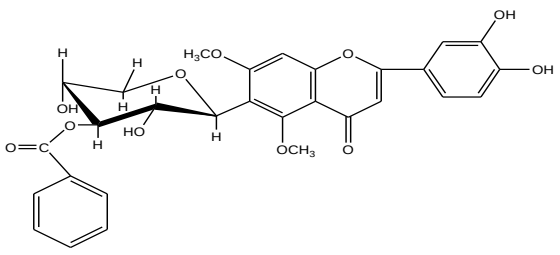
Presence of Catechol type of substitution in B ring is evidenced by the peak seen at m/z 110.

Peaks at m/z 522, m/z 394, m/z 267 and at m/z 240 are also in favour of the structure of the compound (K.R. Markham)[12],(K.R. Markham)[15].

Based on the above evidences, the glycoside has been characterized as 5,7 di-O-methyl luteolin-6-C-(3''-O-benzoyl)-β-d-xyloside.

Mass fragmentation pattern



5,7-di-O-methyl luteolin-6-C-(3''-benzoyl-)β-d-xyloside

**Table 1:** $R_f$ X 100 Values of the glycoside and aglycone from the flowers of *Cucumber sativus* (Whatman No.1, Ascending, 30±2°C)

| Compound | Developing solvents | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | f | G | h | i |
| Glycoside | 09 | 10 | 17 | 33 | 62 | 63 | 75 | 76 | 64 |
| Aglycone (Complete hydrolysis) | - | 01 | 05 | 25 | 50 | 88 | 71 | 61 | 86 |

* Solvent Key

a = $H_2O$ d = 30 % aq. HOAc g = Phenol saturated with water

b = 5% aq. HOAc e = 60 % aq. HOAc h = HOAc : Conc. HCl : $H_2O$ = 30:3:10

c = 15% aq. HOAc f = n. BuOH : HOAc : $H_2O$ = 4:1:5 ( Upper case) i = t BuOH : HOAc : $H_2O$ = 3:1:1

**Table 2:** $R_f$ x 100 values of the sugar from the glycoside, from the flowers of *Cucumber sativus* (Whatman No.1, Ascending, 30±2°C)

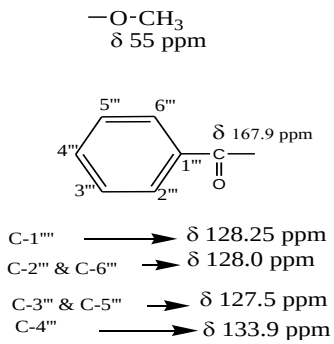
| Compound | Developing solvent | | | | |
|---|---|---|---|---|---|
| | e | f | g | h | i |
| Sugar from the glycoside | 71 | 14 | 49 | 85 | 33 |
| Xylose (from literature) | 73 | 15 | 50 | 86 | 33 |

**Table 3:** $^{13}C$ NMR spectral data and their assignments for the glycoside from the flowers of Cucumber sativus

| Compound | C-2 | C-3 | C-4 | C-5 | C-6 | C-7 | C-8 | C-9 | C-10 |
|---|---|---|---|---|---|---|---|---|---|
| Luteolin from literature (δ ppm) | 164.5 | 103.3 | 182.2 | 162.1 | 99.2 | 164.7 | 94.2 | 157.0 | 104.2 |
| Glycoside (δ ppm) | 165.0 | 104.0 | 182.0 | 171.7 | 108.5 | 173.5 | 94.8 | 157.2 | 104.0 |

**Table 4:** Luteolin from literature

| Compound | C-1' | C-2' | C-3' | C-4' | C-5' | C-6' |
|---|---|---|---|---|---|---|
| Luteolin from literature (δ ppm) | 122.1 | 113.8 | 146.2 | 150.1 | 116.4 | 119.3 |
| Glycoside (δ ppm) | 122.0 | 114.0 | 146.1 | 150.2 | 115.8 | 119.5 |
| **Compound** | **C-1''** | **C-2''** | **C-3''** | **C-4''** | **C-5''** | |
| Glucoside from literature (δ ppm) | 74.6 | 70.3 | 78.5 | 70.0 | 70.0 | |
| Glycoside (δ ppm) | 73.5 | 66.9 | 80.0 | 66.7 | 69.43 | |




## CONCLUSION

Our pharmaceutical industries continuously search new lead molecules having better therapeutic action and fewer side effects. In recent years lead molecules from natural origin had gaining more popularity due to less side effect and better therapeutic action.

Recently, ethno-botanical and traditional use 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.

The best classical approach is the isolation of the compound of flavone glycoside like 5,7 di-O-methyl luteolin-6-C-(3''-O-benzoyl)-β-d-xyloside from flowers of *Cucumis sativus* flowers for management of various diseases.

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**Source of Support:** Nil, **Conflict of Interest:** None.