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Bacteriostatic effect of Coumarin 2-(3,4- Dihydroxyphenyl)-3,5,7-Trihydroxy-4H-Chromen-4-*One* Isolated from the root extract of *Strychnos nux vomica*.

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Abstract

Introduction and Aim: Strychnos *nux-vomica* (S. *nux-vomica*) being Loganiaceae is a well-known herb in India as well in Srilanka, Northern and Southeast Asia America. The present investigation has been carried out to evaluate the bacteriostatic effects of **2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one** isolated from root ethyl acetate extract of S. *nux-vomica*.

Materials and Methods: Structure elucidation was carried out using ^{1}HNMR.The bacteriostatic effect of isolated compound at 75 and 100 µg/ml was evaluated using agar well diffusion method against MRSA (Methicillin Resistant Staphylococcus aureus, B. *subtilis*, B. *cereus*, P.*aeruginosa*, E.*coli*, S. *typhi*.

Result: Based on the result, we noted that, MRSA was most susceptible bacterial strain with 5.4mm and 11.3mm zone of inhibitions recorded at 75 and 100µg/ml respectively. Result is compared with known standard gentamycin sulphate.

Conclusion: In accordance to the result of the investigation, here we conclude that, the isolated compound is coumarin derivative compound and it exhibit significant activity against MRSA.

Keywords: Strychnos *nux-vomica,* MRSA, Agar well diffusion, ^{1}HNMR, Bacteriostatic

1.0 **Introduction**

Strychnos *nux-vomica* (S. *nux-vomica*) being Loganiaceae is a well-known herb in India as well in Srilanka, Northern and Southeast Asia America. This plant is extensively used as herbal and Ayurvedic medical system against different human alignments (1). The Ayurvedic system of medicine prescribes the purified form of S. *nux-vomica* for treatment of several diseases (2). The anti-inflammatory activity of S. *nux-vomica* has been reported in folk system of medicine. In addition, the alleviation of joint pains by the use of S. *nux-vomica* is also an important consideration in the traditional folk medicine (3). Wound healing and anti-ulcer properties of the leaf extracts and anti-cholera property of the root bark extract have been reported (4). Among the parts of this plant, seeds are used in the treatment of bronchitis, anaemia, diabetes, asthma, constipation, skin diseases, paralysis, nervous disorders (5). In addition, it has been also reported that rheumatism (6), eczema (7), chicken pox fever (8) are also alleviated by the use of seed extract of S. *nux-vomica*. The whole plant is used in the treatment of digestive problems, migraine, menopause or its related problems and also works against analgesic, inflammatory, depressant, convulsant problems (9-15). At the level of compound isolation, the different alkaloids belonging to Brucine and Strychnine group have isolated

from this plant and are reported for different pharmacological properties (16-19). With reference to the above text, most of the pharmacological evaluation of S. *nux-vomica* has been reported on the crude extracts or its crude drug formulations. The drug discovery requires novel compounds that show high therapeutic activity. Thus, the isolation of active principle responsible for a particular activity holds great importance in the plant medicinal chemistry. Now-a-days, bacterial infections and their subsequent multidrug resistance are the serious threats worldwide and made an urgent drug discovery with high therapeutic function. Bacteriostatic property (making bacterial growth inhibition and stay in the stationary phase is a basic assay to ascertain the antibacterial activity of any crude extracts or drugs. In the present investigation, we have determined the bactericidal efficiency of coumarin 2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one isolated from the root extract of S. *nux-vomica*.

2.0 **Materials and Methods**

2.1 Collection and Authentication of plant material

The S. *nux-vomica* root material was collected from Siddhapuram village, Warangal rural, Telangana. The authentication of the plant was carried out by the Dr. Sateesh Sutari, Botanical Taxonomist, Department of Botany, Kakatiya University, Warangal. The roots were bought to the laboratory and the associated soil was separated by gentle washing under running tap water. The material was dried under shadow. Approximately after 45 days, the dried root material was made fine powder.

2.2 **Extraction Procedure**

The fine root powder of about 250g of was packed in the thimble of soxhlet apparatus and extracted with the Petroleum ether, Ethyl acetate, toluene, Acetone and Methanol. The solvent was separated using rotary evaporator to collect crude extract. The crude extracts are preserved at -20 ^{0}C till their usage.

2.3 **Bio assay guided fractionation**

2.3.1 Elution of S. n*ux-vomica* Ethyl acetate Extract of Root (EER)

The column was filled with silica gel (100-200 mesh) by slurry method. Initially the silica gel was run with petroleum ether for 1 to 2h to set the column bed. The top of the bed approximately 2g of the EER was placed and eluted with tri mobile phase (Toluene (6mL): Ethyl acetate (1mL): Acetone (3mL). The total number of fractions collected was 8 and were denoted as (EER-1-EER-8). All the fractions were tested for antibacterial activity against MRSA (Methicillin Resistant Staphylococcus aureus, B. *subtilis*, B. *cereus*, P.*aeruginosa*, E.*coli*, S. *typhi*. This is an important method for separation of the non-active fractions.

2.3.2 Structure elucidation of the isolated compound

The structure of isolated compound was carried out using ^{1}HNMR

2.4 **Bacteriostatic assay of isolated compound**

2.4.1 Chemicals

Chemicals necessary for preparation of Nutrient Agar Medium (NAM) for bacteria cultivation were bought from Hi-media, Mumbai, India. Other chemicals required are purchased from Sd-fine chemicals Mumbai, India.

2.4.2 Procurement of Bacterial Strains

Bacterial strains for the screening of anti-bacterial capabilities of isolated compound were bought from Department of Microbiology, Kakatiya University, Warangal, Telangana, India. The selected strains are *B.subtilis*, MRSA (NCTC 13616), (ATCC 6633), *B. cereus*, (ATCC 14579), *S.typhi* (ATCC 19430), *E.coli* (ATCC 8739), *P. aeruginosa* (ATCC 27853). All the strains are inoculated on suitable nutrient media and maintained at 37^{o}C for further use.

2.4.3 Agar well Diffusion method

Agar well diffusion was performed to find out the bacteriostatic effect of the isolated compound. According to Chung *et al*. (1990), (20) 1 mL fresh culture of bacterial strains were transferred and spread on the Nutrient Agar Medium (NAM). Using sterile borer, approximately 6mm wells are created. All the wells are filled with isolated compound extract at 75, 100 µg/ml concentrations. The plates are incubated at suitable temperature for 24h. The zones of inhibition were measured in mm. The results are compared with known standard Gentamycin sulphate.

3.0 **Results**

3.1Elution of S. n*ux-vomica* Ethyl acetate Extract of Root (EER)

Among the collected fractions (EER-1-EER-8), the EER-5 showed significant activity against tested bacterial strains. 1.0 represents the antibacterial activity of the collected fractions. The active fraction (EER-5) was evaluated for TLC using Toluene (6mL): Ethyl acetate (1mL): Acetone (3mL) mobile phase. We found the single spot on the TLC sheet. Further, this compound was sent for structure elucidation by ^{1}HNMR spectra.

Table 1.0 Antibacterial activity of the fractions collected from Ethyl acetate extract of root

Fraction	MRSA	B. *subtilis*	B. *cereus*	P. *aeruginosa*	E. *coli*	S. *typhi*
EER-1	---	---	---	---	---	---
EER-2	---	---	---	---	---	---
EER-3	---	---	---	---	---	---
EER-4	---	---	---	---	---	---
EER-5	11.8	17.4	15.2	9.7	13.0	8.1
EER-6	---	---	---	---	---	---
EER-7	---	---	---	---	---	---
EER-8	---	---	---	---	---	---

3.2 Structure elucidation of the isolated compound

Based on the 1HNMR spectra data the isolated compound is identified as **2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one** (Fig 1.0).

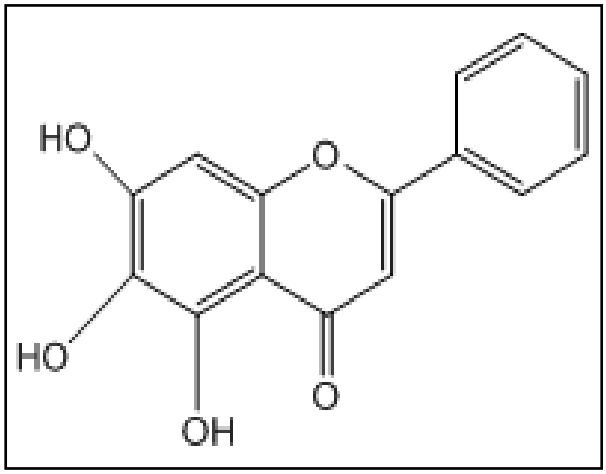

Fig 1.0 Structure of 2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one.

3.2.1 [1]HNMR Spectra

6.61 (s, 1H, Ar-H), 6.92 (s,1H, Ar- H),7.54-7.57 (m, 3H, Ar-H), 8.04-8.06 (d,2H,Ar-H) 8.80 (br, s,1H, -OH), 10.5 (br,s,1H,-OH), 12.62 (s, 1H, -OH) (Fig 2.0).

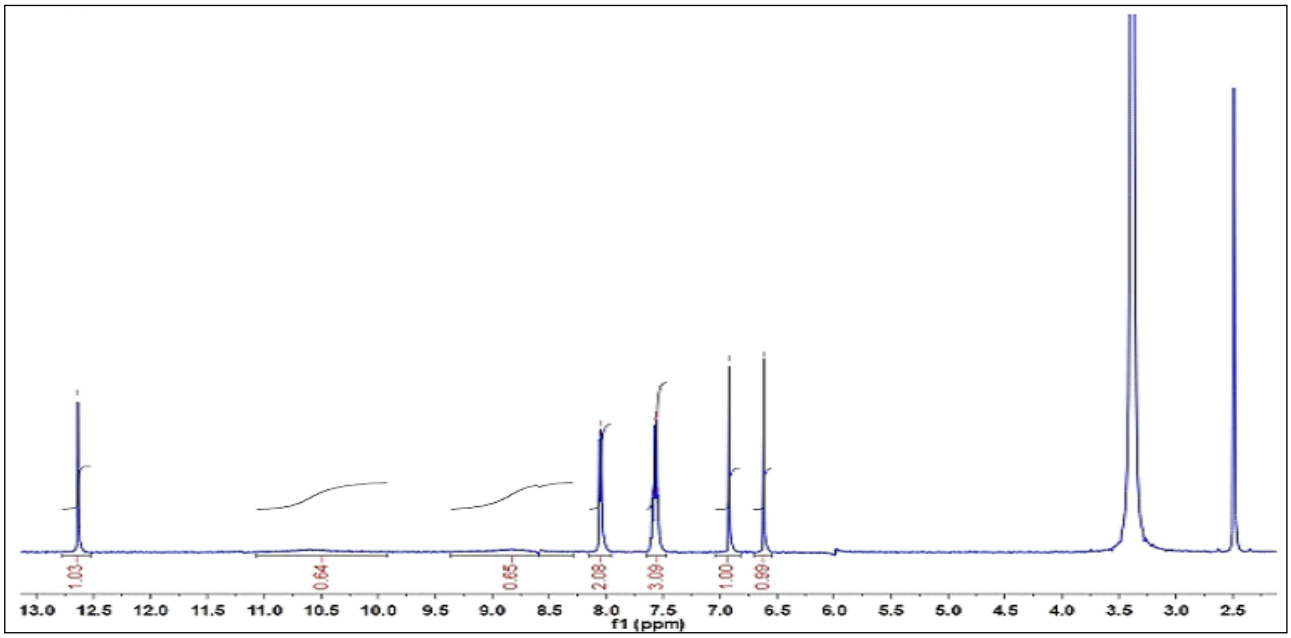

Fig2.0 [1]HNMR spectra of the 2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one.

3.3 Bacteriostatic assay of isolated compound

The bacteriostatic effect of the isolated compound 2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one was found concentration dependent against the tested bacterial stains. In accordance to our result, the bacteriostatic property of the isolated compound was wound significant against Methicillin Resistant Staphylococcus *aureus* (MRSA) with 5.4mm and 11.5mm zone of inhibitions recorded at 75 and 100µg/ml respectively. Following, B. *subtilis* and B. *cereus* are also highly susceptible towards the isolated compound with 9.2mm, 7.3mm and 6.9mm and 8.5mm zone of inhibitions recorded at 75 and 100µg/ml respectively. Whereas, E. *coli* was found susceptible with 6.5mm and 7.3mm zone of inhibitions recorded at 75 and 100µg/ml respectively. On the other hand, P. *aeruginosa* and S. typhi are found least susceptible towards the isolated compound (Table 2.0)

Table 2.0 Bacteriostatic activity of the 2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one against human pathogenic bacteria.

Zone of Inhibition (mm)							
Compound	**Conc (µg/ml)**	**MRSA**	***Bacillus subtilis***	***Bacillus cereus***	***Pseudomonas aeruginosa***	**E. *coli***	**S. *typhi***
Isolated compound	75	5.4	9.2	6.9	4.5	6.5	3.6
	100	11.5	7.3	8.5	5.0	7.3	4.3
Gentamycin sulphate	10	18.9	22.3	26.1	21.0	24.8	19.5
MRSA-Methicillin resistant *Staphylococcus aureus*							

4.0 **Discussion**

The present investigation was the extension of our previous work reported on the antibacterial activity of sliver nano particles prepared using S. *nux-vomica* crude extract (21). In this investigation coumarin derivate compound 2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one is isolated from the root ethyl

acetate extract of S. *nux-vomica* was evaluated for its attributed bacteriostatic efficiency against human pathogenic bacteria. With reference to the results obtained, the susceptibility nature of MRSA towards isolated coumarin derivate was found significant. It is evident that MRSA was highly susceptible towards root ethyl acetate AgNP's (21) and as well towards coumarin derivate isolated from ethyl acetate extract. The result is correlated with antibacterial activity of root ethyl acetate AgNP's against MRSA (22). The activity might be attributed to that hydroxyl groups of the compound possess the significant antibacterial or bacteriostatic properties. The bacteriostatic activity of the isolated coumarin derivative was probably attributed to its function groups. Naturally derived drugs such as from plants exhibit high therapeutic functions and are safer, affordable comparing to chemically derived drugs. Bacteriostatic effect of plants is majorly relay on their crude extracts. However, the isolation of the responsible agent for the activity is the first step in the development of novel drug. In future we will focus on the Bactericidal activity of the isolated compound.

5.0**Conclusion**

Basing on the result of the investigation, here we conclude that, the isolated compound is coumarin derivative compound and it exhibit significant activity against MRSA.

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Contributions

Thupurani Murali Krishna (TM) designed the work, Galla Praveen Kumar (GP) have carryout the work

Conflicts of Interest

The authors declare no conflicts of interest.

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