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Formulation of an antibacterial crop protectant using the acetylated derivative of 2,7,(14),10-Bisabolatriene-1,9,12-triol isolated from *Curcuma longa* L.

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ABSTRACT

The paper includes a comparative account of antibacterial assay of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol and its acetylated derivative isolated from Curcuma longa L. The results indicated that acetylated derivative of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol showed positive results in antibacterial assay. The antibacterial assay was also done with the 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol and no antibacterial acivity was found for this compound.

Key words: Antibacterial assay; Crop protectant; *Curcuma longa* L.; 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol; acetylated derivative of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol.

INTRODUCTION

The plant kingdom is a treasure house of potential drugs and there has been an increasing awareness about their importance of medicinal plants [1]. Plant is man's friend in survival, giving him food and fuel and medicine from the days beyond dawn of civilization [2]. Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health [3]. According to World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [4]. The pharmaceutical industry has come to consider them as a source of bioactiveagents, which have gained considerable importance due to their potential as antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti inflammatory activities [5].

The plant pathogenic bacterium studied in this paper mainly causing spots, blight, gall, rot etc of vegetables and crop plants. The bacterium *Serratia marcescens*, is a phloem inhabiting bacterium causing yellow vine disease of Cucurbits [6]. Another bacterium, *Erwinia herbicola* causes fire blight of pear and apple, Stewart's wilt in corn, and soft rot of fleshy vegetables [7]. The genus *Xanthomonas* causing numerous leaf spots, fruit spots, blights of annual and perennial plants, vascular wilts and citrus canker [8]. *Arthrobacter chlorophenolicus* causing bacterial blight of holly, is the cause of Douglas fir bacterial gall [9]. Turmeric has immuno enhancing properties [10]. The antimicrobial activity of ethanolic extract of turmeric was evaluated against several strains of bacteria and fungi [11, 12, 13, 14, 15]. The rhizome extract was effective against bacteria *Staphylococcus albus*, *E. coli*, and *Pseudomonas yocyanea*. The methanolic extract of *C. longa* inhibited the growth of *Helicobacter pylori* [16]. Singh et al. 2002 [17] evaluated the antibacterial potential of *C. longa* rhizome extracts against pathogenic strains of Gram positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram negative (*E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) bacteria. Here we report for the first time the antibacterial potentialities of acetylated derivative of 2, 7, (19), 10 Bisabolatriene- 1,9,12 triol [18], a bisabolane type sesquiterpene isolated from *C. longa* L. against four plant pathogenic bacterium.

MATERIALS AND METHODS

From the shade dried rhizomes of the plant 2, 7, (19), 10 Bisabolatriene- 1,9,12 triol was isolated, characterised and indentified by usual physicochemo spectroscopic methods [19]. The compound was acetylated and the process of acetylation was also explained in details [19].

Antibacterial assay of 2, 7, (19), 10 Bisabolatriene- 1,9,12 triol and its acetylated derivative

Microorganisms, culture media and incubating teparatures

The compound and its acetylated derivative were individually tested against a panel of microorganisms including Gram negative *Serratia marcescens* (MTCC NO. 7298) incubated at 30^0C, *Erwinia herbicola* (MTCC NO. 3609) incubated at 37^0C, *Xanthomonas* sp. (MTCC NO. 7444) incubated at 30^0C and Gram positive *Arthrobacter chlorophenolicus* (MTCC NO. 3706) incubated at 28^0C. All the bacterial strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference strains of bacteria were maintained on nutrient agar medium and LB medium slants at 4^0C with a subculture period of 30 days.

Composition of the media

Contituents	**Weight / Volume**	**Nutrient agar medium (pH 7.0)**
Beef extract	1.0g	After adjusting the pH, volume of the medium was adjusted to 1 liter by adding double distilled sterile water. Nutrient broth medium has the same composition without agar.
Yeast extract	2.0g	
Peptone	5.0g	
NaCl	5.0g	
Agar	15.0g	
Contituents	**Weight / Volume**	**LB agar medium (pH 7.0)**
Tryptone	10.0g	After adjusting the pH, volume of the medium was adjusted to 1 liter by adding double distilled sterile water. LB broth medium has the same composition without agar.
Yeast extract	5.0g	
NaCl	10.0g	
Agar	15.0g	

Preparation of McFarland standard

The turbidity standard was prepared by mixing 0.5 ml of 1.75% (w/v) $BaCl_2.2H_2O$ with 99.5 mL of 1% H_2SO_4.$BaSO_4$ (v/v). The standard was taken in screw cap test tube to compare the turbidity. The bacterial culture of selected strains were grown for 48- 72 hours and subsequently mixed with physiological saline. Turbidity was corrected by adding sterile saline until McFarland 0.5 $BaSO_4$ turbidity standard 10^8 Colony Forming Unit (CFU) per ml was achieved. These inocula were used for seeding of the nutrient agar medium, LB medium respectively.

Disc diffusion assay

1 mg of the compound and its acetylated derivative were separately dissolved in 1 ml of propylene glycol and then the volume was adjusted to 10 ml by adding sterile water. The ultimate concentration reaches to 10^3 µg/ ml and sterilized by filtration (0.22 µm millipore filter). The concentrations at 100 µg/ ml, 200 µg/ ml, 250 µg/ ml, 300 µg/ ml, 400 µg/ ml, 500 µg/ ml were taken in each case. The sterile paper discs (6 mm diameter) were saturated with 10 µl of the solution of the compound at a concentration of 100 µg/ ml, 200 µg/ ml, 250 µg/ ml, 300 µg/ ml, 400 µg/ ml, 500 µg/ ml and placed on the inoculated agar of 10^8 cfu/ml. Antibacterial tests were then carried out by disc diffusion method [20] using 100 µl of suspension containing 10^8 CFU/ml of bacteria on nutrient agar medium, LB medium respectively. Negative controls were prepared using propylene glycol. Gentamicin (10 µg/ disc) was used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 30^0 C, 37^0 C, 30^0 C and 28^0 C respectively for 48 h, 24 h, 48 h and 72 h. Antibacterial activity was evaluated by measuring the zone of inhibition and the diameters of these zones were measured in millimeters against the test organisms.

Determination of Minimum inhibitory concentration

The minimal inhibitory concentration (MIC) values were studied for the bacteria strains, being sensitive to the acetylated derivative in disc diffusion assay. The inocula of the bacterial strains were prepared from 24-72 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The compound and its acetylated derivative was dissolved in 1 ml of propylene glycol, were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial dilutions were made in order to obtain a concentration range from 500 to 100 µg/ml in 10 ml sterile test tubes containing nutrient broth and LB broth medium respectively. MIC values of the acetylated derivative against bacterial strains were determined based on a micro well dilution method as previously described [21]. The plate was covered with a sterile plate sealer and then incubated at appropriate temperatures for 24 - 72 h at 30^0 C, 37^0 C, 30^0 C and 28^0 C respectively. Bacterial growth was determined by absorbance at 600 nm and confirmed by plating 10 µl samples, forming clear wells on nutrient agar medium or LB medium respectively. The

MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. Each test in this study was repeated, at least, thrice.

RESULTS AND DISCUSSION

Antibacterial assay

Antibacterial assay was performed with 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol and its acetylated product. Results in table number 1 indicate that acetylated derivative of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol was positive in antibacterial assay and the MIC value was 220 µg/ml, 232 µg/ml, 245 µg/ml and 353 µg/ml for the bacterium *Serratia marcescens* (MTCC NO. 7298), *Erwinia herbicola* (MTCC NO. 3609), *Xanthomonas* sp. (MTCC NO. 7444) and *Arthrobacter chlorophenolicus* (MTCC NO. 3706) respectively (Table 2 & Figure 1), where as 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol was inert in its antibacterial property (Table 1).

Figure 1: MIC values (indicated by arrows) of the acetylated derivative of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol against four plant pathogenic bacteria

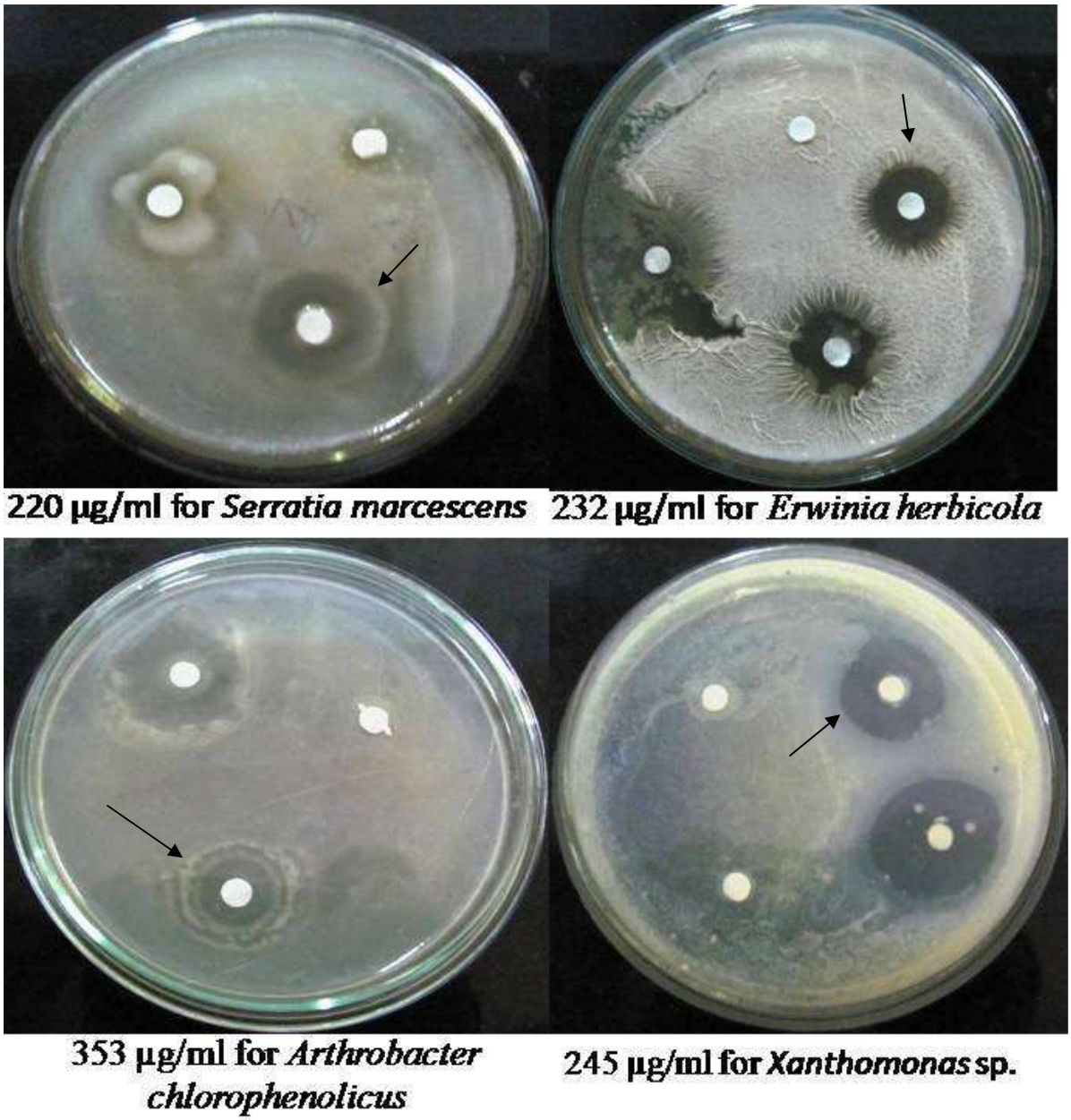

Table: 1 Antibacterial potentiality of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol

Test sample	Test bacterial strains			
2, 7, (14), 10 Bisabolatriene- 1,9,12 triol	Diameter of inhibition zone in mm			
Concentration (µg/ml)	*Serratia marcescens*	*Erwinia herbicola*	*Xanthomonas* sp.	*Arthrobacter chlorophenolicus*
500	No activity	No activity	No activity	No activity
400	No activity	No activity	No activity	No activity
300	No activity	No activity	No activity	No activity
250	No activity	No activity	No activity	No activity
200	No activity	No activity	No activity	No activity
100	No activity	No activity	No activity	No activity

Table: 2 Antibacterial potentiality of the acetylated derivative of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol (Mic values are indicaied within bracket)

Test sample	Test bacterial strains			
Acetylated derivative	Diameter of inhibition zone in mm			
Concentration (µg/ml)	*Serratia marcescens*	*Erwinia herbicola*	*Xanthomonas* sp.	*Arthrobacter chlorophenolicus*
500	20.7	18.3	19.1	16
400	20	17.1	16	14.2
353	19.1	16.9	15.1	12.3 **(MIC value)**
300	18.2	16.2	13	No activity
253	17.5	13	11.5	No activity
250	14.3	12.7	11	No activity
245	11.2	12	9.2 **(MIC value)**	No activity
232	8	10.5 **(MIC value)**	No activity	No activity
220	6.3 **(MIC value)**	No activity	No activity	No activity
200	No activity	No activity	No activity	No activity
100	No activity	No activity	No activity	No activity

Hence the paper may be cited as a formulation of antibacterial crop protectant against the common pathogens causing rot, blight and gall diseases in some vegetables as well as some crop plants. So it was concluded that the acetylated derivative of 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol possess antibacterial property.

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