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ANTI BACTERIAL ACTIVITY OF BENZOXADIAZINES DERIVED FROM EMBELIN

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ABSTRACT

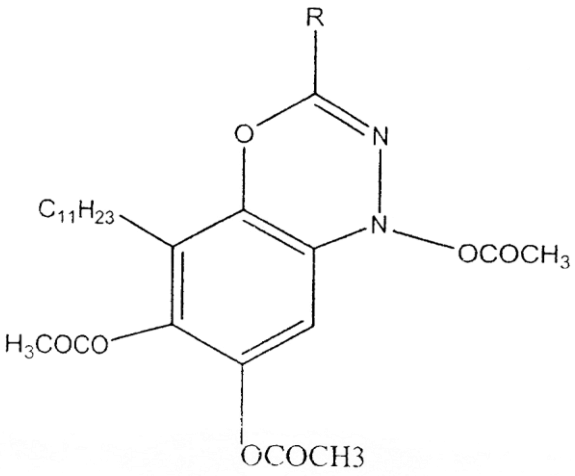
Embelia ribes is a woody shrub, belonging to the family Myrsinaceae and it is considered as medicinal plant .This is distributed in the most deciduous forests of the Western Ghats in india. It is a naturally occurring quinonoid compounds. Benzoxadiazine is a heterocyclic ring which was built on this compound is also a biologically important compound .It has anti bacterial and anti fungal activity.

KEYWORDS

Benzoxadiazine, Embelia ribes, Antibacterial activity.

INTRODUCTION

Quinone constitutes one of the well known groups of naturally occurring organic compounds. One of the major attractions among researchers towards quinone compounds is their color and biological activities[1]. Benzoquinones are the simplest representatives of quinone group.Embelin (2, 5-dihydroxy-3-undecyl-p-benzoquinone), is found to be the active principle of Embelia ribes and reported to possess a wide spectrum of biological activities. Chemical structure of embelin is having quite resemblance with that of natural Coenzyme Q10 (ubiquinones) and the role of this is well defined in various biochemical protective mechanism[2].



a) $R=C_6H_5$,

b) $R-CH_3$,

c) $R-COCH_3$,

d) $P-NO_2-C_6H_4$

Fig 1: The antibacterial activity of substituted N-Acetyl-2-Aryl-6, 7-diAcetoxy-8- Undecylbenzoxadizine.

E.ribes is widely used as traditional herbal medicine in india.Especially, the berries of E.ribes are reported to contain mainly benzoquinone derivatives such as embelin (2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1,4 benzoquinone) and viliangn.In addition ,dried berries have been used in India since ancient times as an antihelmanthic..In addition, dried berries are also reported to inhibit enzymes such as pancreatic lipase[3], alpha amylase[4]and trypsin[5] Embelin, as such as, evaluated against Heligmosomoides polygyrus in mice and found to reduce the total worm count[6,7] reported embelin as potent oral contraceptive having 85.7% anti-implantation activity in rats when administered at 50mg kg-1 for 7 days and it also inhibited pregnancy at single dose regimen. In addition, embelin inhibited pregnancy and possessed anti–estrogenic and weak progestationalactivity[8.] Embelin also reported to have anti-inflammatory[11], antibacterial[12], antitumor[10], antioxidant and free radical scavenging activities[9].However minimum inhibitory concentration index (MIC index) and minimum bactericidal concentration (MBC) for embelin have not been reported so far. Therefore, the present work deals with extraction, characterization and identification of minimum inhibitory concentration (MIC) and bactericidal/bacteriostatic of embelin against gram +ve and –ve bacteria.

MATERIALS AND METHODS

Extraction of embelin

Extraction of embelin was carried out according to 14.100g of the powdered berries of E.ribes was extracted with n-hexane using a soxlet extractor for 6 hours. The extract was then evaporated on rotator and recrystallised using ethanol and chloroform and characterized by using UV-Visible, FT-IR, NMR, DSC, TGA and X-ray diffraction analyses according to the standard methods.

The compound 5- Undecyl-4, 1, 2-benoxadiazines are synthesized in a three step reaction[13], on this compound. In this compound the oxadiazine ring is fused to Benzene ring of quinone skeleton.

N-5-hydroxy-6-undecyl-p-benzoquinone-2-yl) benzhydrazides ,undergoes reductive cyclization with simultaneous acetylation in the acetic anhydride, zinc powder Triethyl amine which on deactivation the desired product 6,7-di hydroxyl -3-aryl-5-undecyl-4,1,2-benzoxadzine was obtained .

Chemicals

Nutrient Agar medium; Peptone, Beef extract, Sodium chloride (Nacl), Agar-Agar (Bacteriological), research grade, were purchased form sigma Aldrich, chemical laboratories, Mumbai. The other chemicals were purchased from domestic market.

Bacterial cultures

The antimicrobial activity was carried by using the bacterial cultures, which were obtained from department of biotechnology, Chaitany Post Graduate College, Hanamkonda, Andhra Pradesh, India.

Two gram negative and gram positive bacterial strains were used in the study viz., *Bacillus subtilis* MTCC 441, *Escherichia coli* ATCC 8739, Bacillus Polymyxa, Proteus vulgaris

Antibacterial activity

The antibacterial activity of leaf and stem bark methanolic extract was carried out according to the method described by elsewhere with slight modifications. Each selective medium was innoculated with the microorganism suspended in nutritive broth. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the test compounds of various concentrations and corresponding wells used as blank. The concentration of the acetone extracts employed at concentrations 400 and 600 µg/ml simultaneously; gentamycin sulfate is used as positive control at a concentration of 10 µg /ml respectively. The dilutent medium for the positive control is sterile distilled water. The test was

carried out in triplicate. The plates were incubated at 35 ± 2°C for 24 h. The antimicrobial activity was calculated in terms of inhibition zone (mm).

RESULTS AND DISCUSSION

Among the tested compounds, the aryl substituted benzoxadiazine produced, significant zones of inhibition against all tested organisms, excluding *Escherichia coli* at both the concentrations tested. Methyl substituted benzoxadiazine doesn't possesses any activity due to inductive effect, while the compounds -$CONHNHCOCH_3$ and P-$NO_2C_6H_4$ are active against only Bacillus subtilis at both the concentrations and for proteus vulgaries at 600µg/ml. The phenyl and Para-nitro phenyl substituted benzoxadiazines have maximum antibacterial activity against Bacillus subtilis.

Table: l Antibacterial ActivityofN-Acetyl-2-Aryl-6, 7 diAcetoxy-8-undecyl Benzoxadiazines.

Compound R	Concentration (µg/disc)	Inhibition zone (mm)			
		B.Polymixa	B.Subtitis	E.Coli	Proteusvalgaries
C6H5	400	7.6	9	-	6.0
	600	8.0	11.0	-	9.0
CH3	400	-	-	-	-
	600	-	-	-	-
CONHNHCOCH3	400	-	10.0	-	-
	600	600	12.0	-	12.2
P-NO2C6H4	400	-	10.0	-	-
	600	-	12.0	-	10.0

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