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Research Article

ANTIBACTERIAL ACTIVITY OF A NEW FLAVONE GLYCOSIDE FROM THE SEEDS OF *CASSIA SOPHERA* LINN.

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ABSTRACT
A new flavone glycoside 1, m.p. 248-251°C, m.f. $C_{33}H_{40}O_{20}$, $[M]^+$ 756 (FABMS) has been isolated from methanol soluble fraction of defatted seeds of *Cassia sophera* Linn.,which was characterized as 5,7,3',4'-tetrahydroxy-3-methoxyflavone-5-O-α-L-rhamnopyranosyl-7-O-β-D-glucopyranosyl (1→3)-O-β-D-xylopyranoside by various colour reactions, chemical degradations and spectral analyses.In present paper, it was screened against *Bacillus coagulas, Staphylococcus aureus, Escherichia coli.* and *Pseudomonas 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, flavone glycoside, *Cassia sophera* Linn.

INTRODUCTION

The natural products play an important role against microorganisms. Plants are unlimited source of natural products. They still form a major part of ingredient in almost all system of therapeutics. There are more than 350 thousand species of higher plants out of which a small portion has been investigated for their biologically active constituents.

The biological screening of indigenous drugs from plants was started in India by Col R.N. Chopra[1]. Kurup[2] studied the presence of antibacterial agent in Ayurvedic system of medicine. The antimicrobial activity of extracts of leaves of *Spondias mombrin* and *Alchornea cordifolia* have been carried out by Ajao and his coworker[3].Among the numerous products identified from medicinal plants, flavonoids represent one of the most interesting groups of biologically active compounds. Flavonoids are polyphenolic compounds isolated from wide varieties of vascular plants with over 8000 individual compounds known[4]. 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. Flavonoids 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[5]. They are important constituents of the non energetic parts of the human diet, the average intake being around of 600 mg/day[6]. Protective role of flavonoid intake against coronary heart diseases has been reported in epidemiological studies[7]. Several flavonoids have been considered valuable phytomedicines for different body system: urinary, digestive, cardiovascular, nervous and skin[8]. Various flavonoids isolated from the plants have been shown to antimicrobial[9-10], antiviral[11], antiinflammatory[12], antimutagenic[13-14], anticancer[15], antiulcerogenic[16], antioxident[17] and antitumor[18] activity. *Cassia sophera* Linn.[19-20] (Leguminosae) is commonly known as 'Kasundi' or 'Banar' in Hindi. It is a shrub or under shrub 2.4-3 m high, annual or perennial. It is distributed throughout India and in most tropical countries. Its bark and seeds are useful in diabetes. Its leaves are used externally in ringworm. Decoction of plant is used in acute bronchitis. Its bark, leaves and seeds are used as cathartic. We have already reported the isolation and structural elucidation of a new flavone glycoside[21] from the seeds of this plant. The methanol fraction of the defatted seeds of the plant afforded a new flavones glycoside**(1)**, m.p. 248-251°C, $C_{33}H_{40}O_{20}$ and $[M]^+$ 756 (FABMS), which was charecterised as 5,7, 3', 4'-tetrahydroxy-3-methoxyflavone-5-O-α-L-rhamnopyranosyl-7-O-β-D-glucopyranosyl(1→3)-O-β-D-xylopyranoside by various colour reactions chemical degradations and spectral analyses[21]. In the present paper, we report the antibacterial activity of this new flavone glycoside **(1)**.

MATERIAL AND METHODS

Plant Material

The seeds of *Cassia sophera* were procured from M/S United Chemicals and Allied Products Kolkata and were taxonomically authenticated by the Department of Botany, Dr. H.S. Gour University Sagar (M.P.), India. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar (M.P.), India.

Extraction and Isolation

Air-dried powdered seeds (3 kg) of the plant were extracted with petroleum ether (40-60°C) in a Soxhlet apparatus for seven days. The defatted seeds successively extracted with C_6H_6, $CHCl_3$, CH_3COOCH_3, CH_3COCH_3 and methanol. The methanol soluble fraction of the plants was concentrated under reduced pressure which showed two spots on TLC examination using solvent system $CHCl_3$:MeOH (4:6) indicating it to be a mixture of **1** and **2**. These compounds were separated and by column chromatography over silica gel and purified by preprative TLC and studied separately. Compound **1** was charecterised as 5,7,3',4'-tetrahydroxy-3-methoxyflavone-5-O-α-L-rhamnopyranosyl-7-O-β-D-glucopyranosyl (1→3)-O-β-D-xylopyranoside by various colour reactions chemical degradations and spectral analyses[22].

Test microorganism

The test microorganism used were: *Bacillus coagulas, Staphylococcus aureus, Escherichia coli*. and *Psedomonas*

aerugenosa. Paper disc agar diffusion method[22] 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 litre. 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°C for 8 hrs.

Preparation of agar plates

The sterilized media was cooled to 45°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

Petri plates were pre-seeded with 15 ml of growth agar medium and 1.0 ml of inoculum[22-23]. 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-I** and compared with the standard reference antibiotic[24]. 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.

TABLE-I ANTIBACTERIAL ACTIVITY OF COMPOUNDS

Compound	Diameter of Zone of inhibition (mm)* against															
	(+) *Bacillus coagulas*				(+) *Staphylococcus aureus*				(-) *Escherichia coli*				(-) *Psedomonas aerugenosa*			
	Concentration of compound				Concentration of compound				Concentration of compound				Concentration of compound			
	100%	**80%**	**60%**	**40%**	**100%**	**80%**	**60%**	**40%**	**100%**	**80%**	**60%**	**40%**	**100%**	**80%**	**60%**	**40%**
Pa	17.2	14.3	10.1	3.5	18.3	15.0	12.7	9.4	11.1	8.3	4.8	3.0	9.8	7.0	3.1	-
****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.

RESULTS AND DISCUSSION

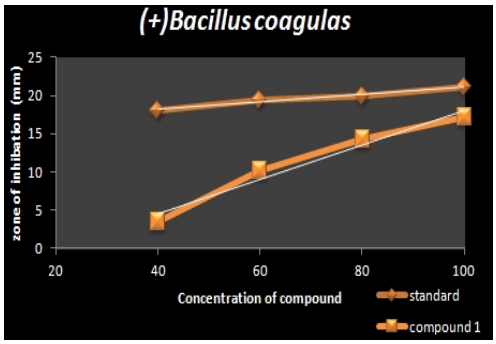

Fig 1. Screening activity of compound 1 with the spatial reference to streptomycin against *Bacillus coagulas*

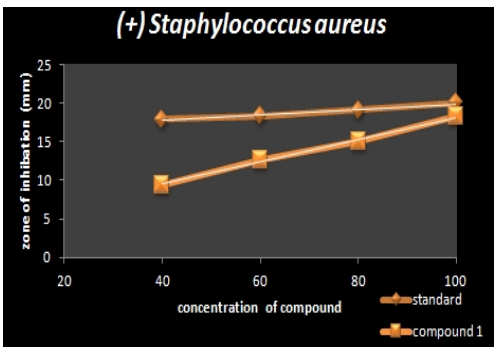

Fig 2 Screening activity of compound 1 with the spatial reference to streptomycin against Staphylococcus aureus

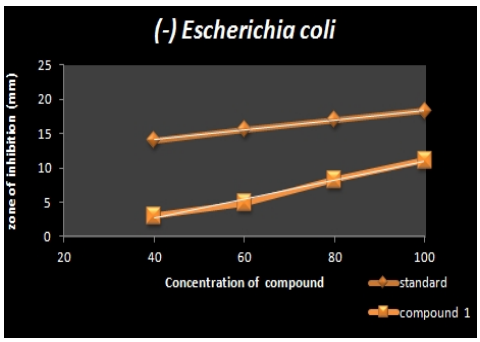

Fig 3. Screening activity of compound 1 with the spatial reference to and streptomycin against *Escherichia coli.*

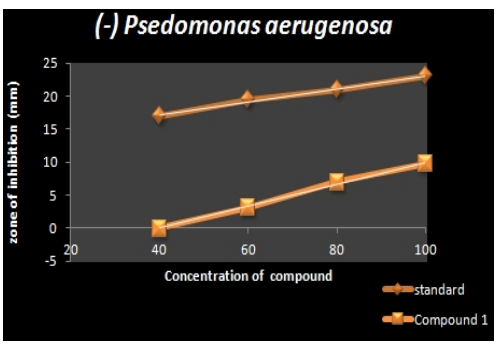

Fig 4. Screening activity of compound 1 with the spatial reference to streptomycin against *Psedomonas aerugenosa*

The results reported in **Table-1** showed that the antibacterial activity of compound **1**, was found to be fairly good against gram positive bacteria *Staphylococcus aureus* and *Bacillus coagulas*. It also exhibited good activity against gram negative bacteria *Escherichia coli* and it showed significant activity against gram negative bacteria *Psedomonas aerugenosa* only on higher concentration.Thus on the basis of above results, it is obvious that the above flavonoid glycoside **(1)** may be potentially used as antibacterial agent.

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