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Evaluation Of Anti Bacterial Activity and Anti-Inflammatory Activity of Diastase Conjugated Naringin

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Abstract: *The flavonoids comprise a large class of low-molecular-weight plant metabolites ubiquitously distributed in food plants. These dietary antioxidants exert significant antitumor, antiallergic, and anti-inflammatory effects. Narngin is a naturally occurring chemical found in fruits and vegetables. Flavonoids such as naringin, are antioxidants, antibacterial and anti inflammatory agents. The molecular mechanisms of their biological effects remain to be clearly understood. Flavonoids have more therapeutic activity and less solubility. So, its activity can be enhanced through enzymatic conjugation or sulfation by Arylsulfotransferases or by other hydrophilic enzymes. In the present work, we have taken Diastase conjugated Naringin enzyme to increase its solubility and bioavailability. Enzyme conjugation was analysed by HPLC method. HPLC at 285nm using an isocratic mobile phase (50:50, acetonitrile water). HPLC was evaluated through high accuracy, precision, recovery And that enzymatic conjugation may enhance the antibacterial, anti inflammatory was found to be less.*
Keywords: *Flavonoids, HPLC, enzymatic conjugation, bioavailability, diastase , naringin.*

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I. INTRODUCTION

The most important dietary sources of flavonoids are citrus fruits (rutin, naringin and hesperidin), apple (quercetin), tea and soybean. Flavonoids are becoming very popular because they have much health promoting effects. Some of the activities attributed to flavonoids include anti-allergic [1] anti-cancer [2], antioxidant [3], anti-inflammatory [4] and anti-viral [5] The flavonoids naringin is known for its ability to relieve to relieve asthma and Parkinson's. Soy flavonoids (isoflavones) can also reduce blood cholesterol and can help to prevent osteoporosis.[6] Flavonoids have been shown to have direct antibacterial activity, synergistic activity with antibiotics, and the ability to suppress bacterial virulence factors in numerous *in vitro* and a limited number of *in vivo* studies[7][8] Noteworthy among the *in vivo* studies[9-11] is the finding that is the finding that oral naringin protects rats against the carcinogen, Compound naringin has potential as an anti-carcinogen drug.

1.1Naringin

Naringin is the flavonoid widely distributed in nature. Naringin has GRAS (Generally Recognized as Safe) status, and no side-effects have yet been noted in doses of a few grams a day in either humans or animals. Naringin has anti-oxidant [13], anti-artherogenic [14], and anti-carcinogenic [15] properties. Naringin is the chemical compound in grapefruit that is responsible for its bitter taste. It is classified as a phytochemical, which is a naturally occurring plant compound with potential nutritional benefits. Specifically, naringin is apartially water soluble flavonoid. Naringin is the aglycone, occur naturally in citrus fruits.

An enzyme conjugated naringin influences its absorption rates. At least intestinally, naringin glycosides (food source) were found to have a 52+/-15% uptake, , and supplemental naringin aglycone had a 24+/-9% uptake ([16]) So, in the present research work we conjugated naringin with diastase enzyme, conjugation was estimated by HPLC analysis and we studied the antibacterial, anti inflammatory activity of conjugated naringin.

II. MATERIALS AND METHODS:

Materials of enzyme conjugation (Diastase with Naringin)

2.1Chemicals, animals and cultures

Flavonoid naringin was provided as a powder extract from yucca enterprises, Inc. in a standardized, Good Manufacturing Practice formulation. The enzyme diastase also purchased from yucca enterprises, Inc. The mixture contained naringin (75%), diastase conjugated naringin [2%; verified by high-performance liquid chromatography (HPLC) assay in our laboratory]. All common chemicals used in this study were purchased from LOBA chemie, INDIA. The microorganisms and media components used in the bioassay were procured

from national collection of industrial microorganisms (NCIM), Pune and Hi media Ltd, Mumbai, India. Sprague dawley rats (four in a group) were collected from mahaveer enterprises and plethismometer (HTC life sciences, USA).

2.2Procedure for conjugation

Synthesis of the diastase conjugated naringin was achieved by modified method of *vijay kumar et al.* Naringin (0.25gm) was dissolved in 1 ml of Dimethyl Sulfoxide (DMSO) with constant stirring, to which a phase transfer catalyst (0.1 M of benzyl triethyl ammonium chloride) dissolved in 10 ml of sodium acetate buffer (0.01 M, pH 7.5) was added. To the above reaction mixture, glucose (0.048 gm) and diastase (0.024 gm) were added and incubated in shaking incubator at 40 ± 2^0 C, 150 rpm for 35 hrs. The product formation was intermittently monitored by HPLC. After completion, the reaction was quenched by keeping in a boiling water bath for 10 min. The product formed in the reaction mixture was concentrated by vaccum evaporation to get the crude product. The concentrated product was tested by HPLC.

2.3Preparation of standard Stock solution

Naringin**:** Weigh 2mg of naringin in a 10 mL volumetric flask. Add 8mL of diluent, sonicate to dissolve and dilute it with diluents at required volume.

2.4Preparation of standard solution

Further, dilute each 5mL of the standard stock solution to 10 mL with the diluent.

2.5HPLC

The concentrations of naringin and diastase conjugated naringin were analyzed by HPLC (Gilson LC system), using analytical Column: Luna C18, 250mm x 4.6mm, 5µm particle size, Injection Volume: 10µL, Column Temperature: Ambient, The system was run isocratically using mobile phase Acetonitrile and Water (50:50) (pH adjusted to 3.5with acetic acid) at a flow rate of 1.0mL/min and the sample detection was done using UV/VIS detector at 285 nm. The mobile phase was filtered through 0.45µ membrane filter and the solvent was degassed ultrasonically before use. Record the chromatograms and measure the peak responses for Naringin. The System suitability parameters should be met. From the peak responses, calculate the content of Naringin in the sample Retention time of Naringin is about 9.6 min.

2.6Evaluation of system suitability

1. Relative Standard Deviation of five replicate injections of Standard preparation for naringin peak should not be more than 2.0%.
2. Tailing factor for naringin peak should not be more than 2.0%.
3. Theoretical Plate count for naringin peak should not be less than 2000.

2.7Antimicrobial Assay

The antimicrobial activity of naringin and diastase conjugated naringin was tested against *Staphylococcus Aureus (ATTC-2901)* is used to cultivate the bacteria. Each culture was transferred from agar medium and incubate overnight at 370c. 12hrs old bacterial cultures were used for determining minimum inhibitory concentration (MIC).

2.8Determination of MIC

The paper disc method was used to assay the naringin and diastase conjugated naringin for antimicrobial activity. The above prepared test cultures were seeded into sterile nutrient agar medium by uniformly mixing 1 ml of inoculums with 20 ml melted nutrient agar medium, cooled to $48\text{-}50^0$ C. and then allow it to solidify. Six wells (7 mm each) were bored in each plate using an aseptic well borer. The test compounds (naringin and diastase conjugated naringin) and control samples (gentamicin against bacteria) were dissolved in 90% DMSO and serially diluted to get concentration of 2-100 ug/ml. an equivalent amount of DMSO (0.1mL) was added to each plate as a negative control. Each experiment was performed in duplicates and repeated thrice. The MIC was reported as the lowest concentration of test sample capable of inhibiting the growth of each bacteria.

2,9Anti inflammatory activity:

Animals used:

Sprague dawlwy of either sex weighing between (220-290 gms) were divided into groups of four animals and each group consists of four animals.

- Group 1: Positive Control
- Group 2: Negative Control

- Group 3: Standard Ibuprofen
- Group 4: Test (Enzyme-conjugated naringin)

The animals were maintained under laboratory condition and kept in standard cases at room temperature of 30± 2^0 C and 60 to 65% relative humidity and provided with standard diet and water.
The enzyme conjugated naringin was given to group 4 and the anti inflammatory activity studies are conducted using Ibuprofen as standard. The method used is carageenan induced paw oedema method (plethismometer).

2.10Carrageenan Induced Paw OEdema Method

Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay. Edema was induced by subplantar injection of 100 μL of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws of each rat of all the groups except the group A. Animals of group B/C, D/E were treated with the single dose of vehicle, cultures, and drug, respectively; 30 minutes prior to carrageenan injection. Paw thickness were measured just before the carrageenan injection, that is, at "0 hour" and then at 1, 2, 3, 4, and 24th hour after carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at "0 hour" and paw thickness at respective hours.

2.11Statistical analysis

All experiments were conducted in triplicates and the results were presented as the mean of
Three independent experiments +_ standard error.

III. RESULTSAND DISCUSSION

In the present study naringin and conjugated naringin were synthesized and charcterised by spectral analysis HPLC. Thus from the retention time and area under peak, it was confirmed the enzymatic conjugation was confirmed and reports were also given in the chromatogram 1and 2. The enzyme conjugated naringin can acts as potent prodrug as glucosyl bond gets hydrolysed at the target sites to liberate pharmacological active molecule naringin. The present methodology develops involved a one step glucosyl; conjugation to naringin by diastase, which showed good yield (25%) and enantioselectivity. Despite naringin demonstrated therapeutic efficacy and safety, the poor bioavailability of its into systemic circulation continues to be highlighted as a major concern in wider pharmacological therapeutic appilications. From the literature, it is well known that the glycosylation of hydrophobic compounds allow the conversion of water insoluble compound into soluble compound, which could improve the bioavailability and pharmacological properties of the compound.

IV. TABLES AND GRAPHS

4.1Anti microbial activity:

Naringin is a bioactive compound , has been shown to have several biological active properties, such as anti oxidant, anti infectious, antiviral and anticancer .*In vitro* antimicrobial activity of naringin and enzyme conjugated naringin was compared with standard antibacterial and such as gentamycin sulfate. Both the molecules naringin and enzyme conjugated naringin showed antibacterial activities with a range from. 0.7 -1.0 mm respectively against the bacterial test culture.enzyme conjugated naringin shown the best result [Fig:1], having lower MIC values compared to naringin[Fig:2] and control[Fig:3]and results were shown in the [Table:1]Therefore the improved antibacterial activity of enzyme conjugated naringin may because of its increased solubility, enhanced cellular uptake, reduced metabolism and better binding to cell components.The zone inhibition diameter for sample (enzyme conjugated naringin) is more than that of standard naringin and control. Thus sample (the enzyme conjugated naringin) shows the effective anti-bacterial activity.

4.2Anti-Inflammatory activity of enzyme conjugated Naringin:

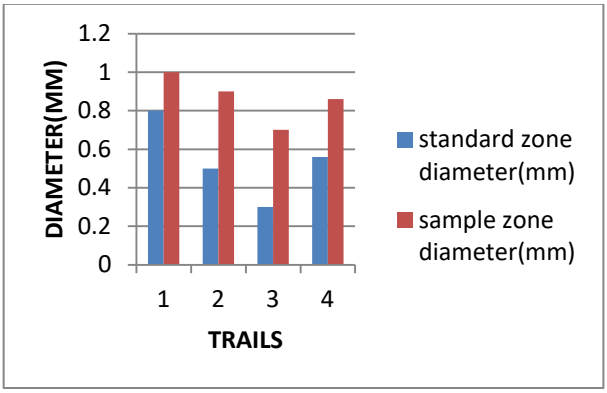
The time of response of sample (enzyme conjugated naringin) is more than that of standard, positive control and negative control. Thus sample shows effective anti inflammatory activity.

Table 1: Anti bacterial activity- naringin vs enzyme conjugated naringin

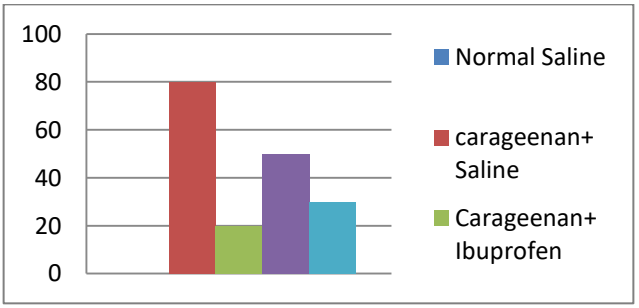
Trails	Standard zone inhibition diameter (mm)	Sample zone inhibition diameter (mm)
1	0.8	1.0
2	0.5	0.9
3	0.3	0.7
4	0.56	0.86

Table 2: anti inflammatory activity – percentage decreased paw oedema

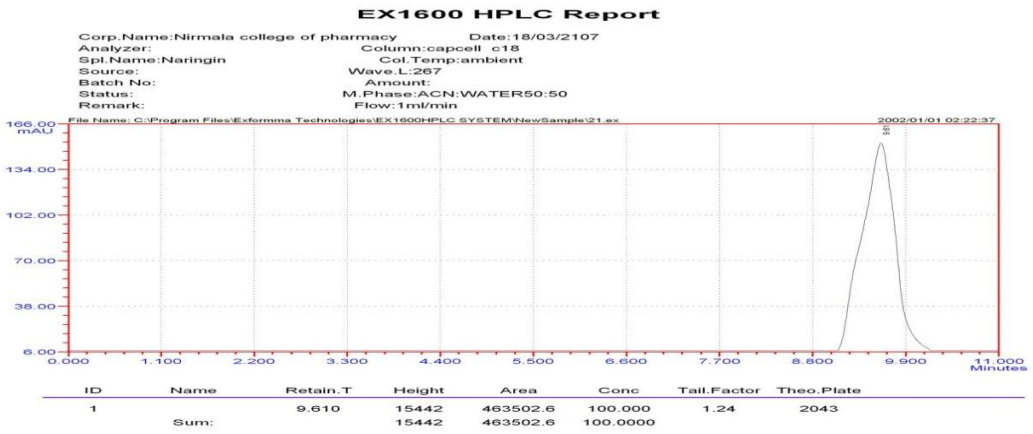
GROUP	TREATMENT	Time(min)	% decrease in paw oedema
Positive control	**Normal saline**	**60**	**0**
Negative control	Saline +carrageenan	60	80
Standard	Ibuprofen	60	20
Sample T1 T2	 Carageenan+Naringin Carageenan+Enzyme conjugated naringin	 60	 50 30



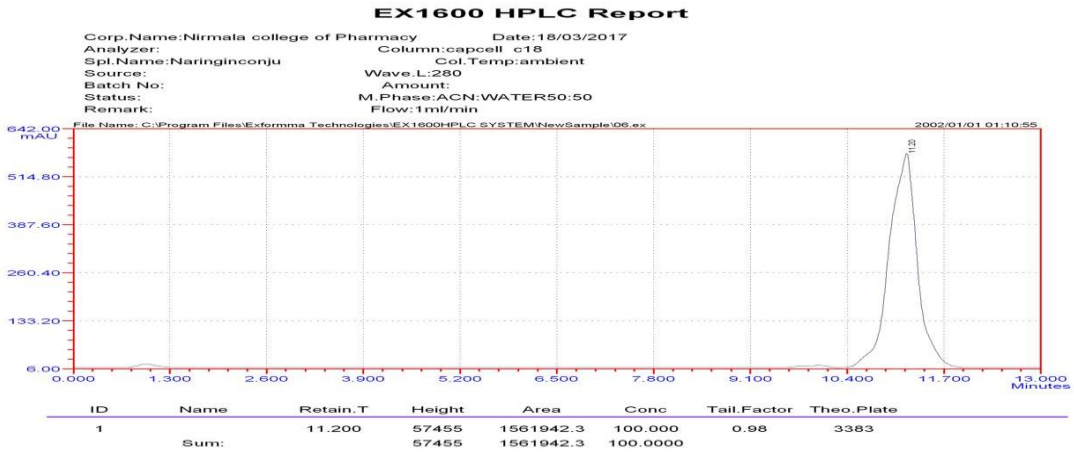
Graph 1: Anti - bacterial activity of naringin vs Conjugated naringin



Graph 2: **Anti inflammatory activity naringin vs Conjugated naringin**



Chromatogram 1 : Free naringin



Chromatogram 2: Diastase conjugated naringin

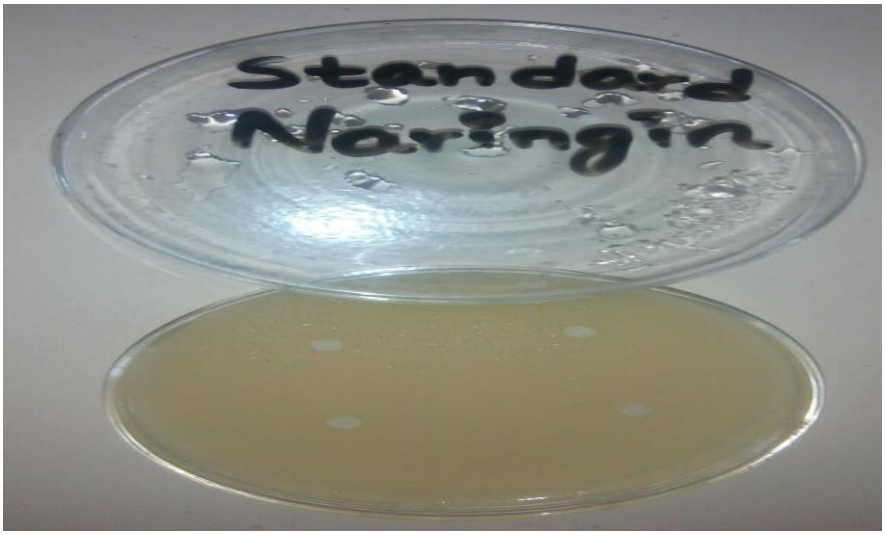


Fig: 1 Free Naringin Shows Growth Inhibition Diameter against Staphylococcus aureus.

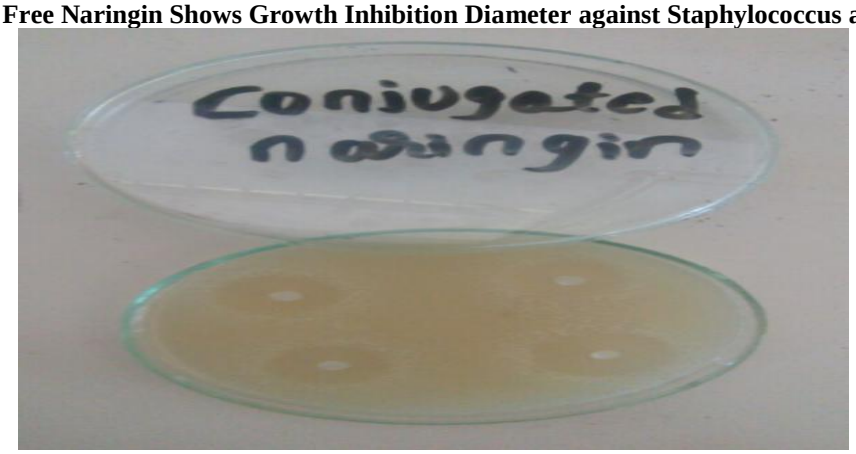


Fig: 2 Enzyme Conjugated Naringin Showing Growth Inhibition Diameter against Staphylococcus aureus.

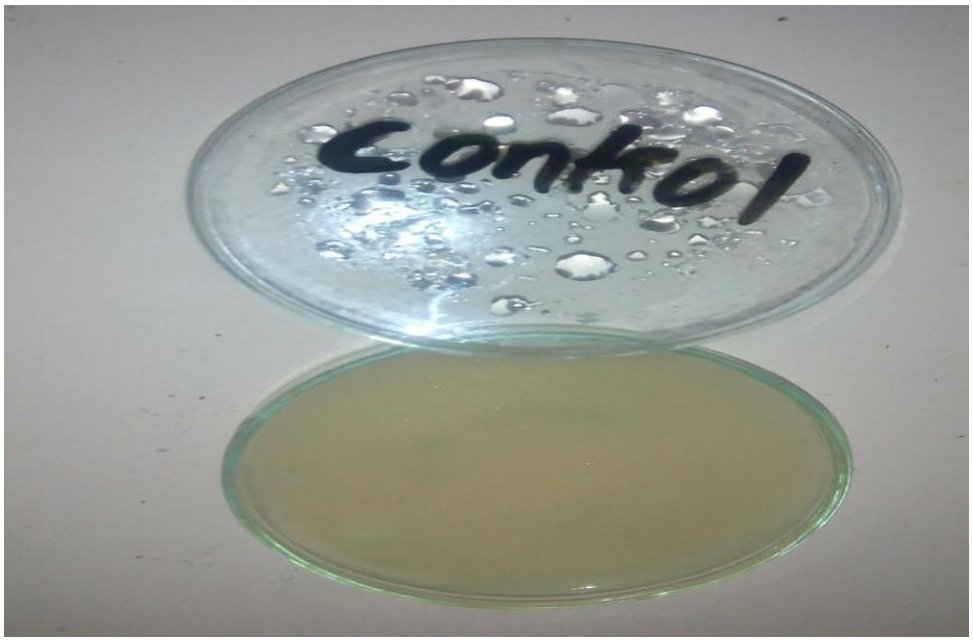


Fig: 3 Control Showing growth of staphylococcus aureus

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