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BIODETERIORATION OF CHEMICAL CONSTITUENTS IN DRUG *GMELINA ARBOREA* LINN. UNDER STORAGE OF DIFFERENT RELATIVE HUMIDITIES DUE TO SPOILAGE OF FUNGI

M Rashidi* and SS Deokule

Department of Botany, University of Pune, Pune-411007- India

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*Corresponding author

E-mail: rashidi_129@yahoo.com

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ABSTRACT

The roots of plant *Gmelina arborea* Linn. were selected for the present investigation. The roots were stored at different relative humidities 30, 50, 75, 96 and 100% RH for 90 days. Total 15 fungal species were isolated from these roots. Quantitative estimation of phenols, proteins, alkaloids, glycosides and sugars in relation to fungi were done. Maximum percentage incidence of storage fungi as well as deterioration of chemical constituents in the roots was showed at above 75% RH.

Key words: Deterioration, relative humidity, quantitative estimation, mycoflora

INTRODUCTION

Gmelina arborea is commonly called as "Shivan" or "Shewun", belonging to family "Verbenaceae". The roots contain yellow viscid oil, resin, alkaloid, a trace of benzoic acid, gmelinol, hentria contanol-1 ceryl alcohol, n- octacosanol- β sitosterol and sesquiterp candidane type of furanoses quiterp gmelofuran and ash, free from manganese. The roots are acidic, bitter, tonic, stomachic, laxative, galactagogue, demulcent antibilious, febrifuge and anthelmintic. They are useful in hallucination, fever, dyspepsia, hyperdipsia, haemorrhoids, stomachalgia, heart diseases, nervous disorders, piles and burning sensation^{1, 7, 14}. Bark is bitter, hypoglycaemic, antiviral, anticephalgic and tonic. It is used in fever and dyspepsia¹⁴. The leaves are demulcent and antigonorrhoeic. Extract of the root is bitter and tonic⁷.

The traditional methods of collection, storage and marketing coupled with humid climatic condition make them victim to the fungal infestation. Our this work is in continuation of earlier investigations reported on association of various fungi with herbal drugs under storage.

During storage, the fungal organisms thrive on drug plants by utilizing various components including the active ingredients and changes in active material content such as carbohydrates, alkaloids, phenol, glycosides and protein. Contents of stem, seed, fruit and root are influenced both by fungi as well as physical factors^{10,13}. However, the information is still scanty; therefore, it was decided to investigate the isolation of mycoflora from the roots of herbal drug *Gmelina arborea* and changes in chemical constituents under influence of Relative humidity.

MATERIAL AND METHODS

The root samples of drug *G. arborea* were collected from different shopkeepers of Pune and Mumbai, then authenticated by the help of Botanical survey of India (BSI); they were brought to the laboratory in polyethylene bags to avoid other aerial contaminations. Samples were stored in small muslin clothes in desiccators at 30, 50, 75, 96 and 100 % RH for 90 days in the room temperature¹⁵. The root samples were taken out on interval of 15, 30, 45, 60, 75 and 90 days, thoroughly washed with distilled water. According International Seed Testing Association⁴, Moist Blotter test and Agar plate method were adopted for isolation and identification of associated fungi. For isolation of associated mycoflora, small pieces of root samples sterilized with 2% sodium hypochlorite solution and thoroughly washed with 100 ml of sterilized distilled water. After developing, colonies were counted and average of 10 Petri plates were calculated from first day to 60th day of storage. Fungi were identified by using references such as Raper and Thom⁹ Barnet and Hunter² and Nelson *et al.*⁸. Some part of washed root samples after drying in oven, finely grinded for evaluation of changes in chemical constituents related to mycoflora. Quantitative estimation of chemical constituents was carried out from first day to 90th day of incubation. Chemical analysis were estimated by the procedure described by Lowry *et al.*⁶ for total protein, Singh *et al.*¹² for total phenols, Harborne³ for total alkaloids and Kokate *et al.*⁵ for glycosides. Anthrone methods for Total sugars (TS) and Dinitrosalicilic acid (DNSA) method for Reducing sugar (RS) amount¹¹ were also followed. Simple correlation were run between selected parameters using Statistical Package for Social Science (SPSS) software in which statistical significance was determined at 0.05 % probability levels.

Table 1: Percentage incidence of fungal isolated from the market root of *Gmelina arborea* stored at various relative humidity

Mycoflora	con	30%				50%				75%				96%				100%			
		15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
<i>Aspergillus niger</i>	0.57	0.57	0.57	0.76	0.95	0.38	0.57	0.95	1.71	0.95	1.33	1.71	2.09	0.38	0.57	0.95	1.52	0.95	1.71	2.85	5.71
<i>A. flavus</i>	0.19	0.38	0.38	0.57	0.76	0.19	0.57	1.33	1.90	0.19	0.38	0.57	0.76	0.57	0.76	0.95	1.52	0.57	0.76	1.33	2.85
<i>A. fumigatus</i>	-	-	-	-	0.19	-	-	0.19	0.19	-	0.19	0.38	0.57	0.19	0.57	0.76	1.14	0.38	0.57	1.33	1.71
<i>A. parasiticus</i>	-	-	-	-	0.19	-	-	0.19	0.19	-	-	0.19	0.38	-	0.19	0.19	0.38	-	0.38	0.57	0.76
<i>A. tamaritii</i>	-	-	-	-	0.19	-	-	-	0.19	-	-	-	0.19	-	-	0.19	0.19	-	0.19	0.19	0.38
<i>A. terreus</i>	-	-	-	-	0.19	-	-	0.19	0.19	-	-	0.19	0.19	-	0.19	0.19	0.38	-	0.38	0.57	0.76
<i>Penicillium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-	0.19	0.38
<i>Trichoderma</i> sp.	0.19	0.19	0.19	0.19	0.38	0.19	0.19	0.38	0.67	0.19	0.19	0.57	0.76	0.19	0.38	0.57	0.95	0.57	0.95	1.33	2.095
<i>Fusarium solani</i>	-	-	-	-	-	-	-	-	0.19	-	-	0.19	0.19	-	-	0.19	0.38	-	0.57	0.76	1.33
<i>F. oxysporum</i>	-	-	-	-	0.19	-	-	-	0.19	-	-	-	0.19	-	-	-	0.19	-	0.19	0.38	0.57
<i>Scytalidium thermophilum</i>	-	-	-	-	0.19	-	-	0.19	0.19	-	-	0.19	0.38	-	-	0.19	0.38	-	0.38	0.57	1.33
<i>Alternaria</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-	-	0.19	-	0.19	0.38	0.57
<i>Curvularia lunata</i>	-	-	-	0.19	0.38	-	-	0.19	0.38	-	0.19	0.38	0.57	-	0.19	0.38	0.57	0.38	0.57	0.95	1.33
<i>Chaetomium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-	0.19	0.38	-	0.19	0.38	0.57
<i>Monilia sitophila</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19	0.38
Total	0.95	1.14	1.14	1.71	3.61	0.76	0.76	3.61	5.99	1.33	2.28	4.37	6.65	2.28	2.85	4.75	9.31	2.85	7.03	11.97	20.72

Table 2: Deterioration of Total sugar (TS) and reducing sugar (RS) content (mg/100mg) in root *Gmelina arborea* at different relative humidities

Incubation days	control		30%		50%		75%		96%		100%	
	TS	RS	TS	RS	TS	RS	TS	RS	TS	RS	TS	RS
1 day	49.53 ±0.072	22.62 ±16.14	49.53 ±0.072	22.62 ±16.14	49.53 ±0.072	22.62 ±16.14	49.53 ±0.072	22.62 ±16.14	49.53 ±0.072	22.62 ±16.14	49.53 ±0.072	22.62 ±16.14
15 days	49.53 ±0.072e	22.62 ±16.14e	49.49 ±0.56d	22.62 ±16.14d	49.24 ±0.45c	22.62 ±15.36cd	49.07 ±0.12bc	22.17 ±15.40bd	48.48 ±0.29a	21.26 ±15.45a	48.27 ±0.45	20.81 ±15.40a
30 days	49.53 ±0.072e	22.62 ±16.14e	48.65 ±0.56d	21.71 ±16.19d	48.10 ±0.70c	21.26 ±14.95cd	47.011 ±0.40b	20.81 ±15.15b	46.63 ±0.19ab	18.09 ±15.37	45.41 ±1.14	15.74 ±13.99a
45 days	49.53 ±0.072e	22.62 ±16.14e	47.72 ±0.56d	19.90 ±17.08d	47.13 ±0.26c	19.45 ±15.15c	46.17 ±0.26b	19.004 ±14.71b	45.48 ±0.98ab	16.74 ±12.88ab	44.61 ±0.72ab	13.57 ±13.05a
60 days	49.53 ±0.072e	22.62 ±16.14e	47.01 ±0.40d	18.099 ±16.88d	46.67 ±0.19c	18.099 ±13.61c	45.58 ±0.12bc	18.09 ±12.02b	45.15 ±1.36a	13.57 ±11.94a	44.19 ±1.19	12.21 ±10.56a
75 days	49.53 ±0.072e	22.62 ±16.14e	45.74 ±0.89d	14.47 ±15.43d	45.91 ±0.69c	14.47 ±13.73c	43.39 ±0.69b	14.02 ±10.65b	41.32 ±0.69a	13.12 ±10.48a	39.89 ±0.57	11.76 ±8.84a
90 days	49.53 ±0.072e	22.62 ±16.14e	45.07 ±0.76cd	13.57 ±14.31d	43.81 ±0.57c	13.57 ±13.53c	42.63 ±0.83b	12.66 ±9.96b	40.10 ±0.38a	11.31 ±9.55a	38.69 ±0.69a	11.28 ±0.62a

Data in tables and are the mean of three replicates ± standard deviation. P- Value denoted the significance of differences between the mean by univariate comparison statistics. The value followed by different letters differ significantly by Duncan's multiple rang test at P=Sig= 0.05

Table 3: Deterioration of protein content (mg/100mg) in root of *Gmelina arborea* at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	29.02±0.86	29.02±0.86	29.02±0.86	29.02±0.86	29.02±0.86	29.02±0.86
15days	29.02±0.86d	28.75±0.78cd	28.05±0.60d	27.77±0.040bc	26.66±0.65a	26.25±0.21a
30days	29.02±0.86d	27.91±0.36cd	27.50±0.21cd	26.38±0.71bc	25.13±0.13a	24.72±0.27a
45 days	29.02±0.86d	27.36±0.13cd	25.97±0.28cd	25±0.036bc	23.61±0.21a	23.05±0.13a
60 days	29.02±0.86d	26.38±0.24cd	25.13±0.44cd	24.30±0.76b	23.33±0.28a	22.22±1.04a
75 days	29.02±0.86d	25±0.036cd	23.61±0.21c	22.22±0.34b	20.97±0.52a	20.41±0.90a
90 days	29.02±0.86d	23.75±0.86c	22.22±0.34c	20.83±0.65b	19.86±0.57a	19.02±0.71a

Data in tables and are the mean of three replicates ± standard deviation. P- Value denoted the significance of differences between the mean by univariate comparison statistics. The value followed by different letters differ significantly by Duncan's multiple rang test at P=Sig= 0.05

Table 4: Deterioration of total phenols content (mg/100mg) in root of *Gmelina arborea* at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	4.05±0.070	4.05±0.070	4.05±0.070	4.05±0.070	4.05±0.070	4.05±0.070
15days	4.05±0.070d	3.97±0.022cd	3.93±0.011cd	3.79±0.052bc	3.74±0.011ab	3.62±0.030a
30days	4.05±0.070d	3.91±0.030c	3.81±0.30c	3.68±0.34b	3.54±0.090ab	3.40±0.049a
45 days	4.05±0.070d	3.74±0.079c	3.58±0.070c	3.40±0.090b	3.28±0.010a	3.07±0.074a
60 days	4.05±0.070d	3.68±0.099c	3.46±0.090c	3.30±0.11b	3.01±0.15a	2.81±0.079a
75 days	4.05±0.070d	3.54±0.15c	3.36±0.069c	3.14±0.13b	2.95±0.17a	2.71±0.30a
90 days	4.05±0.070d	3.34±0.12c	3.16±0.030c	3.01±0.13b	2.86±0.19a	2.55±0.16a

Data in tables and are the mean of three replicates ± standard deviation. P- Value denoted the significance of differences between the mean by univariate comparison statistics. The value followed by different letters differ significantly by Duncan's multiple rang test at P=Sig= 0.05

Table 5: Deterioration of total alkaloids content (mg/100mg) in root of *Gmelina arborea* at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	25.53±1.14	25.53±1.14	25.53±1.14	25.53±1.14	25.53±1.14	25.53±1.14
15days	25.52±1.024e	25.52±1.14d	25.51±0.094c	25.50±1.011c	25.49±1.25ab	25.47±1.04a
30days	25.50±1.04e	25.49±1.14d	25.44±1.17c	25.39±0.13c	25.32±1.11ab	25.28±1.12a
45 days	25.48±1.038e	25.44±1.070d	25.39±0.19c	25.28±1.15bc	25.20±1.068a	25.15±1.26a
60 days	25.46±1.047e	25.39±1.072cd	25.32±1.10c	25.17±1.17bc	25.08±1.01a	24.96±1.07a
75 days	25.46±1.035e	25.29±1.064cd	25.21±1.10c	25.04±1.17bc	24.91±1.11a	24.79±1.12a
90 days	25.45±1.034e	25.16±1.092cd	25.08±1.048bc	24.96±1.076b	24.82±1.094a	24.68±1.14a

Data in tables and are the mean of three replicates ± standard deviation. P- Value denoted the significance of differences between the mean by univariate comparison statistics. The value followed by different letters differ significantly by Duncan's multiple rang test at P=Sig= 0.05

Table 6: Deterioration of total glycosides content (mg/100mg) in root of *Gmelina arborea* at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	16.71 ±0.090	16.71 ±0.090	16.71 ±0.090	16.71 ±0.090	16.71 ±0.090	16.71 ±0.090
15days	16.71 ±0.090d	16.71±0.097cd	16.71±0.10c	16.70±0.095bc	16.69±0.010ab	16.68±0.10a
30days	16.71 ±0.090d	16.71±0.087cd	16.69±0.10c	16.68±0.10c	16.62±0.10ab	16.62±0.015a
45 days	16.69±0.010d	16.68±0.010cd	16.67±0.13c	16.61±0.090bc	16.56±0.12a	16.55±0.12a
60 days	16.80±0.023d	16.64±0.10cd	16.61±0.11c	16.57±0.060bc	16.50±0.10a	16.42±0.05a
75 days	16.69±0.10d	16.58±0.10c	16.53±0.070c	16.50±0.076bc	16.42±0.066a	16.36±0.041a
90 days	16.68±0.017d	16.55±0.10c	16.50±0.11c	16.38±0.03b	16.31±0.01a	16.25±0.026a

Data in tables and are the mean of three replicates ± standard deviation. P- Value denoted the significance of differences between the mean by univariate comparison statistics. The value followed by different letters differ significantly by Duncan's multiple rang test at P=Sig= 0.05

RESULTS

Total 15 fungi from market roots of *G. arborea* were isolated at different relative humidities which include: *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. tamarii*, *A. terreus*, *Penicillium* sp., *Trichoderma* sp., *Fusarium solani*, *F. oxysporum*, *Scytallidium thermophilum*, *Alternaria* sp., *Curvularia lunata*, *Chaetomium* sp. and *Monilia sitophila*. Total percentage incidence of fungi under 30, 50, 75, 96 and 100% RH after 15 days of incubation observed were 1.14, 0.76, 1.33, 2.85 and 2.85%. These percentages increased to 3.61, 5.99, 6.65, 9.31 and 20.72 % after 60 days of incubation (Table 1).

The drug stored under higher relative humidities (75, 96 and 100 %) observed maximum reduction in chemical constituents while percentage incidence of the fungi were also maximum.

After 15 days of incubation period, under 30, 50, 75, 96 and 100% RH, amounts of Total sugar (TS) and Reducing sugar (RS) recorded 49.49, 22.62; 49.24, 22.62%; 49.07,

22.17%; 48.48, 21.26% and 48.27, 20.81% respectively, these amounts deteriorated to 45.07, 13.57% (30 % RH); 43.81, 13.57% (50 % RH); 42.63, 12.66% (75 % RH); 40.10 11.31% (96 % RH) and 38.69, 11.29% (100 % RH) after 90 days of incubation (Table 2).

In case of total protein amount, under 30, 50, 75, 96 and 100% RH after 15 days of storage, total value of protein recorded 28.75, 28.05, 27.77, 26.66 and 26.25% respectively, while theses values decreased to 23.75, 22.22, 20.83, 19.86 and 19.02 % after 90 days of incubation (Table 3).

The control samples contained 4.72 % of total phenol amount. After 15 days of incubation period in all tested relative humidity 30, 50, 75, 96 and 100% RH. Total phenol was recorded 3.97, 3.93, 3.79, 3.74 and 3.62%. These values of total phenols reduced to 3.34, 3.16, 3.01, 2.86 and 2.55% after 90 days of incubation period (Table 4).

At the first day of incubation control of market, sample showed 25.53% total alkaloids (Table 5). After 15 days of

incubation and under 30, 50, 75, 96 and 100 % RH, total amount of alkaloid; recorded 25.52, 25.51, 25.50, 25.49, 25.47%; respectively. These values of total alkaloids deteriorated to 25.16, 25.08, 24.96, 24.82, and 24.68 % after 90 days of incubation period. Deterioration of glycosides amounts also recorded, while after 15 days of storage the values of glycosides showed 16.71, 16.71, 16.70, 16.69 and 16.68 % under 30, 50, 75, 96 and 100% RH, after 90 days of incubation period, these amounts of glycosides decreased to 16.55, 16.50, 16.38, 16.31 and 16.25 % under different relative humidity, respectively (Table 6).

The result were tested statistically which showed the effect of relative humidity and incubation days in reduction of sugar, proteins, phenols, alkaloids and glycosides contents were significant at 5 % level of significance (P value <0.05).

DISCUSSION

Fungal activity can cause changes during storage of herbal plants and their products that are detrimental to nutritive value. Specifically, nutrients are lost because of changes in carbohydrates, protein, phenols and other constituents. Conditions that favour for fungal activity lead to carbohydrate decomposition. Sugars are consumed and converted into CO₂ and H₂O. Reduction of protein contents maybe is due to fungi and their utilization, photolytic enzymes produced by fungi can modify the proteins in tissues by hydrolyzing them into polypeptides and amino acids. Function of phenols and some classes of phenolic compounds are well established. For examples lignins which are as structural material of the cell walls, but generally they may be natural resistance factors for reduction of phenol amount during pathogenesis and it's subsequently utilization as carbon source by fungi for their growth. Experimental result revealed that there was gradual depletion in alkaloids and glycosides concentration under different relative humidity which might be due to its degradation into simple form by microbial enzymes and utilization of them by fungi.

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