Research Article

Antibacterial Activity of a New Flavone Glycoside from the Seeds of *Dolichos lablab* LINN.

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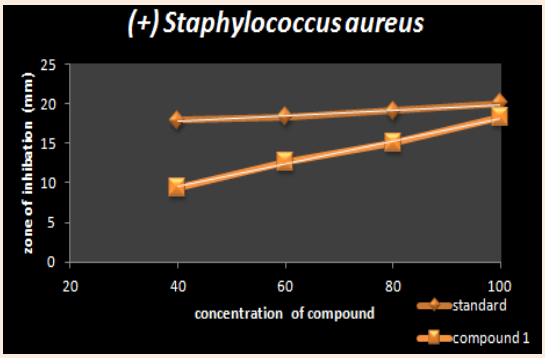
Abstract

A new flavonolglycoside 3,7-dihydroxy-5,6-dimethoxyflavone-7-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-galactopyranoside isolated from the seeds of *Dolichos lablab* Linn. by various chemical degradations, colour reaction and spectral analyses. .In present paper, it was screened against *Bacillus coagulas*, *Staphylococcus aureus*, *Escherichia coli* and *Psedomonas aerugenosa* by using paper disc diffusion method. Results were compared with the zone of inhibition produced by commercially available standard antibiotic. It was observed that it showed good activity against these microbes.



Keywords: antibacterial activity, flavonol glycoside, *Dolichos lablab* Linn.

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Introduction

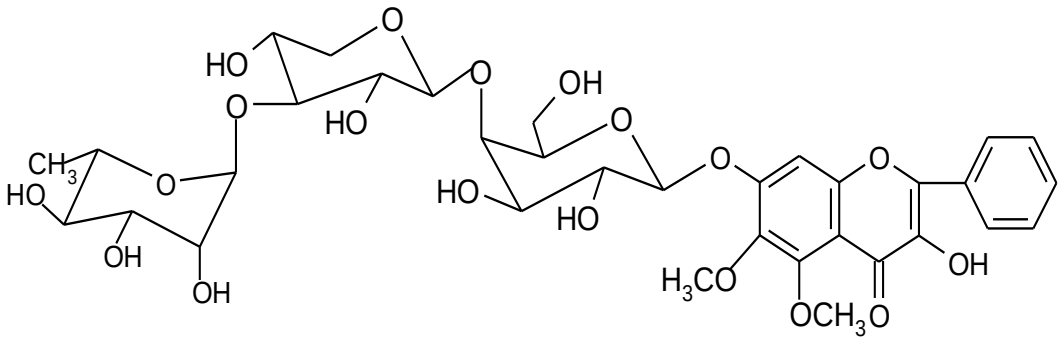
Flavonoids play an important role against microorganisms. Many studies suggested that flavonoids of plants belonging to various families exhibit antimicrobial activity against Gram-positive, Gram-negative bacteria and fungal pathogen [**1-2**]. They have many biological effects including antimicrobial [**3**], antiviral [**4**], anti-inflammatory [**5**], antimutagenic [**6-7**], anticancer [8], antiulcerogenic [**9**], antioxidant [10] and antitumor [**11**] activity and are of great interest in the investigation of disease processes and as potential new drugs.

Flavonoids are polyphenolic compounds isolated from wide varieties of vascular plants with over 8000 individual compounds known [**12**]. Flavonoids are plant pigments which occur in the plant kingdom and occur naturally in free state (aglycone), or as glycosides, or associated with tannins, possessing secretory structures. The most common classes are flavonol, flavones and their dihydroderivatives followed by anthocyanins, flavans and isoflavans. They are found in vegetables, fruits, seeds, nuts, grains, spices and different medicinal plants as well as in specific beverages such as red wine, tea and unfiltered beer [**13**]. They are important constituents of the non energetic parts of the human diet, the average intake being around of 600 mg/day[**14]**.

Dolichos lablab Linn.[**15-17]** belongs to Leguminosae family. It is known as 'Lobiya' in Hindi. It is a tall nearly glabrous twining, perennial or annual herb, with sm oothy or downy stem and contain. Its seeds are globose, ovate or flattened varying in colour from white to dark black. It is cultivated throughout India and tropical and temperate regions of Asia, Africa and America.Its seeds are used as diuretic, stomachic and antiplasmodic. Its leaves are used as alexipharmic and given in colic. Its seeds are useful in inflammations. Ayurvedic system of medicine describes that the seeds of this plant are used in treatment of burning sensation and diseases of the blood.

We have already reported the isolation and structural elucidation of a new flavonol glycoside[18] from the seeds of this plant. The methanol fraction of the defatted seeds of the plant afforded a new flavonol glycoside**(1)**, which had

molecular formula $C_{34}H_{42}O_{19}$, m.p. 203-204°C and $[M]^+$ 754(FABMS). Its structure has been characterized as 3,7-dihydroxy-5,6-dimethoxyflavone-7-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-galactopyranoside by various chemical degradations, colour reactions and spectral analyses[18]. The present work deals with the screening of this new flavonol glycoside(**1)** against *Bacillus coagulas, Staphylococcus aureus, Escherichia coli*. and *Psedomonas aerugenosa*.



(1)

Scheme 1 Structure of compound 1

Material and methods

Plant Material

The seeds of *Dolichos lablab* Linn. were collected from the Sagar region and authenticated by Taxonomist of the Department of Botany, Dr. H.S. Gour University, Sagar (M.P.)

Extraction and Isolation

Powdered air-dried seeds (3 Kg) of the plant were extracted with petroleum ether (40-60°C) in a Soxhlet extractor for 6 days. The total defatted seeds were successively extracted with chloroform, ethyl acetate, acetone and methanol. The chloroform, ethyl acetate and acetone soluble fractions after removal of the solvent gave very small quantity of the residue, therefore, these fractions were discarded. The methanol soluble fraction of defatted seeds of this plant was concentrated under reduced pressure to yield brown viscous mass, which was subjected to TLC examination using solvent system $CHCl_3$:MeOH:H_2O (8:6:2) and I_2 vapours as visualizing agent, gave two spots. Therefore, it was separated by column chromatography over silica gel and eluted with $CHCl_3$:MeOH in various proportions and purified by preprative TLC and studied separately. Compound **1** was analysed for m.f. $C_{34}H_{42}O_{19}$, m.p. 203-204°C and $[M]^+$ 754 (FABMS). It gave positive response to Molisch test for glycoside and characteristic colour reactions of flavonoids[19-20]. It was charecterised as 3,7-dihydroxy-5,6-dimethoxyflavone-7-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-galactopyranoside by various chemical degradations, colour reactions and spectral analyses.

Test microorganisms

The test microorganisms used were: *Bacillus coagulas*, *Staphylococcus aureus*, *Escherichia coli* and *Psedomonas aerugenosa.* Paper disc agar diffusion method[21] were used for in vitro evaluation of antibacterial activity.

Preparation of culture media

Peptone-beef extract has been used for study of antibacterial activity with the following composition:

Peptone	5.0 gm
Beef extract	3.0 gm

Sodium chloride	5.0 gm
Agar	15.0 gm
Distilled water	1000 ml

All the above ingredients were dissolved in distilled water and volume made up to 1 liter. The pH of the medium was adjusted to 7.2 with 1N NaOH.

Preparation of slants

5 ml of sterilized nutrient agar was poured in sterilized culture tube and allowed to cool. The tubes were incubated in electrically heated incubators at different temperature for various periods for different kinds of microorganisms depending upon the optimum growth of microorganisms.

Sterilization

The media and the slants were sterilized by autoclaving for about 15-20 minutes at 15 lb pressure for the preparation of subcultures of the organism. Petridishes were sterilized in an electrically heated oven at 125^{o}C for 8 hrs.

Preparation of agar plates

The sterilized media was cooled to 45^{o}C. Homogeneous suspension of each microorganism was mixed with sterilized media and 15 ml of this medium was poured into each sterilized Petridish and allowed to gel.

Standard drug used

Streptomycin was used as standard antibacterial agent.

Antibacterial activity

Table 1 Antibacterial Activity of Compound 1

Compd	Diameter of Zone of inhibition (mm)* against															
	(+) *Bacillus coagulas*				**(+) *Staphylococcus aureus***				**(-) *Escherichia coli***				**(-) *Psedomonas aerugenosa***			
	Concentration															
	100%	**80%**	**60%**	**40%**	**100%**	**80%**	**60%**	**40%**	**100%**	**80%**	**60%**	**40%**	**100%**	**80%**	**60%**	**40%**
1	18.0	16.2	12.7	8.8	11.4	8.4	3.4	-	10.5	7.8	4.7	1.4	15.3	10.4	8.1	6.0
****Std.**	21.2	20.0	19.5	18.0	20.0	19.1	18.3	17.9	18.3	17.0	15.6	14.0	23.1	21.0	19.5	17.0

*The zone of inhibition (mm) taken as average of four determinations in four different directions.
**Streptomycin used as standard antibacterial agent.
Petri plates were pre-seeded with 15 ml of growth agar medium and 1.0 ml of inoculum [**21-22**]. Paper discs of 6 mm diameter, which absorbs about 0.1 ml of test samples of the compound **1** and known quantity of standard reference antibiotic, were used. The inoculated plates were kept at 5 ºC for 40-45 minutes so as to allow the diffusion of the substances and then incubated at 36±1 ºC for 36 hours. The inhibition zones were measured in mm and the results obtained are recorded in the **Table 1** and compared with the standard reference antibiotic[22]. On the basis of **Table-I,** various graphs has been drawn to show the comparison of antibacterial activity between compound and standard drug against each single bacteria

Results and discussion

The results reported in **Table-1** showed that the compound **1** was found to be highly active against gram positive bacteria *Bacillus coagulas* even on dilute concentration while against gram positive bacteria *Staphylococcus aureus* exhibited significant activity only on higher concentration. The flavone and its different partitionates when subjected

to antimicrobial screening at 500 μg/disc revealed antimicrobial activity against the tested microorganisms having the zone of inhibition ranging from 3.4 to 18 mm for gram positive bacteria whereas 1.4 to 15.3 mm for gram negative. It also showed good activity against gram negative bacteria *Psedomonas aerugenosa* and *Escherichia coli.* Antimicrobial activity of protein of *D. lablab* has already reported by many researchers [24, 25].

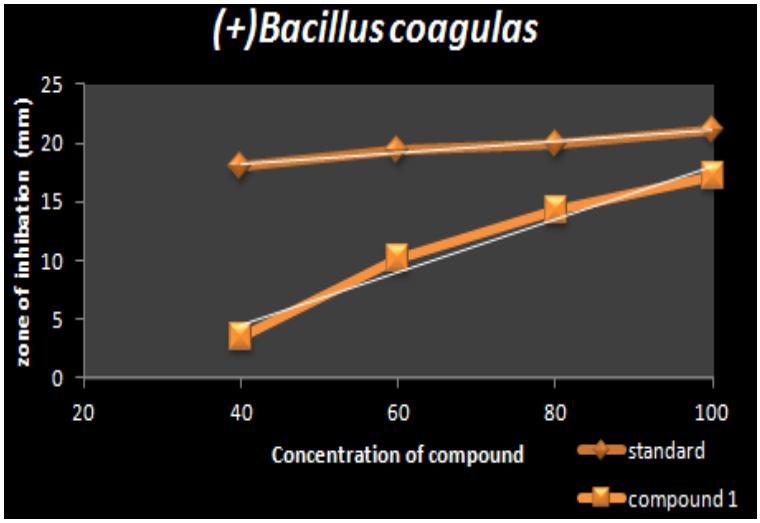


Figure 1 Screening activity of compound 1 with the spatial reference to streptomycin against *Bacillus coagulas*

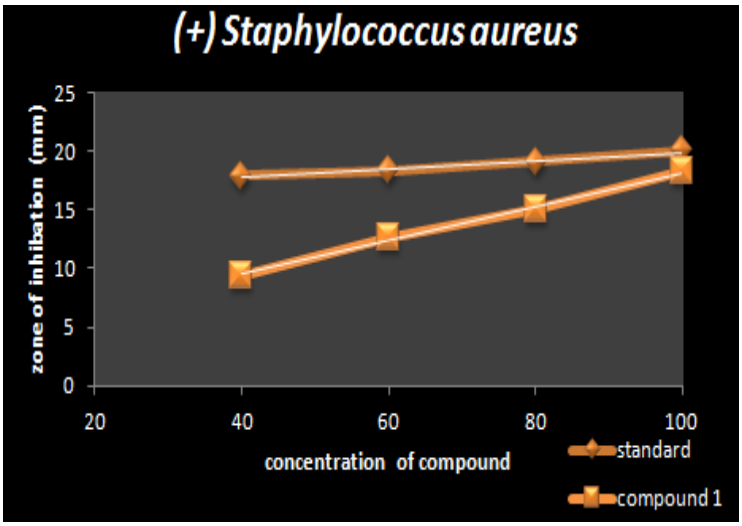


Figure 2 Screening activity of compound 1 with the spatial reference to streptomycin against *Staphylococcus aureus*

Thus on the basis of above results, it can be concluded that the above flavonoid glycoside **(1)** may be potentially used as antibacterial agent. It can be used to design different antimicrobial agents due to its moderate antimicrobial activity. This in vitro study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants in folk medicine suggests that they represent an economic and

safe alternative to treat infectious diseases.

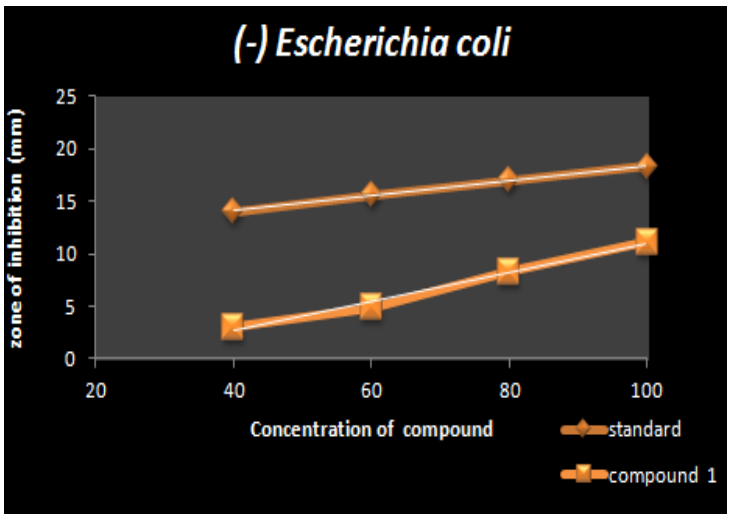


Figure 3 Screening activity of compound 1 with the spatial reference to and streptomycin against *Escherichia coli*

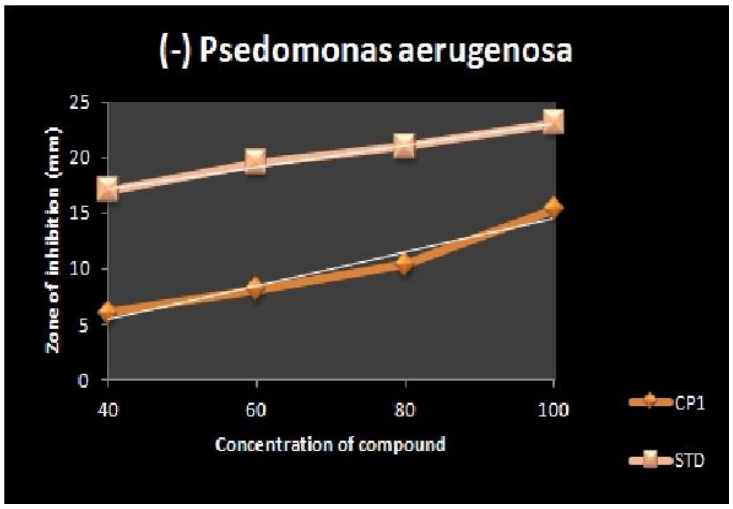


Figure 4 Screening activity of compound 1 with the spatial reference to streptomycin against *Psedomonas aerugenosa*

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