Research Article

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Ergosterol content of several wood decaying fungi using a modified method

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Abstract

Ergosterol is a fungal specific sterol and is known for its medicinal properties. A semi-micro determination method used for yeast to estimate ergosterol content from 18 different wood decaying fungal members. The method was modified to estimate the semi-micro quantity of ergosterol from some of the aphyllophorales. *Antroidia albida* showed the highest ergosterol content amongst the screened, while *Hymenochatae* and *Gloiotheca* species. Amongst the *Ganoderma* samples *Ganoderma lucidum* showed highest ergosterol content.

Key-Words: Ergosterol, Aphyllophorales, *Ganoderma*.

Introduction

Several sterols have been isolated from different mushrooms; ergosterol and its derivatives being the major group having biological activities hence are focused generally. Ergosterol is a fungal specific sterols (a precursor for vitamin D_2; ergocalciferol) and important components of mevalonic acid pathway. Windaus[2] first reported the conversion of ergosterol to vitamin D_2 on ultra violet irradiation, for his work on sterols and their relations with vitamins. Windaus also reported that 5mg of irradiated ergosterol possessed equivalent action that of 1liter good quality cod-liver oil in curing rickets. Later, ergosterol has been accepted as a tool to measure the fungal biomass[3]. Ergosterol being fungal specific sterol found in the cell membrane makes it an important target component in antimycotic drug development[1-4].

There are several methods reported for ergosterol extraction from fungi. Padgett and Posey evaluated several such extraction techniques, concluding that use of alcoholic KOH substantially enhanced ergosterol recovery[5]. Currently HPLC methods are regularly practiced for the estimation/ determination of ergosterol, but a spectrophotometric method is also sensitive for semi micro-determination of ergosterol.

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The method is based on the specific absorption spectrum of ergosterol (4 headed peak), which was mentioned by Windaus[2]. Thereafter Breivik and Owades developed the basic spectrophotometric semi micro-determination protocol of ergosterol from yeast, which is still used with some modifications[1,4-6].

Material and Methods

Several methods have been reported for estimating the ergosterol, but majorities are based on HPLC or similar techniques[5]. Breivik and Owades first reported a method, sterol quantification method (SQM) from yeast[7]. Arthington-Skaags used this method with few modifications for determination of ergosterol from *Candida albincans*[1,4].

The protocol was standardized with some modifications for Aphyllophoraceous fungi, as follows: 200mg of sample powder was macerated with 25% alcoholic KOH (25gm KOH, dissolved in 35 ml of DW, and diluted to 100ml with ethanol). The suspension was transferred to a screw cap tube and vortexed for 1 minute accurately. The suspension was refluxed in water bath at 80 – 100°C for 5hrs. The tubes were then cooled, 1 ml of distilled water and 5ml of n-heptane was added and the suspension was again vortexed vigorously for minimum 3 minutes. These tubes were allowed to stand so as to separate the organic layer, which was transferred to another clear screw cap tube. The spectrum of this organic phase was read in range of 200 – 300nm using spectrophotometer (Shimadzu, UV 1601, Japan). The four headed peak is a characteristic of Ergosterol confirming its presence.

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The quantification was carried out using the formula.
% ergosterol + % 24(28) DHE = [($Abs_{281.5}$ / 290) X F] / pellet weight --- (A)
% 24(28) DHE = [(Abs_{230} / 518) X F] / pellet weight --- (B) % ergosterol = A – B
Where, F = dilution factor (if required).
Note: Presence of ergosterol and late sterol intermediate [24(28) DHE] in extract, results in a characteristic four-peaked curve. A flat line indicates the absence of ergosterol in extract. [24(28)DHE]: is a 24(28) Dehydroxy ergosterol, a sterol pathway intermediate, which shows intense spectra at 230nm and the complex of ergosterol and this intermediate shows maximum absorption at 281.5 nm.

Results and Conclusion

The Ergosterol content for the studied mushrooms were observed in the range with as low as 0.004% and as high as 0.065%, where as the 24 (28) DHE content in the range of 0.010% to 0.026% (table 1). It has also been observed that the 24 (28) DHE content is less than that of ergosterol except in case of *Hymenochaete* species, *Schizopora paradoxa*, *Polyporus gramomocephalus* and *Daedaleopsis* species (Fig 1.).
The 24, 28 DHE, was observed to be less than that of ergosterol content except for GA –7, GA –11, GA –27 and GA –28 with 0.0324%, 0.0304%, 0.0314% and 0.0257% respectively. The highest 24, 28 DHE content was observed in the isolate GA –36 (0.0329%), while the lowest was observed in GA –39 with 0.0241% (Table & Fig 1).

Table: 1 Ergosterol and 24 (28) DHE content of different *Ganoderma* samples.

Sample code	Ergosterol [%]	24 (28) DHE [%]
GA –7	0.0300 ± 0.0009	0.0324 ± 0.0015
GA –11	0.0295 ± 0.0003	0.0304 ± 0.0009
GA –12	0.0373 ± 0.0011	0.0325 ± 0.0011
GA –19	0.0343 ± 0.0015	0.0304 ± 0.0012
GA –27	0.0303 ± 0.0019	0.0314 ± 0.0015
GA –28	0.0196 ± 0.0018	0.0257 ± 0.0022
GA –36	0.0381 ± 0.0011	0.0329 ± 0.0015
GA –37	0.0264 ± 0.0016	0.0246 ± 0.0012
GA –38	0.0394 ± 0.0016	0.0301 ± 0.0008
GA –39	0.0267 ± 0.0016	0.0241 ± 0.0014

GA –7 - *Ganoderma stipitatum*(Murrill) Murrill, GA –36- *G. resinaceum* Boudier, GA –11- *Ganoderma* sp., GA –37- *G. praelongum* Murrill, GA –12- *G. multiplicatum*(Mont.)Pat.. GA –38- *G. lucidum* Lloyd, GA –19- *G. lipsiense* (Batsch) Atk., GA –39- *G. Chalceum* (Cooke) Steyaert, GA –27- *G. multiplicatum* (Mont.)Pat., GA –28- *G. multicornum* Ryverden

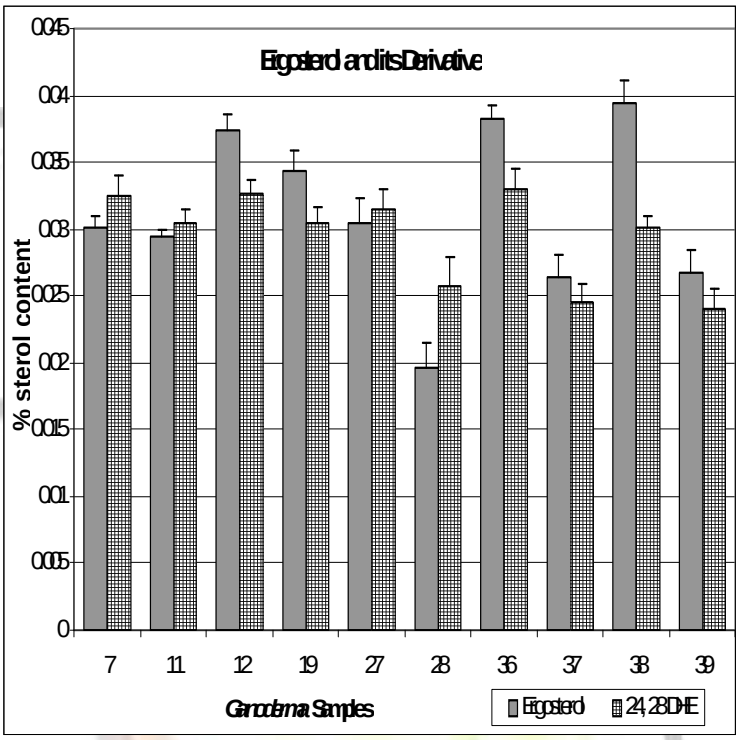

Fig 1: Ergosterol and 24, 28, DHE content of *Ganoderma* samples

Table 2: Ergosterol and 24 (28) DHE content of some wood decaying fungi

Code	Mushroom sample	Ergosterol [%]	24 (28) DHE [%]
WDF-1	*Favolus brasiliensis* Fr.	0.016 (±0.0012)	0.014 (±0.0017)
WDF-2	*Hymenochaete rubiginosa* Dickson ex	0.004 (±0.0033)	0.015 (±0.0045)
WDF-3	*Schizopora paradoxa* (Schrad.) Donk	0.014 (±0.0009)	0.026 (±0.0020)
WDF-4	*Lopharia papyracea* (Jungh.) D.A. Reid	0.013 (±0.0028)	0.010 (±0.0029)
WDF-5	*Amauroderma sp.*	0.028 (±0.0020)	0.021 (±0.0039)
WDF-6	*Clavariella alutacea* P. Karsten.	0.038 (±0.0032)	0.022 (±0.0026)
WDF-7	*Schizophyllum commune* Fries	0.049 (±0.0014)	0.013 (±0.0005)
WDF-8	*Clavulina cinerea* Bulliard J schroten	0.025 (±0.0007)	0.021 (±0.0015)

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WDF -9	*Microporus xanthopus*(Fries) Kuntze	0.025 (±0.0010)	0.019 (±0.0012)
WDF -10	*Gloeocystidiellum citrinum* (Persoon) Donk	0.028 (±0.0040)	0.002 (±0.0001)
WDF -11	**Podoscypha petaloides, *(Berk.) Pat.***	0.034 (±0.0009)	0.024 (±0.0029)
WDF -12	**Antrodia albida *(Fries) Donk***	0.065 (±0.0021)	0.025 (±0.0005)
WDF -13	*Hyphoderma roseocremeum* (Bres.) Donk	0.028 (±0.0024)	0.015 (±0.0015)
WDF -14	*Polyporus grammocephalus* Berk.	0.007 (±0.0008)	0.013 (±0.0009)
WDF -15	*Gloeocystidiellum luridum* (Bresadola) Boidin	0.015 (±0.0031)	0.014 (±0.0010)
WDF -16	*Daedaleopsis flavida* (Lev.) Roy & Mitra	0.018 (±0.0027)	0.017 (±0.0029)
WDF -17	*Daedaleopsis confragosa* (Fr.) shroet.	0.015 (±0.0033)	0.017 (±0.0026)

Ergosterol is an important component, specific to fungal group only and hence can be used as tool to estimate fungal biomass from any kind of mixtures. A semimicro determination method for Ergosterol was standrardized to an accuracy of 0.001% (10µg/gm). Estimation of ergosterol along with its derivative 24 (28) DHE, was also estimated for the first time from 17 different wood decaying fungi.

Ergosterol and some of its derivatives are known to possess medicinal properties. Ergosterol derivatives from *Ganoderma lucidum* exhibit potent antitumor activity against KB cells and human PLC/PRF/5 cells *in vitro* [7]. Jain and Gupta reported ergosterol derivatives from *G. austral*[8]. While the ergosterol peroxide and ergosterol from *Polyporus* species strongly inhibited (100% inhibition) the bladder tumor promoters in a dose dependent manner[9]. Only ergosterol has the ability to inhibit two or more tumor promoters having different mechanism [9]. The ergo peroxide from *Meripilus giganteus* was identified as immunosuppressive component[10].

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