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Triterpenoids from *Quercus suber* and their antimicrobial and phytotoxic activities

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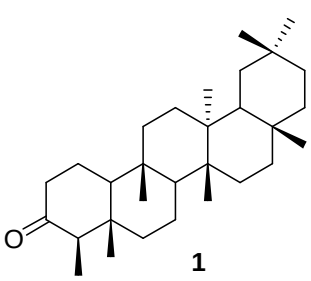
ABSTRACT

*In continuation of our studies on the phytochemical investigation of natural products and evaluation of the biological activities of the isolated compounds, recently we have isolated and characterized two triterpenoids, friedelin (**1**) and cerin (**2**) from the bark cork (Quercus suber). Biocidal activities of each of them were tested on some fungi and bacrerial plant pathogens, particularly available in this art of west Bengal, India. In addition phytotoxicity of each of them was also tested on some selected specimens. The results of our investigation were encouraging and will serve a lot to the pharmaceutical chemists.*

INTRODUCTION

Plants serve as medicines to treat human remedies form prehistoric ages. It is also found that the parts of the plant used sometimes contain alkaloids, steroids and terpenoids [1-2], the secondary metabolites that functions as a defense against harmful pathogens [3].Thus, it is assumed that these compounds can also be used to treat human ailments. Following this same argument, people from all over the world made some attempt to isolate these classes of natural products from the medicinal plants and evaluated their biological activities [1-3] to give the folk medicine culture a scientific basis. Our laboratory is very much involved to isolate newer chemicals from nature that have pronounced biochemical activities as was reported very recently [4]. In continuation of our studies in this direction recently we have isolated two triterpenoids namely, friedelin and cerin from cork and screened for their preliminary biological activities. The

structures of these two triterpenoids were established from physical (IR, NMR) data as well as by comparison with that reported in literature [5]. Furthermore, we examined both the antimicrobial and phytotoxic activities of the compounds isolated from cork. Biological activities of friedelin and cerin are reported earlier [5] but their application against plant pathogens is limited. Here, we are reporting the anti fungal and anti bacterial activities of these triterpenoids against some fungal and bacterial plant pathogens that are naturally abundant in North Bengal, India. With best of our knowledge, this is the first report of biocidal activities of friedelin and cerin against these plant pathogens.



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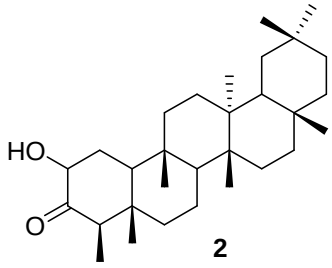

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RESULTS AND DISCUSSION

First we isolated friedelin and cerin from cork. The structure was assigned as **1** and **2** by physical data as well as comparison with that reported earlier. The compound **1** gave white crystalline solid from CH_2Cl_2-MeOH, m.p. 258-260^oC, IR (KBr) $\square_{max}$: 1715 (C = O stretching of ketone), 2927, 2870, 1463, 1390 cm^{-1}. 1H NMR spectrum showed seven sharp singlets at 0.76, 0.82, 0.88, 0.94, 1.00, 1.18 and 1.22 for seven tertiary methyl groups. A doublet centered at 0.99 of J = 6.5 Hz, for the secondary methyl at C-4. All other peaks are consistent with that reported for friedelin [5]. 13C NMR data was as follows 22.3 (C-1), 41.5 (C-2), 210.3 (C-3), 58.2 (C-4), 42.1 (C-5), 41.2 (C-6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 60.2 (C-10), 35.6 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.4 (C-15), 36.2 (C-16), 30.0 (C-17), 42.7 (C-18), 35.3 (C-19), 28.1 (C-20), 32.7 (C-21), 39.2 (C-22), 12.8 (C-23), 14.6 (C-24), 17.9 (C-25), 20.2 (C-26), 18.6 (C-27), 32.1 (C-28), 35.2 (C-29) and 31.7 (C-30). All data are in good agreement to that of friedelan skeleton [6] and the compound was identified as friedelin, **1**.

Cerin, **2** was purified by crystallization from the cold chloroform solution followrd by by recrystallization from ethyl acetate solution, that gave slightly yellowish needle shaped crystals of m.p.248-250^o C. The IR data are in good agreement with that of cerin. Because of its solubility problem we could not able to take its NMR spectrum.

Fungal pathogens used were *Fussarium equisitae*, *Curvularia eragrostidies*, *Colletrichum Gleosproides* and for antibacterial study we used *Ralstonia solanacearum*, TS3 (*Pseudomonas syringae*), P8 (*Eruniya caratovora*), OS (*Xanthomonas* sp). Suitable strains of these organisms were procured from the microbiology laboratory of our institute. All these microorganisms were native in North Bengal, India and are responsible for causing wilt diseases in tomato, pine apple and different varieties of citrus fruits cultivated traditionally in this region. Every year farmers in this area found a serious financial set back due to the wilt caused by these micro organisms. To cope with, farmers used pesticides available in the local market severely without knowing the impact of those chemicals on the fruits as well as on the environment. To this end, we applied naturally occurring triterpenoids, isolated in and purified in our lab friedelin and cerin against

these microorganisms to evaluate their activities to control the growth of the above microorganisms. Our aim is to use triterpenoids as environmentally benign biocontrol agent.

All antimicrobial experiments were performed by filter paper disc diffusion method in Petri dishes and were incubated at 37^{o}C for reported period. Bacterial nutrient media (NA) was prepared by using agar, beef extract and bacto peptone in distilled water and the pH of the solution (6.8 - 7.0) was adjusted. Solvent control (DMSO) was also maintained throughout the experiment. Filter paper disc having 5 mm diameter were prepared from Whatmann 40 filter paper. The whole process was carried out in inoculation chamber. The results were summarized in table 3.

Antifungal activities were determined following two different processes viz filter paper disc diffusion method and spore germination through wet chamber method to examine the order of effectiveness of our compounds in the two separate methods. Both the processes gave attracting results as elaborated in table 1. Culture media for fungal strains were prepared by mixing in suitable proportions of potato extract, dextrose and agar powder. All glass apparatus, culture media were autoclaved before use.

In order to show phytotoxic activities of the compounds (table -3), solutions of different concentrations of different compounds were prepared and applied to check germination of both root and shoot of the germinating seeds. Phytotoxicities of the isolated compounds were determined on the healthy seeds of wheat (sonalika variety), rice (Ratna variety) and pea purchased from local market. Some healthy seeds of rice, wheat and pea were dipped in DMSO (30 µL) used as control in Petri dish covered with filter paper (Whatman 40). In another set of experiment healthy seeds were dipped in about 30 µL of experimental compound of different concentrations and left for 24, 48 and 72 hours.

Antifungal activities of cerin and friedelin (table -1) showed comparable activity against all the fungal pathogens. Both the compounds showed full inhibition in spore germination of three fungal pathogens. As both the compounds have same basic skeleton their activities were similar too. Both showed highest activity at 500 ppm concentration. But, friedelin was slightly more active than cerin (table 1). However, the antibacterial activities were not similar (table -2), cerin and friedelin showed a variation in their properties (table 2). Cerin was active against Os only at 100 and 200 ppm, in which remarkably, at 100 ppm concentration its activity was higher. Friedelin on the other hand showed better affectivity against all the bacteria. In case of Ralstonia 400 and 500 ppm concentration showed much effectively than others. Whereas, against T3 friedelin showed affectivity gradually from higher to lower concentration, i. e. at 100 ppm concentration it has higher activity than 200 and 300 ppm concentrations. At 500 ppm concentration it did not show any activity. For P8 the results were in reverse order, here friedelin has higher activity at 500 ppm concentration and the rest followed similar trend as concentration. Activity data of Os was quite similar to that of TS3, showing its highest activity at 100 ppm concentration.

Additionally, we determined the phytotoxicity of these two triterpenoids against rice, wheat and pea. Both the compounds totally inhibited the germination of both root and shoot (table 3).

Table 1 Antifungal activity of cerin[a] and friedelin[b]

Organism	Conc in ppm	Total spore per Mfv	GP	GTL (O.D)	%G	RI	DIZ (CM)
Carvularia[a]	Control	151.1	144.2	13-25	90	-	-
	100	140.6	Nil	-	-l	-	1.3
	200	147.2	,,	-	-	-	1.3
	300	131.2	,,	-	-	-	1.5
	400	151.2	,,	-	-	-	1.8
	500	161.0	,,	-	-	-	1.9
Fusarium[a]	Control	174.6	172.8	2-4.6	98	-	-
	100	145.4	Nil	-	-	-	1.6
	200	147.1	,,	-	-	-	1.7
	300	142.6	,,	-	-	-	1.8
	400	142.0	,,	-	-	-	1.8
	500	148.0	,,	-	-	-	1.9
Colletriticum[a]	Control	156.0	144.4	3.4-10	90	-	-
	100	126.0	Nil	-	-	-	1.4
	200	144.4	,,	-	-	-	1.6
	300	124.2	,,	-	-	-	1.6
	400	138.6	,,	-	-	-	1.7
	500	122.8	,,	-	-	-	1.8
Carvularia[b]	Control	151.1	144.2	13-25	90	-	-
	100	121.8	Nil	-	-	-	1.7
	200	139.2	,,	-	-	-	1.7
	300	131.0	,,	-	-	-	1.7
	400	128.6	,,	-	-	-	1.8
	500	129.0	,,	-	-	-	1.8
Fusarium[b]	Control	174.6	172.8	2-4.6	98	-	-
	100	122.6	Nil	-	-	-	1.5
	200	137.0	,,	-	-	-	1.7
	300	125.4	,,	-	-	-	1.7
	400	129.1	,,	-	-	-	1.8
	500	129.6	,,	-	-	-	1.9
Colletriticum[b]	Control	156.0	144.4	3.4-10	90	-	-
	100	124.0	Nil	-	-	-	1.6
	200	123.8	,,	-	-	-	1.6
	300	123.8	,,	-	-	-	1.8
	400	123.6	,,	-	-	-	1.9
	500	123.1	,,	-	-	-	2.0

All data were average of five readings, Mfv- Microscopic field vision, GP- Germinated spore, GTL- Germ tube length, %G- Percent of germination, RI- Rate of Inhibition, DIZ- Diameter of inhibition zone.

Table 2 Antimicrobial activity of cerin[a] and friedelin[b]

Organism	Conc. (ppm)	Inhibition zone (cm)	
		24 hr	48 hr
Ralstonia[a]	Control	Nil	Nil
	100	,,	,,
	200	,,	,,
	300	,,	,,
	400	,,	,,
	500	,,	,,
Ralstonia[b]	Control	Nil	Nil
	100	,,	,,
	200	,,	,,
	300	,,	,,
	400	0.9	1.2
	500	1.2	1.5
TS_3[a]	Control	Nil	Nil
	100	,,	,,
	200	,,	,,
	300	,,	,,
TS_3[a]	400	Nil	Nil
	500	,,	,,
TS_3[b]	Control	Nil	Nil
	100	1.2	2.0
	200	1.0	1.5
	300	0.8	1.2
	400	Nil	Nil
	500	,,	,,
P_8[a]	Control	Nil	Nil
	100	,,	,,
	200	,,	,,
	300	,,	,,
	400	,,	,,
	500	,,	,,
P_8[b]	Control	Nil	Nil
	100	,,	,,
	200	,,	,,
	300	0.8	1.3
	400	1.0	1.8
P_8[b]	500	1.3	2.0

All data were average of five readings, Ralstonia – Ralstonia, TS_3 – Pseudomonas syringae, P_8 – Eruniya caratovora, O_s – Xanthomonas sp.

Table 3 Phytotoxicity of Cerin[a] and Friedelin[b]

All data were average of five readings, LR- Length of root and LS- Length of shoot.

Specimen	Conc in(ppm)	LR(cm)		LS(cm)	
		48(hr.)	72(hr.)	48(hr.)	72(hr.)
Rice[a]	Control	1.0	1.5	0.6	1.0
	100	Nil	Nil	Nil	Nil
	200	,,	,,	,,	,,
	300	,,	,,	,,	,,
	400	,,	,,	,,	,,
	500	,,	,,	,,	,,
Wheat[a]	Control	0.8	1.2	0.5	0.9
	100	Nil	Nil	Nil	Nil
	200	,,	,,	,,	,,
	300	,,	,,	,,	,,
	400	,,	,,	,,	,,
	500	,,	,,	,,	,,
Pea[a]	Control	1.0	1.6	0.9	1.3
	100	Nil	Nil	Nil	Nil
	200	,,	,,	,,	,,
	300	,,	,,	,,	,,
	400	,,	,,	,,	,,
	500	,,	,,	,,	,,
Rice[b]	Control	1.0	1.5	0.6	1.0
	100	Nil	Nil	Nil	Nil
	200	,,	,,	,,	,,
	300	,,	,,	,,	,,
Rice[b]	400	Nil	Nil	Nil	Nil
	500	,,	,,	,,	,,
Wheat[a]b	Control	0.8	1.2	0.5	0.9
	100	Nil	Nil	Nil	Nil
	200	,,	,,	,,	,,
	300	,,	,,	,,	,,
	400	,,	,,	,,	,,
	500	,,	,,	,,	,,
Pea[a]	Control	1.0	1.6	0.9	1.3
	100	Nil	Nil	Nil	Nil
	200	,,	,,	,,	,,
	300	,,	,,	,,	,,
	400	,,	,,	,,	,,
	500	,,	,,	,,	,,

EXPERIMENTAL

General experimental detail

All the melting points were determined by open capillary method and are uncorrected. The NMR spectra were recorded in $CDCl_3$ solutions at ambient temperature on a Bruker Avance 300 MHz-FT NMR spectrometer using 5mm BBO probe. The chemical shift δ are given in ppm related to

tetra methyl silane (TMS) as internal standard. The coupling constants (*J*) are reported in Hz. The IR spectra were recorded in Shimadzu FT-IR spectrophotometer in KBr discs.

Plant material

Fresh corck were collected from the local market and were cut into small pieces. These were then used for further study.

Preparation of plant extract

The cork material was extracted with pet ether in a soxhlet apparatus for 72 hours. The solvent were recovered that yielded a deep brown gummy residue. This crude extract was dissolved in minimum volume of hot chloroform, crocked up and stored for 24 hours. Being less soluble in cold chloroform, cerin crystallized out at the bottom of the container as slightly yellowish crystals. It was then filtered over a sintered Buckner funnel and washed thrice with cold chloroform. All the washings and the supernatant liquid were then mixed and purified over a column of silica gel.

Biological activity

Antifungal activity

Antifungal activities were determined following two different processes *viz*. filter paper disc diffusion method and spore germination through wet chamber method to examine the order of effectiveness of our compounds in the two separate methods. Both the processes gave attracting results as elaborated in table 1. Culture media for fungal strains were prepared by mixing in suitable proportions of potato extract, dextrose and agar powder. All glass apparatus, culture media were autoclaved before use.

Antibacterial activity

All antimicrobial experiments were performed by filter paper disc diffusion method in Petri dishes and were incubated at 37^{o}C for reported period. Bacterial nutrient media (NA) was prepared by using agar, beef extract and bacto peptone in distilled water and the pH of the solution (6.8 - 7.0) was adjusted. Solvent control (DMSO) was also maintained throughout the experiment. Filter paper disc having 5 mm diameter were prepared from Whatmann 40 filter paper. The whole process was carried out in inoculation chamber.

Phytotoxicity

In order to show phytotoxic activities of the compounds, solutions of different concentrations of different compounds were prepared and applied to check germination of both root and shoot of the germinating seeds. Phytotoxicities of the isolated compounds were determined on the healthy seeds of wheat (sonalika variety), rice (Ratna variety) and pea purchased from local market. Some healthy seeds of rice, wheat and pea were dipped in DMSO (30 µL) used as control in Petri dish covered with filter paper (Whatman 40). In another set of experiment healthy seeds were dipped in about 30 µL of experimental compound of different concentrations and left for 24, 48 and 72 hours.

CONCLUSION

In conclusion, we have isolated two important triterpenoids of friedelan skeleton namely friedelin and cerin. Both the compounds were isolated from cork. We applied both compounds against seven different micro organisms that are naturally abundant in North Bengal, India. All are plant pathogens harmful against tomato, in an attempt to evaluate their biological activities against these organisms, we have determined antifungal, antibacterial and phytotoxicity of the isolated compounds and all the data as reported in table 1, 2 and 3 are encouraging. Our study also revealed that against the tested organisms friedelin is more active than cerin.

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