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Isolation, identification and anti-microbial studies of Baicalein-7-O-glucuronide from *Gymnema sylvestre*

Kannan Thangaiyan Department of Chemistry, Government Arts College, Karur, Tamilnadu, India - 639 005

Srinivasan Ramanujam Department of Chemistry, Government Arts College, Karur, Tamilnadu, India - 639 005

ABSTRACT

Objective: To isolate a flavonoid glycoside from flowers of *Gymnema sylvestre* using chromatography separation techniques, spectral characterization and its anti-microbial activity.
Method: The structure of the isolated compound was purified and characterized by chemical tests, HPLC and spectroscopic methods such as UV, IR, ^{1}H-NMR and ^{13}C-NMR. Anti-microbial activity of a drug determined by using disc diffusion method.
Results: HPLC analysis showed the presence of a flavone glycoside and it's identified peak with the retention time of 13.82 min and exhibited two major peaks at 330 nm (band-I) and 230 nm (band-II).
Conclusion: The flowers of *Gymnema sylvestre* have been found to contain Baicalein-7-*O*-glucuronide. The drugs are effective at a concentration of 200 mg/ml for *Streptococcus pyogenes* and concentration of 100 mg/ml *Pseudomonas aeruginosa* consider to better results were observed. The resulting information will contribute to a better appreciative of the anti-microbial activity of the plant *Gymnema sylvestre*.

KEYWORDS Anti-microbial activity, Baicalein-7-*O*-glucuronide, *Gymnema sylvestre*, HPLC

INTRODUCTION

Medicinal plants are important sources for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents[1]. Nowadays, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. Secondary metabolites like alkaloids, terpenoids, phenolics, steroids and flavonoids play an important role in interaction of the plant with its environment[2].

Microbiological growth commonly induces unwanted organoleptic and appearance change. In classifying the anti-bacterial activity as gram-positive or gram-negative, it would generally be expected that a much greater number would be active against gram-positive than gram-negative bacteria[3]. However, treatment of infections has been remarkably effective since the discovery of anti-bacterial drugs of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases[4].

Gymnema sylvestre (Asclepiadaceae) a vulnerable species is a slow growing, medicinal woody climber found in India. It has been used as a natural treatment for diabetes for nearly two millennia and commonly known as gudmar. The plant has been reported to possess antiviral effect[5]. Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory and central nervous system activities [6, 7]. More recently, drug discovery techniques have been applied to the standardization of herbal medicines to elucidate analytical marker compounds[8]. By considering these above and other factors the present investigation was undertaken to screen the anti-microbial activity of Baicalein-7-O-glucuronide isolated from the flower extracts of *Gymnema sylvestre*.

MATERIALS AND METHODS

Collection of plant material

The fresh flowers of *Gymnema sylvestre* were collected in the month of September - November from the area of Kollidam river basin, Thanjavur, Tamilnadu, India. These Plants were identified and authenticated by Dr. S. Sambathkumar, Assistant Professor, Department of Botany, Government Arts College, Thiruvannamalai, Tamilnadu, India. The voucher specimen (GACBOT-124) was maintained in our research laboratory for future reference. The collected fresh flower materials were washed properly and dried in shade. Dried plant material was subjected to reduction to coarse powdered and stored in airtight container for further use.

Isolation and Identification

The important stage in the experimental work includes first the isolation of chemical substances from the chosen plant and secondly, the characterization of those isolated compounds. The flowers *Gymnema sylvestre* (2.5 Kg) were extracted with 90% methanol (MeOH) (5 X 500ml) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was fractionated with peroxide free ether (4 x 250 ml) and ethyl acetate (6 x 250 ml) (Sigma Aldrich Co., India).

The residue from ethyl acetate fraction was taken up in Me_2CO and left in an ice-chest for two days when a yellow solid separated. It came out as yellow plates a recrystallization from hot Mooch. It developed a greenish brown colour with alc. Fe^{3+}, formed yellow precipitate with basic lead acetate solution and reduced ammoniacal $AgNO_3$ but not Fehling's solution. It gave yellow colour with aqueous NaOH intense yellow with Con.H_2SO_4 and magenta colour with Mg/HCl. It appeared deep purple under UV which turned yellow an exposure to NH_3. It responded to Wilson's Boric Acid, Gibbs and Molisch's tests but not answer to Horhammer- Hansel tests.

Supporting evidence for the structure of the flavonol glycoside is provided by the HPLC (Shimadzu, Columbia), UV (Perkin Elmer Spectrophotometer), IR (Perkin - Elmer spectrometer) and NMR (500 MHz, DMSO-d_6 and TMS) spectral data were recorded on a Bruker AMX 500 NMR spectrometer. Chemical shifts were reference to the respective residual solvent peaks and the values were recorded in δ.

Baicalein-7-*O*- glucuronide

Yellow solid crystals; m.p. 204-206°C; RT 13.82 min; UV *MeOHmax*l (log ε) 230, 260 sh, 330 nm; IR (KBr): ν_{max}3367, 2990, 2372, 2088, 1763, 1623, 1458, 1377, 1242, 1056, 854 and 634 cm^{-1}; ^{1}H - NMR spectrum δ (500 MHz, DMSO-*d*6): Figure 1; ^{13}C - NMR (125 MHz, DMSO-*d*6): Figure 2.

Hydrolysis of the glycoside

The glycoside dissolved in hot aqueous methanol was hydrolyzed with H_2SO_4 (5%) at 100°C for about 2 hrs. The excess of alcohol

was distilled off *in vacuo* and the resulting aqueous solution was extracted with ether. The residue from ether fraction was isolated as described below. The glycoside was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexane and the resulting solution extracted with ethyl acetate.

Phytochemical screening of plant extract

A small amount of the dry extract was used for the phytochemical tests[9] for compounds which include alkaloids, flavonoids, saponins and glycosides while steroids and tannins are absent in all the crude extracts.

Anti-microbial activity by disc diffusion method

Anti-microbial activity of a drug determined by using disc diffusion method.[10] The test organisms were sub-cultured using nutrient agar medium.[11] The tubes containing sterilized medium were inoculated with the respective bacterial strain. After incubation at 37ºC ±1ºC for 18 hours, they were stored in a refrigerator. The nutrient agar medium was sterilized by autoclaving at 121ºC for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot-air oven at 160 ºC, for an hour. Into each sterilized petriplates (20 cm diameter), was poured about 125 ml of molten nutrient agar medium which was already inoculated with the respective strain of bacteria (5 ml of inoculum to 250 ml of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow the solidification. After solidification, the paper discs containing the derivatives were placed at different areas on the surface of each plate and labelled accordingly. The isolated compounds from chosen plants have been investigated for their anti-microbial activity. This informs the basis for testing the anti-microbial activity against the three Gram-positive bacteria viz., *Streptococcus pyogenes, Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria viz., *Escherichia coli* and *Pseudomonas aeruginosa* by using the disc diffusion method. Ciprofloxacin was used as reference standard for comparing the results.

Each test compound was dissolved in dimethyl sulfoxide (5 ml) to give a concentration of 1000 µg/ml. Ciprofloxacin solution was also prepared to give a concentration of 1000 µg/ml in sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 to 3 by using conc.HCl. The solutions of each test compound, control and reference standard were added separately in the cups and the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37±1^{0}C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

RESULTS AND DISCUSSION

Chemical constituents

Baicalein-7-*O*-glucuronide has been isolated from the fresh flowers of *Gymnema sylvestre*. Its melting points (204-206°C) were compared using pure chemical of Baicalein-7-*O*-glucuronide (95%) purchased from Sigma Aldrich Co (India) as external standard and found to have the same values. The UV spectrum of the compound exhibited two absorption peaks at 330 nm and 230 nm showing a flavone skeleton. The flavone oxygenated in A - ring, but not in the B - ring, tends to stretch spectra in methanol with an predictable band II and a weak band I. The IR spectrum shown the presence of ν_{max}3367, 2990, 2372, 2088, 1763, 1623, 1458, 1377, 1242, 1056, 854 and 634 cm^{-1}. HPLC analysis showed the presence of a flavone glycoside. The identified peak with the retention time of 13.82 min. Analysis of ^{1}H and ^{13}C-NMR data (Figure 1 & 2) shown that the aromatic signals are close to those reported for baicalein-7-*O*-glucuronide. The 5-OH proton resonates at δ 12.355 ppm. The signals at δ 7.041-7.898 ppm corresponds to the aromatic protons. The ^{13}C NMR data showed that were 21 carbons in this structure, 15 of which were typical for a flavone skeleton and the others were assigned to glycoside. The sugar carbon signals exhibited 119.53, 114.64, 89.84, 76.81, 76.61 and 50.80 ppm are comparable with those reported for *O*-glucoside[12]. The ^{1}H NMR spectrum revealed the presence of anomeric proton signal at δ 5.132 ppm indicated the presence of *O*- linked sugar. It has ascertained that the sugar moiety bonded to hydroxyl group at C-7 of the aglycone as deduced from the correlation between the anomeric proton at δ 5.132 ppm and the C-7 at δ 138.31 ppm. We have referred for these flavonoid extracted from *O. indicum*[13].

By comparing all the above mentioned physical and chemical part of evidences the flavonoid obtained from *Gymnema sylvestre* flowers has been characterized as the Baicalein-7-*O*-glucuronide (Figure 3)

Anti-microbial activity

The anti-microbial activity of Baicalein-7-*O*-glucuronide isolated from *Gymnema sylvestre* was studied in three different concentrations (50, 100 and 200 mg / ml) and methanolic extract at 300 mg / kg against five pathogenic bacterial strains (Figure 4). All the bacterial strains were selected for the basis of its application purpose of further formulation study. Anti-microbial potential of test samples were assessed in terms of zone of inhibition of bacterial growth and the results were compared with standard (Ciprofloxacin). The results of the anti-microbial activities are presented in Table 1.

The test samples doses at 50, 100 and 200 mg revealed moderate anti-microbial activity with zone of inhibition ranging from 2.0 to 11.0 mm and had shown to all pathogens. The results revealed that in the methanol extracts for bacterial activity shows inhibition zone measured from 2.0 to 9.0. These observations may be due to the nature of biological active component and the stronger extraction capacity of methanol could have been produced active constituent that are responsible for anti-bacterial activities. It may be due to the presence of broad spectrum of anti-microbial compounds in the flowers of *Gymnema sylvestre*. The present study reported, isolated compound Baicalein-7-*O*-glucuronide and methanol extracts of *Gymnema sylvestre* showed considerable inhibitory activity against both *Streptococcus pyogenes* and *Pseudomonas aeruginosa*.

CONCLUSION

On the basis of the results reached in the present investigation, it is possible to conclude that the Baicalein-7-*O*-glucuronide was found to have anti-microbial activity for both gram positive and gram negative organisms. Our results indicate the potential utility of *Gymnema sylvestre*, in the treatment of various bacterial infections. The drugs are effective at a concentration of 200 mg/ml for *Streptococcus pyogenes* and concentration of 100 mg/ml *Pseudomonas aeruginosa* consider to better results were observed. The resulting information will contribute to a better appreciative of the anti-microbial activity of the plant *Gymnema sylvestre.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

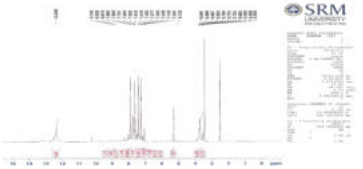

Figure 1: ^{1}H - NMR spectrum of Baicalein-7-*O*- glucuronide from *Gymnema sylvestre*

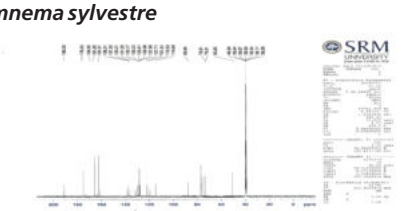

Figure 2: ^{13}C - NMR spectrum of Baicalein-7-*O*- glucuronide from *Gymnema sylvestre*

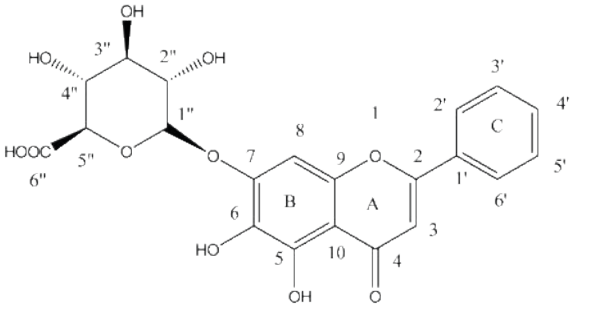

Figure III: Structure of Baicalein-7-O-glucuronide

Table 1. Anti-microbial activity of Baicalein-7-*O*-glucuronide extracted from *Gymnema sylvestre*

S. No.	Micro organisms	Zone of inhibition mm in diameter (M ± SD)				
		Baicalein-7-*O*-glucuronide			Methanolic Extract (300 mg)	Standard Ciprofloxacin (30 mg)
		50 mg	100 mg	200 mg		
1	*Streptococcus pyogenes*	03	10	11	09	18
2	*Bacillus subtilis*	04	01	02	02	16
3	*Staphylococcus aureus*	03	02	04	05	21
4	*Escherichia coli*	03	-	02	-	12
5	*Pseudomonas aeruginosa*	04	09	06	08	22

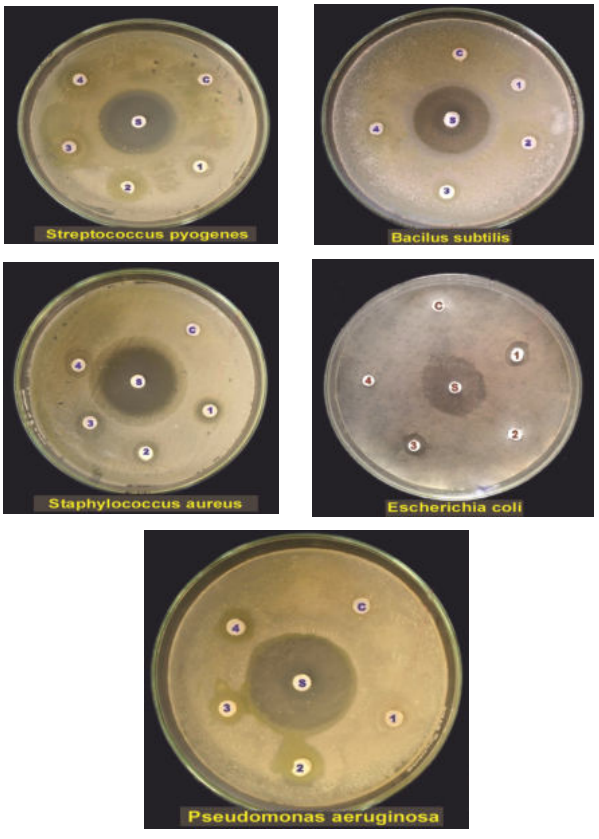

Figure 4. Anti-microbial activity of Baicalein-7-*O*-glucuronide extracted from *Gymnema sylvestre*

S – Standard; 1 – 50 mg; 2 – 100 mg; 3 – 200 mg; 4 – Methanolic extract (300 mg)

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