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Isolation and biological evaluation of steroid from stem of *Costus igneus*

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ABSTRACT

Isolation of the ethanol extract of the plant Costus igneus has been done. The single compound was isolted in a particular fraction (206-218) were characterized by UV, IR ,[1]HNMR [13]C NMR, Dept 135, ESI-MS spectroscopy. The antimicrobial activity of the isolated compound was evaluated, on Staphylococcus aureus, Eschericia coli and Candida albicans. The present investigation deals with the isolated compound possessing good antibacterial and anti fungal activity.

Key Words: Isolated compound, UV, IR ,[1]HNMR [13]C NMR, Dept 135, ESI-MS spectroscopy Antimicrobial activity,

INTRODUCTION

costus ingneus , Family costaceae is used as expectorants, treating diabetes, lung diseases, liver ailments, rabies and intermittent fevers .It is available in the forest of Tamil nadu, Karnataka and Kerala. The cytotoxicity of this plant has been already reported in mice in other methods. Seed contains Dioscin, prosapogenis – A, and – B of dioscin, protodioscin, methyl protodioscin, gracillin, costusoside, B-sitosterol.Leaves contain Tigogenin, gracillin, sitosterol, D-Glocoside. Rhizomes having Diosgenin, Prosapoenin B of dioscin, Diosgenone , Cycloartanol, 25-en-cycloartenol and octacosanoic acid [1,2,3,4,5,6]. The main constituents reported are sterols, aminoacids, glycosides, proteins and flavanoids. Antioxidant principles derived from plants are

reported to have antitumour activity .Hence plants containing flavanoids are constantly being screened for antitumour activity. So it was decided to illustrate the ethnobotanical use of the plant and the study was planned to evaluate the anti microbial activity of the plant.

EXPERIMENTAL SECTION

All the chemicals are analytical grade and were purified by the established methods. Melting points and were determined by open capillary tubes method purity and homogeneity of the compounds was routinely determined by thin layer chromatography on glass plates using silica gel G as absorbent and solvent system. Benzene: Ethylacetate: Methanol (8.5:1.4:0.1). Spots were visualized by iodine vapor by irradiation with UV light.^{1}HNMRspectra was recorded on Bruker Ultra shield (300MHZ) spectrometer using DMSO (TMS as internal standard). DEPT stands for Distortionless Enchancement by Polraization Transfer. It is useful method for determining the presence of primary, secondary and tertiary carbon atoms. The anti microbial activities of the synthesized compounds were evaluated on *S.aureus* and *E.coli*[7,8].

Isolation method

The plant *costus ingneus* was collected in Nagarkovil District in Tamil Nadu during the second week end of November authenticated by Dept. of Botany, Devaswom board college, Kollam, Kerala, India. The plants were cleaned thoroughly with running water and dried in shade. The stem were shade dried and powdered. The stem were extracted with ethanol in soxhlet apparatus by simultaneous extraction for 72 hrs.The extrct was concentrated in vacumm. On concentration it yielded ethanol extract 20 gm of residue. The ethanol extract was chromotographed for the isolation of components. The 80% ethanol extract of *costus ingneus* was chromotographed in silica gel(60-120 mesh, merk, India) column built in n hexane.The column was eluated with n hexane and chloroform mixture of increasing polarity .The fraction of 150 ml were collected each time. The fractions eluted from 206-218 yielded single compound and responded positively for the steroidal test.

Antimicrobial activity

Anti bactetrial activity[9,10,11,12]

Assay was carried out by “diffusion plate method”. The method followed was “spread plate technique”. The agar plates free from contamination were spread with 50µl of 48hr old culture of bacterial test organism using sterile buds. The standard disc of Amikacin (sterile) of 5mm diameter was in the petriplates. Then the discs (sterile) of 5mm were soaked in 1ml of test solution and in solvent DMSO. After evaporating the solvent in a sterile atmosphere, the drug impregnated discs were placed in petriplates. The plates were refrigerated for 1h to arrest the growth and for easier diffusion of test compounds. Then the plates were removed from refrigerator and incubated at 37^{0}C over night in an inverted position. The clear zones of inhibition were measured using Hi media zone reader scale. The values are tabulated. The zones of test solutions were compared with standard Amikacin.

Antifungal activity[13,14,15,16]

Assay was carried out by diffusion plate method. The method followed was spread plate technique. The agar plates free from contamination were spread with 50µl of 48hr old culture of fungal test organism using sterile buds. The standard disc of Ketoconazole (sterile) of 5mm

diameter was in the petriplates. Then the discs (sterile) of 5mm were soaked in 1ml (1mg/ml) of the test solution and in solvent control DMSO. After evaporating the solvent in a sterile atmosphere, the drug impregnated discs were placed in petriplates. The plates were refrigerated for 1h to arrest the growth and for easier diffusion of test compounds. Then the plates were removed from refrigerator and incubated for 72 hrs at 28^{o}C in an inverted position. The clear zones of inhibition were measured using Hi media zone reader scale. The values are tabulated. The zones of test solutions were compared with standard Ketoconazole.

RESULTS AND DISCUSSION

Physical and Chemical method

In column chromatography a single compound was isolated in the fraction of 206-218 (Choloroform 70%, Hexane 30%) from ethanol extract. It was designated as isolated compound. Isolated compound white colur neddle shape from ethanol.The melting point of isolated compound was found to be 136-137° c.

UV Spectrum

The isolated compound showed UV absorption at λ 275 nm in methanol which is the characterstic of the substituted ester group.

IR Spectrum

In the IR spectrum of compound contain adsorption band due to hydroxyl group at 3448.19 cm^{-1},2930.66 cm^{-1} bands indicates CH stretching of CH_3,CH_2 grouping. The bands at 1457.34 cm^{-1} indicates the C-H bending vibration of CH_3,CH_2 group.

^{1}HNMR Spectrum

The signals at δ 0.660, 1.015, 0.853, 0.820, 0.806 ppm were assigned for five three proton signals of position H-18, H-19, H-21, H-26, H-27, H-28, CH_3, groups respectively. The signals at δ 1.843, 1.005, 1.834, 2.293, 2.221, 0.900, 1.009, 1.502, 0.910, 1.834, 0.928, 1.497, 1.177, 1.370, 1.366, 1.028, 0.779, 1.150 ppm were assigned for proton signals of position H-1a, H-1b, H-2, H-3, H-4a, H-4b, H-11a, H-11b,H-12a, H-12 B, H-15a, H-15b, H-16a, H-16b, H-20, H-22a, H-22b, H-23a, H-23b CH_2 groups respectively. The signals at δ 3.533, 5.391, 1.933, 1.732, 1.620, 1.617, 1.083, 0.998, 1.366, 0.846, 1.512 ppm were assigned for H-3, H- 6, H-7a, H-7 b, H-8, H- 9, H-17, H-24, H-25 CH groups respectively.

$^{13\,c}$NMR Spectrum

The carbon signals at δ 37.25, 31.25, 71.85, 42.29,140.76, 121.72, 31.90, 26.40, 36.51, 56.06, 32.41,51.23,39.68,21.21,50.60, 14.52, 19.71, 36.14, 19.92, 33.80, 28.24, 45.84, 28.95, 20.03, 20.12, 23.02, 14.82 ppm were assigned to C-1 ($CH_{2),}$ C-2 ($CH_{2),}$ C-3 (CH),C-4 ($CH_{2),}$ C-5 (C), C-6(CH), C-7 (CH),C-8(CH), C-9(CH),C-10(C), C-11($CH_{2),}$ C-12 $CH_{2),}$ C-13 (C), C-14 (CH), C-15 ($CH_{2),}$ C-16($CH_{2),}$ C-17 (CH), C-18 ($CH_{3),}$ C-19 ($CH_{3),}$ C-20 ($CH_{2),}$ C-21($CH_{2),}$ C-22 (CH), C-23 ($CH_{2),}$ C-24 (CH), C-25 (CH), C-26(CH_3), C-27(CH_2), C-28 (CH_2), C-29 (CH_3) respectively.

DEPT-135 Spectrum

In DEPT 135 showed the 10 negative signals at δ 37.75, 31.65, 42.29, 56.06, 32.41, 39.68, 21.21, 36.14, 28.24, 234.03 ppm .This confirms the presence of 10 CH_2 groups in the isolated

compound. It also shows 10 positive signals. The signals at δ 71.85, 121.72, 31.90, 26.09, 25.40, 42.21, 51.23, 50.06, 33.80, 28.95 ppm confirms the 10 groups and the signals at δ 14.52,19.71, 19.92, 20.03, 20.12, 14.82 ppm confirms the six CH_3 groups.

ESI-MS Spectrum

The mass spectrum that the presence of molecular ion peak at m/z 414 and base peak at m/z 186 in addition fragment ion peak of m/z (M+ -c8H17) .The peak at m/z 414 consistant with molecular formula $C_{29}H_{51.}$ ^{13}CNMR, DEPT- 135, ^{1}HNMR signals confirm the 6 CH_3, 10 CH_2 , 10 CH groups and 2 quaternary carbon atoms in the isolated compound.

Anti bacterial activity

The antibacterial activity of isolated compound revealed that in the test organism such as *S.aureus* and *E.coli* showed moderate activity in different concentrations when compared to Amikacin as standard.

Anti fungal activity

The antibacterial activity of isolated compound revealed that in the test organism such as *Candida albicans* showed moderate activity in different concentrations when compared to Ketacanozole as standard.

Table 1: Anti bacterial activity of isolated compound

S.no	Concentration µg	*Zone of inhibition*	
		E.coli	*s.aureus*
1.	50	8mm	7mm
2.	30	6mm	5mm
3.	10	5mm	4mm
4.	StandardAmikacin	10mm	10mm
5.	Control	0mm	0mm

Table 2: Anti fungal activity of isolated compound

S.no	Concentration µg	*Zone of inhibition Candida albicans*
1.	50	7mm
2.	30	6mm
3.	10	5mm
4.	Standard Amikacin	8mm
5.	Control	0mm

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