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Evaluation of antibacterial activity of bioactive compound from ethanolic leaves extract of *Erythrina indica*

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Abstract: **The present study was to assess the antimicrobial activity of isolated bioactive compound from the ethanolic leaves extract of *Erythrina indica*. Bioactive fraction was identified as 13Docosenamide,(Z). Dosage dependent antibacterial activity of 13Docosenamide,(Z) was assessed against clinical isolates such as gram positive bacteria (*Bacillus subtilis, Streptococcus pneumonia* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Salmonella typhi, Salmonella paratyphi, Vibrio parahemolyticus* and *Vibrio cholera*). It was observed that, 13Docosenamide,(Z) was able to inhibit *Salmonella typhi* and *Salmonella paratyphi* completely at 400µg/ml concentration. Above 79% inhibition was seen in *Vibrio parahemolyticus* and *Vibrio cholera* and less than 50% inhibition was observed in *Escherichia coli* and *Bacillus subtilis* with the maximum tested concentration of 400µg/ml. Among the tested microorganisms *Klebsiella pneumonia, Proteus mirabilis, Streptococcus pneumonia* and *Staphylococcus aureus* were not inhibited by the 13Docosenamide,(Z). This study confirmed the antibacterial effect of isolated compound against selected microorganisms establishing the medicinal activity of *Erythrina indica*.**

***Keywords: Erythrina indica*, ethanol extract, 13Docosenamide,(Z), *Salmonella typhi, Salmonella paratyphi, Vibrio parahemolyticus* and *Vibrio cholera*.**

I. INTRODUCTION

Infectious agents like bacteria, virus and fungi are the causative agents for many life threatening diseases even with the advancement of medications and treatment [1]. These pathogenic microorganisms have been developing multi drug resistance, as a result there is a surge in research for a new anti microbial drug [2]. Due to the toxic side effects of modern (chemical) medicines, researchers turned aside their focus towards plants for discovering new compound with potential medicinal action [3]. Traditional utilization of plants for the treatment of common infectious microbial diseases is an age old practice [4]. The non toxic nature, easy availability and century old practice in culinary attracted the attention of scientific community to investigate the medicinal compounds present in herbal plants for the identification and the development of new drug.

The plant *Erythrina indica* belongs to Fabaceae family. It is commonly known as Indian coral tree; Paribhadra in Sanskrit [5] and Kalyana murungai in Tamil language. Traditionally the leaves were used as poultice to alleviate joint pain, oral injestion of leaves juice used to kill parasites, to stimulate menstruation and lactation and to heal wounds [5]. The antioxidant, anticancer [6], thrombolytic [7], anti alopecia [8] and antibacterial and antifungal [1] activity of *Erythrina indica* were reported. The progress in pharmaceutical research occurs on the identification, isolation and structural elucidation of compounds for the treatment [9]. So, the present study was aimed to test the antibacterial potency of a purified molecule from the leaves extract of *Erythrina indica*.

II. MATERIALS AND METHODS

Plant Collection:

The leaves of the plant of *Erythrina indica* were collected in and around the villages of Orathanadu and Pattukkottai taluk of Thanjavur district of Tamil Nadu. After identification by a botanist, the experimental processes were carried out. The leaves were examined carefully, any infected, damaged leaves were manually removed washed in tap water and distilled water, dried under shades and ground in a electrical grinder. The leaf powder was extracted thrice with 99% ethanol and the extract was lyophilized and the dried sample content was purified by using High Performance Liquid Chromatography and the bioactive fraction was identified, structural characterization was studied by FT-IR, NMR, GC and MS/MS and identified as 13Docosenamide,(Z) [10].

Microorganisms:

This purified compound 13Docosenamide,(Z) was tested against clinical isolates such as gram positive bacteria (*Bacillus subtilis, Streptococcus pneumonia* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Salmonella typhi, Salmonella paratyphi, Vibrio parahemolyticus* and *Vibrio cholera*). The above said pathogens were obtained from Sharmila Institute of Medicinal Products Research Academy, Thanjavur Dt, Tamil Nadu, India. The culture of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Antibacterial activity:

The antibacterial activity of 13Docosenamide (Z).was tested against bacterial strains by the microdilution method in 96 well flat bottom plastic tissue culture plates [11]. Nutrient broth medium (pH 7.0; 37°C) was used for culture condition. Sterile double strength culture medium of volume 125µl were placed in the first column of the 96 well plates and 125µl of single strength culture medium (sterile) in the remaining wells. Stock solution of 13Docosenamide,(Z) (3200µg/ml) in phosphate buffered saline, pH 7.0 was prepared, from this 125µl were added to the first column and mixed resulting in a stock concentration of 400µg/ml. Followed by the serial transfer of 125µl to the subsequent wells till tenth column, so the final volume of each well is 125µl. The concentration of stock substance in the first 10 columns were 400µg/ml to 780ng/ml. There was no test substance in column 11 and 12 and they serve as negative and growth controls. 2.5µl of overnight culture (10^8 cfu/ml) was inoculated in all wells except 11^{th} column. Microtitre plates were covered and incubated for 48h under appropriate growth conditions. Experiments were performed thrice for each strain and absorbance at 600 nm was measured, then growth inhibition percentage for each strain at different concentrations were calculated. The results were expressed as mean ±SD.

The density of viable pathogenic cells after treatment with 13Docosenamide,(Z) was visualized using Confocal laser scanning microscopy. After 48h incubation, bacterial smear from 20µl was prepared from the positive microtitre plates on glass microscopic slides and fixed with 2% glutaraldehyde in phosphate buffered saline for 15 minutes. 0.01% acridine orange in phosphate buffered saline was used to fix. The stained bacterial smears were visualized by Confocal laser scanning microscopy [12].

III. RESULTS AND DISCUSSION

Table 1 presented the percentage of growth inhibition effect of 13Docosenamide,(Z) on tested microorganisms. Among the tested 10 microorganisms, the purified 13Docosenamide,(Z) showed antimicrobial activity on six of the ten tested bacterial strains and the percentage of growth inhibition was dependent on the 13Docosenamide,(Z) concentration used in this study. Complete growth inhibition was observed in gram negative micro organisms *Salmonella typhi and S paratyphi* with the concentration of 400 µg/ml, whereas 87.1% and 79.4% of growth inhibition was achieved with *Vibrio cholerae* and *V. parahemolyticus* at the maximum concentration of 400µg/ml, respectively Furthermore, *Escherichia coli* (gram –ve) and *Bacillus subtilis* (gram +ve) showed less than 50% growth inhibition even at the maximum concentration of 400 µg/ml. Four bacterial strains revealed no inhibition activity with the use of 13Docosenamide,(Z) even at the maximum concentration (400 µg/ml), they were *Klebsiella pneumoniae, Proteus mirabilis, Streptococcus pneumoniae and Staphylococcus aureus.* Based on these observations, the antimicrobial activity of 13Docosenamide,(Z) and its application in antimicrobial therapy was affirmed.

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Microscopic examinations done at all the pathogenic strains susceptible to antimicrobial activity of 13Docosenamide,(Z) at the concentrations of 100µg/ml and 400µg/ml were represented in the Figure 1 and 2. The presence of dense viable bacterial cells at the control and decreased viable cells visualized at the tested concentrations with respect to 100 and 400 µg/ml, proved the antimicrobial effect of 13Docosenamide,(Z) against this pathogenic bacterial strains studied in this investigation. 13Docosenamide,(Z) isolated from the ethanol leaves extract of *Erythrina indica* have shown a promising antimicrobial activity against 60% percentage of the tested human pathogenic bacteria. This phytochemical compound revealed a complete growth inhibition of *Salmonella typhi* and *S. paratyphi* proving its potential possible therapy against these pathogens. Earlier studies of antibacterial activity of various leaves extract (hexane, ethyl acetate and ethanol) of *Erythrina variegata* against *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *E.coli* were reported [13]. Nagaraja *et al* [1] reported the antibacterial activity of chloroform and ethanol extract of *E.mysorensis* against gram positive and gram negative microorganisms. Tanaka *et al* [4] observed the antibacterial activity of isolated compound isoflavonoids from *E.variegata* against methicillin resistant *Staphylococcus aureus*. Various crude solvent extracts of leaves of *E.variegata* exhibited antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* were reported by Preetikumari *et al* [14].

From the study it was confirmed that the isolated bioactive compound 13Docosenamide,(z) has antimicrobial activity and play an important role in the medicinal activity of leaves of *E.indica.*

Table 1: Microbial growth inhibition percentages of 13Docosenamide,(Z) from *Erythrina indica* at different concentrations. Experimental values are expressed as mean ± SD (n=3).

Test organism	13Docosenamide,(Z)(µg/ml)					
	12.5	25	50	100	200	400
Gram Positive Organisms						
Bacillus subtilis	2.1 ± 0.1	7.6 ± 0.2	13.9 ± 0.2	25.3 ± 0.3	37.7± 0.1	47.8 ± 0.2
Streptococcus pneumoniae	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-
Gram Negative Organisms						
Escherichia coli	3.5 ± 0.2	12.8 ± 0.2	19.1 ± 0.2	28.4 ± 0.2	41.7 ± 0	49.8 ± 0.3
Salmonella typhi	19.5 ± 0.1	36.6 ± 0.1	53.5 ±0	72.4 ± 0.1	89.6 ±0.3	100 ± 0.1
S. paratyphi	39.8 ± 0.1	50.9 ± 0.1	62.2 ± 0.1	74.1 ± 0.1	89.4± 0.2	100 ± 0.2
Klebsiella pneumoniae	-	-	-	-	-	-
Vibrio para hemolyticus	22.0 ± 0.1	30.4 ± 0.2	38.7 ± 0	49.9 ± 0	63.2± 0.1	79.4 ± 0.1
V. cholerae	29.6 ± 0.3	38.8 ± 0.3	49.8 ± 0.3	60.8 ± 0.2	73.2± 0.2	87.1 ± 0.3
Proteus mirabilis	-	-	-	-	-	-

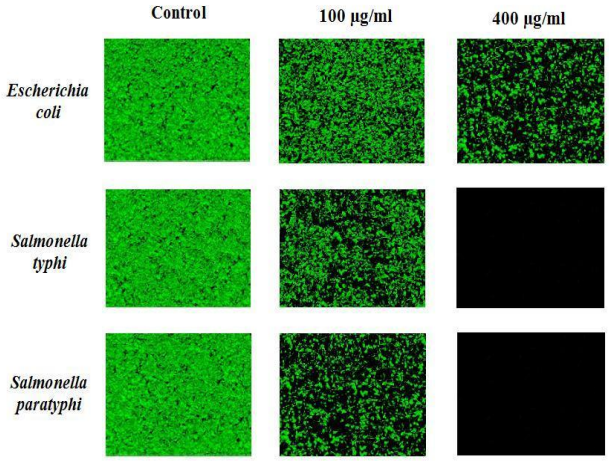

Figure 1: Effect of 13Docosenamide,(Z) of *Erythrina indica* at a concentration of 100µg/ml and 400 µg/ml against *Escherichia coli, Salmonella typhi and Salmonella paratyphi*

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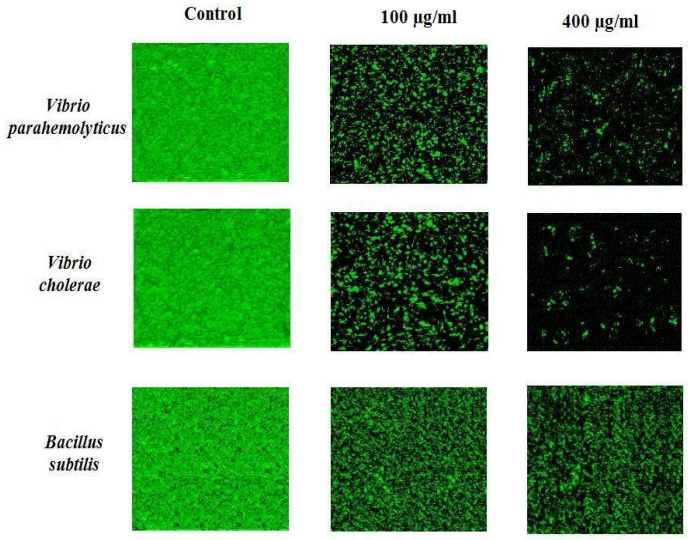

Figure 2: Effect of 13Docosenamide,(Z) of *Erythrina indica* at a concentration of 100µg/ml and 400 µg/ml against *Vibrio parahemolyticus, Vibrio cholerae and Bacillus subtilis*

IV. CONCLUSION

This study was mainly focused on the antibacterial activity of isolated 13Docosenamide,(Z) from the ethanolic leaves extract of *Erythrina indica*. It was found to inhibit the human pathogens *Salmonella typhi* and *Salmonella paratyphi* completely at a tested concentration, thereby establishing the medicinal value of the plant.

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