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Antimicrobial study of luteolin 7-O-α-L-rhamnopyranoside from heartwood of *Terminalia bellerica* (Roxb.)

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***Terminalia bellerica* (Roxb.) is an important indigenous deciduous medicinal plant and its fruit is an important ingredient of herbal formulation '*Triphala*'. In our study, we isolated compound luteolin 7-O-α-L-rhamnopyranoside from the chloroform: ethyl acetate [in the ratio of 70:30] fraction of 95% ethanolic extract of *Terminalia bellerica* heartwood. Further studies indicated that luteolin 7-O-α-L-rhamnopyranoside has an anti-bacterial effect against *Escherichia coli, Salmonella Stanley, Bacillus anthracis* and *Klebsiella pneumonia*. In addition to that luteolin 7-O-α-L-rhamnopyranoside has an anti-fungal activity against *Aspergillus niger, Aspergillus flavus, Chrysoporium tropicum*. The results in the present study clearly indicate that the isolated compound possess potential broad spectrum antimicrobial activity.**

Keywords: *Terminalia bellerica*, antimicrobial, luteolin 7-O-α-L-rhamnopyranoside.

Terminalia bellerica (Roxb.) plant is used as medicine since ancient times. Traditionally it was used in Asian countries as a constituent of '*Triphala*'. '*Triphala*' is commonly used in the disease related to eyes, liver and gastrointestinal tract[1]. *Terminalia bellerica* (Roxb.) belongs to natural order Combretaceae[2]. The plant is common throughout India in the plains and lower hills, chiefly in the deciduous forests, at 90 m elevation where the climate is not very dry. It is also found in forests of Burma and Sri Lanka[3]. Experimental studies have also proven that the fruit of this plant has anti-diabetic, anti-oxidant and anti-microbial properties[4]. Many groups of researchers have isolated and identified many flavonoids possessing antifungal, antiviral and antibacterial activity. An extensive review on the biochemistry and medical significance of flavonoids has been produced[5]. Numerous flavonoids with antimicrobial activity have been isolated from the genus *Terminalia*[6]. In the present study, we isolated luteolin 7-O-α-L rhamnopyranoside from the heartwood of *Terminalia bellerica* (Roxb.) and it was analyzed for its antimicrobial activity.

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MATERIAL AND METHODS

Plant material

The heartwood of *Terminalia bellerica* (Roxb.) was collected from the Gond berasia forest division, Bhopal, Madhya Pradesh, India in the middle of March, 2008 and identified by Professor Archna Shotriya, Government post graduate College Guna, Madhya Pradesh, India. A voucher specimen (TBH 73) has been deposited in the department of chemistry, Government M.L.B post graduate college, Bhopal, Madhya Pradesh, India.

Extraction and isolation

The dried and powdered heartwood (6.0 kg) of the plant were exhaustively extracted with 95% ethanol (Merck UN 1170, 99.9%) at 80°C using soxhlet apparatus, The solvent was evaporated under reduced pressure to obtain a crude residue (410 g), which was chromatographed on a column of silica gel (60-120 mesh LR) using chloroform (Merck HPLC, grade 99.0-99.4%), and ethyl acetate (Rankem HPLC grade 99.8%) in varying proportions Isolated noval compound luteolin 7-O-α-L-rhamnopyranoside from chloroform:ethyl acetate fraction (70:30) has been tested for antimicrobial activity.

Antibacterial and antifungal screening

The antimicrobial testing of luteolin 7-O-α-L-rhamnopyranoside was done by "Disc Diffusion Method". It is a commonly used method[7], it has been used by other workers with some modifications[8,9] . Bacterial strains used were *Escherichia coli, Salmonella stanley, Bacillus anthracis* and *Klebsiella pneumoniae.* Fungal strains used were *Aspergillus flavus, A. niger* and *Chrysrporium tropicum*. All microorganisms were supplied by the Institute of Microbial Technology, Chandigarh, India.

In vitro antimicrobial and antifungal assays were carried out by adopting the disc diffusion methods for the noval compound luteolin 7-O-α-L-rhamnopyranoside on nutrient agar medium (NAM) for bacteria and Potato dextrose agar medium (PDAM) for fungus. After solidification of culture medium, petriplates were seeded with organism suspension uniformly with the help of rotator and sterilized cotton ball held up by sterilized forceps on the surface of media. The sterilized filter paper discs (6 mm) moistened with test compound luteolin 7-O-α-L-rhamnopyranoside (10 mg/ml) and solvent was left to evaporate. Different concentrations (eg. 50 µg/disc, 100 µg/disc, 200 µg/disc, 400 µg/disc) of test compound were used in the experiment and each test concentration had three replications. The incubation period for antibacterial and antifungal activity was 24 and 72 hours respectively. After incubation, the result was recorded as the diameter of the zones of growth inhibition surrounding the discs[10].

Standard antibiotic discs were also used to test the sensitivity of the bacterial strains towards standard antibiotics used for treatment. Streptomycin was used as positive controls for the bacteria while for the fungal species β-napthol was used as positive control.

RESULTS AND DISCUSSION

The in vitro antimicrobial activity of luteolin 7-O-α-L-rhamnopyranoside against microorganisms was qualitatively assessed by disc diffusion assay. In this screening, zone diameters and the presence or absence of inhibition zones were analyzed. The results of the antibacterial and antifungal activity are presented (Table 1 & 2). Luteolin 7-O-α-L-rhamnopyranoside showed considerable antibacterial and antifungal activity at concentrations of 200 and 400 µg/disc. Results demonstrated that the luteolin 7-O-α-L-rhamnopyranoside showed considerable growth inhibition of *Salmonella* (18.3) at higher concentrations (Table 1). Earlier workers reported that the another glycoside of luteolin (luteolin-7-O-glucoside) had displayed the highest antifungal activity against *Klebsiella pneumoniae, Salmonella typhimurium* and *Aspergillus niger*[11].

In the present study, luteolin 7-O-α-L-rhamnopyranoside showed significant inhibition of *Salmonella stanley, Klebsiella pneumoniae, Escherichia coli* and *Bacillus anthracis*. However, low concentrations of luteolin 7-O-α-L-rhamnopyranoside showed poor activity. This suggests that luteolin 7-O-α-L-rhamnopyranoside is one of the biologically active principles.

The antibacterial activity of flavonoid is probably due to their quealting ability of flavones to form complex with extracellular and soluble

Table 1. Antibacterial activity of luteolin 7-O-α-L-rhamnopyranoside

Diameter of the zone of inhibition (mm) luteolin 7-O-α-L-rhamnopyranoside

Name of the organism	200 µg/disc	400 µg/disc	Standard
Escherichia coli	16.5 ±0.32	15.4 ± 0.35	24.4 ± 0.40
Salmonella stanley	15.7 ± 0.61	18.7 ± 0.56	23.4 ± 0.40
Bacillus anthracis	13.7 ±0.51	16.8 ±0.45	21.5 ± 0.32
Klebsiella pneumoniae	11.5 ± 0.35	14.7 ±0.43	16.7 ±0.56

Values are given in the mean of triplicates ± SE. Zone of inhibition with diameter of the disc (6.0 mm). Standard, Streptomycin (400 ppm) for bacteria; Luteolin-7-*O*-glucoside (10 mg/ml).

Table 2. Antibacterial activity of luteolin 7-O-α-L-rhamnopyranoside

Diameter of the zone of inhibition (mm)luteolin 7-O-α-L-rhamnopyranoside

Name of the organism	200 µg/disc	400 µg/disc	Standard
Aspergillus flavus	11.4 ± 0.35	16.4 ± 0.42	19.3 ± 0.25
Aspergillus niger	11.8± 0.82	15.7 ± 0.75	17.3 ± 0.26
Chrysoporium tropicum	12.3 ± 0.46	14.5 ± 0.74	16.3 ± 0.18

Values are given in the mean of triplicates ± SE. Zone of inhibition with diameter of the disc (6.0 mm). Standard, β- napthol (1000 ppm) for fungi; Luteolin-7-O-glucoside (10 mg/ml).

proteins and with bacterial cell walls hence disrupting the microbial membranes (12). Among the fungal species tested, luteolin-7-O-glucoside exhibited maximum zone of inhibition on *Aspergillus flavus* (16.5) and *A. niger* (15.7).

The current study showed that the inhibitory zones produced by luteolin 7-O-α-L-rhamnopyranoside against bacteria was considerably lesser than that of the standard, streptomycin, a broad-spectrum antibacterial drug to which most gram-negative bacteria are highly susceptible and many gram-positive bacteria are susceptible or moderately susceptible (13). The inhibitory zones produced by luteolin 7-O-α-L-rhamnopyranoside against *A. niger* and *A. flavus* was considerably similar to that of the standard β-napthol, the most commonly used standard antifungal agent[14].

Earlier different glycoside of luteolin has gained economic importance due to their anti-malarial, anti-inflammatory and anti-oxidant properties. In the present study, This is the first study of novel compound luteolin 7-O-α-L-rhamnopyranoside showed as a antibacterial and antifungal agent.

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