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A Fungi Toxic Quinone Derivative from *Anisochillus carnosus*

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Abstract: A Quinone derivative was characterized from the chloroform extract of the aerial part of *A.carnosus* by employing chemical and spectral methods. Further the chloroform extract was characterized for its antifungal activity against the species like *Aspergillus, Penicillium* and *Fusarium*. The extract possessed an effective control on the growth of all the organisms tested. The maximum percentage of inhibition was found at 125 µg/mL, might be due to the presence of Quinone functionality.

Key words: Phytochemistry; Fungi toxic, Quinone derivative Chloroform extract, *Anisochillus carnosus*

INTRODUCTION

Plant and plant product have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to extract various natural products for screening microbial activity [1]. The plant *Anisochillus carnosus* belonging to the family of Lamiaceae [2, 3] is medicinal plant found throughout India. The leaf juice of the plant was reported to use traditionally against liver disorders, allergic symptoms, muscle relaxation, cold and fever [4-6].

Hence there is no much scientific evidence for the phytochemical analysis and pharmacological activity. Mycotoxins are structurally diverse fungal metabolites that can contaminate the ingredients of animal food and human food. Spurred by the discovery of the aflatoxins in the early 1960's, the search for mycotoxins in food has led to the identification of over 100 toxigenic fungi and more than 300 mycotoxins [7, 8]. Among most of the mycotoxin, aflatoxin B1, a potent hepatatoxin is produced by *Aspergillus* species, while ochratoxin A, involved in porcine nephropath is produced by *Aspergillus* and *Penicillium* species [9]. The flavonoids like Letuolin and Apigenin were reported by Subramanian and Nair [10]. In our previous studies we have characterized a flavonoid 3, 5, 4, 4'-tetrahydroxy-8-isoprenyl flavonoid [11]. Thus an attention was drawn, to characterize a new compound from the chloroform extract of the aerial part. Further chloroform extract was subjected to study the effect on inhibition of the growth of pathogenic fungal species like *Aspergillus*, *Penicillium* and *Fusarium*.

MATERIALS AND METHODS

Plant Material: The aerial part of *Anisochillus carnosus* were collected from Palani Hills,Tamilnadu, India in June 2006.A voucher specimen is deposited in the Rabinet Herbarium, St.Joseph College, Tiruchirappalli, India.

Extraction and Isolation: The shade dried plant material was extracted by cold perculation method [6]. About 1500 gm of the plant material was soaked and homogenized in the solvent mixture alcohol: water (4:1) for 48 hrs. The extract was then filtered and concentrated by distillation.The concentrated extract was acidified using 2M Hydrochloric acid and extracted with chloroform. The active principle present in this chloroform extract was identified by micro TLC using chloroform: benzene (3:2) as mobile phase and silica gel as stationary phase. There are three different compounds identified of which one is isolated and characterized using preparative TLC. This compound was labeled as ACC. (*Anisochillus carnosus* chloroform extract) Fig. 1.

Characterization: An orange solid (850 mg/1500g) from chloroform extract was recrystallized from acetone. The melting point was found to be 137-139°. The molecular mass point was found by Cryoscopic method (ΔT_f =14.7). From the melting point of pure Camphor the molecular mass of ACC was calculated to 297.73 g/mol. The compound ACC was taken for C-H-N analysis using the instrument Heraeus for C-H-N Rapid Analyzer. Based on the data the empirical formula was calculated as

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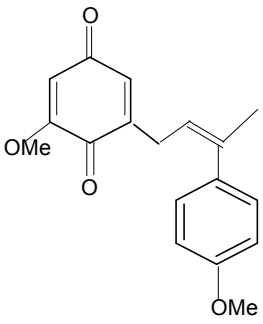

Fig. 1: Compound ACC

$C_{18}H_{18}O_4$. The UV-VIS and IR spectra were taken in Varian Cary Brucker 1 F6-66V, FT-IR spectrometer respectively. H^1 NMR was recorded in AMX 400 instrument and frequencies used were 400 MHz and 100 MHz for ^{13} C NMR.

***In vitro* Antifungal Activity:** The primary isolates of *Alternaria, Aspergillus flavus, Aspergillus fumigatus*, *Fusarium oxysporum*, *Fusarium solani* and *Penicillium crysogenum* were subcultured on glucose-neopentone agar without chloramphenicol, which were incubated at 25°-30°C for 48 hrs. Conidia were arrested from slope with sterile physiological saline, counted using haemocytometer and adjusted to final concentration of 10^5 cfu/ml. The antifungal activity of chloroform extract was measured by serial dilution method. The concentrations used for activity study were ranging from 25 µg/mL to 125 µg/mL.

RESULTS AND DISCUSSION

Compound ACC: The compound ACC answers positively for the quinone functionality with the reagent of 10% potassium iodide in acidic medium, liberating iodine. It reacted with semi carbazide hydrochloride gave the yellow solid of bis-semicarbozone. With the nitrating agent it gave a yellow color indicating the aromatic nature. When it was reacted with bromine in chloroform, it yielded an orange precipitate confirming the presence of unsaturation. The UV-Vis spectrum [12] of the ACC showed the bands at 205, 244, 261, 287, 310 and 432 nm. The λ_{max} at 244 with high absorptivity was due to the π-π* transition of the-C=C-part of the molecule. The band at 310 nm showed the high absorption was characteristic of π-π* transition of-C=C-part involved in extended conjugation. The weak bands at 287 and 432 nm with low absorptivity were suggestive of n-π* transition of C=0 of the quinine and-C=C-of the aromatic ring respectively.

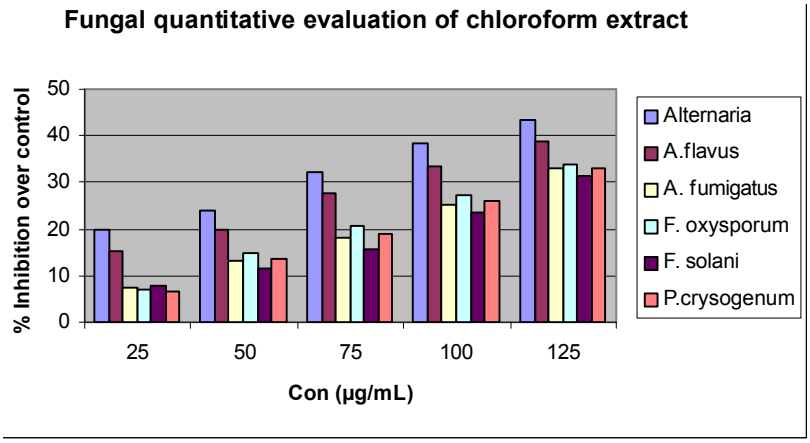

Fig. 2:

Table 1: Analytical and Spectral data for compound ACC

S.No	Type of experiment	Unit	Data
1	Cryoscopic method	Mole. mass	297.73
2	C-H-N analysis	Percentage composition	C-72.064%, H-6.34%, O-21.352%
3	UV-VIS	$\lambda_{max}e_{max\ (10^{-3}}cm^2mol^{-1})$	205, 244, 261, 287, 300, 432.2100, 2309, 4500, 13330, 26940, 2750
4	IR	1/ λ (cm^{-1})	3090, 3035, 3030, 3015, 2960,2940, 2815, 2840, 1680, 1689, 1620, 1628, 1610,1590, 1520, 1495, 1458, 1420, 1360, 1260, 1245, 1060,1040, 840, 815, 720, 720, 706.
5	H^1 NMR	δ (ppm)	1.79, 2.95 d (J6), 3.2 S, 3.4 S, 5.9 t (J9), 6.0S, 6.3 S, 6.78 d (J10), 7.88 d (J8)
6	^{13}C NMR	δ (ppm)	31.6, 46.3, 55.8, 56.5, 74.75, 79.25, 92.1, 114.0, 114.3, 118.1, 133.2, 134.7, 136.5, 158.5, 159.1, 173.6, 193.4, 194.6.

Table 2: Fungal quantitative evaluation of chloroform extract

	% Inhibition over control					
Con (µg/mL)	*Alternaria*	*A.flavus*	*A. fumigatus*	*F. oxysporum*	*F. solani*	*P. crysogenum*
25	19.89	15.16	7.36	7.06	8.03	6.63
50	24.12	19.72	13.02	14.81	11.50	13.50
75	32.31	27.89	18.33	20.62	15.76	19.05
100	38.51	33.33	25.4	27.42	23.47	26.15
125	43.2	39.02	32.9	34.01	31.32	33.19
150	Complete inhibition					

Thus the minimum concentration at which complete inhibition was fixed to be 150-µg/ mL

The IR spectrum [13, 14] showed the bands at 1689, 1680 cm^{-1} in for (C-0) stretching of carbonyl group, bands at 1628, 1620 cm^{-1} stand for (C-C) stretching of alkenes part. Strong bands at 1260,1245,1060 and 1040 cm^{-1} for asymmetric and symmetric vibration of-C-0-C. The moderately intense bands at 2840 and 2815 cm^{-1} are for (C-H) stretching of aromatic ring. The asymmetric and symmetric vibrations of C-H bands of the-CH_3 and-CH_2 group were significant of bands at 2960, 2855, 2930 and 2840 cm^{-1}. The (C-H) bending vibrations of some groups were observed as bands at 1479, 1458 and 1360 $cm^{-1.}$

In the ^1H NMR spectrum [15, 16] the singlet at 1.79 with mild splitting is due to deshielded methyl-CH_3 protons. 2.95 d (J6) is for-CH_2 in alkene part. 3.2 S, 3.4 S are for-OCH_3 proton. 5.9 t (J9) is for alkene-C-C proton, 6.78 d (J10), 7.88 d (J8) were evident of the aromatic (C-H) proton of a para disubstituted system. The signal at 6.0 S, 6.3 S are for the proton present in Quinone system.

In the fully decoupled ^{13}C NMR of ACC [17], the signal at 31.6 ppm was identified as the methyl (-CH_3) carbon of alkene part. The signal at 46.3 ppm was due to methylene (-CH_2) carbon to alkene part. The peaks at 74.75 and 79.25 are for-CH =C< carbon. The peaks at 92.1, 114.0 114.3,118.1 133.2, 158.5 ppm is characteristic of aromatic carbon. The signals at 55.81, 56.4 ppm were elusive of two methoxy carbons. The signals at 134.7, 136.5, 159.1, 173.6, 193.4 and 194.6 were attributed to the quinone system. Thus the compound ACC was proposed to have the following structure from the available data

Antifungal Assay: It was observed that the extract showed remarkable activity on the inhibition of the fungal growth compared to extract free control. The growths of fungus tested were inhibited maximum at 125 µg/mL and minimum at 25-µg/ mL. The growths of the microbes were totally stopped at 150-µg/mL (Fig.2.) The percentages of inhibition of growth of various concentrations against different pathogenic fungi are given in Table 2.

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