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Studies on biodeterioration of some chemical constituents in fresh and market roots of drug *Desmodium gangeticum* DC. under storage

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ABSTRACT

In the present study fresh and market roots of drug Desmodium gangeticum DC. were analyzed for study in changes of chemical constituents under storage. Root samples were stored under different 30, 50, 75, 96 and 100 % relative humidity and different incubation days 15, 30, 45 and 60 days. Quantitative estimation of proteins, phenol and alkaloids in fresh and market roots was done. The results indicated that biodeterioration of selected chemical constituents were observed under high relative humidities 75, 96 and 100% RH and with increased in times of incubation 45 and 60 days. More deterioration of chemical constituents recorded in case of market samples as compared to fresh samples. Analysis of variance also showed that the effect of relative humidity and incubation days on biodeterioration of chemical constituents amount were significant.

Key words: deterioration, relative humidity, incubation days, *Desmodium gangeticum*

INTRODUCTION

D. gangeticum belongs to the family of Leguminosae sub family Fabaceae. It is commonly called as "Sarivan" or "Darh" (Nadkarni, 1954). It is a small shurb found in tropical region and throughout India extending from the Himalayas South Wards to Kerala, in the plains and as an undergrowth of semideciduous forests.

The aqueous extract of the root showed anti-inflammatory, antibacterial and antifungal activities. The drug had mild diuretic effect and it inhibited respiration in moderate doses. It was found to be non-toxic in acute toxicity studies (Prema, 1968). Kurian *et al*. (2010) reported the antioxidant effects of ethyl acetate extract of *D. gangeticum* root on myocardial ischemia reperfusion injury in rat hearts. Salparni has been used in Ayurvedic medicine for centuries, the whole plant, mainly the roots are used in medicine. The plant is a bitter tonic, digestive, antidysentric, alterative, aphrodisiac, antipyretic, anticatarrhal, febrifuge. It is used to cure typhoid and other fevers, asthma, bronchitis, vomiting, dysentery, piles, biliousness, chorela, scorpion sting and snake bite. The roots of this drug are used as one of the ingredients of two very important Ayurvedic preparations, 'Dashmoola Kwatha' and `Dashamootarishta'. If storage of medicinal plant organs is not properly there may be possibility of contamination of different organism and these microorganisms growth are responsible for the deterioration and changes of chemical constituents. Therefore, it is necessary to study the changes in chemical constituents in roots of this drug. So that, fresh and market roots of this plant stored at various relative humidity 30, 50, 75, 96 and 100% RH for different incubation periods 15, 30, 45,

60, 75 and 90 days. The effect of various relative humidity and incubation days on changes in chemical constituents studied.

MATERIALS AND METHODS

The fresh roots of drug *Desmodium gangeticum* DC. were collected in healthy, flowering and fruiting conditions from different localities. Market survey has also been carried out for the collection of the market roots from various Shopkeepers and Kashthaushadhi. Collected roots were brought to the laboratory in polyethylene bag to avoid aerial contamination. For evaluate the deterioration of chemical constituents, organ form of roots cut to small pieces and were stored in small muslin clothes bags under different level of RH i.e. 30, 50, 75, 96 and 100 % RH for 90 days and at 28 ± 3°C temperature (Wink and Sears, 1950). At an interval of 15 days, root samples were taken out and thoroughly washed with distilled water and were dried in oven for chemical analysis. Chemical analysis were estimated by the procedure described by Lowry *et al*. (1951) for total protein**,** Singh *et al*. (1978) for total phenols and Harborne method (1973) for total alkaloids. 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.

RESULTS AND DISCUSSION

The roots of *D. gangeticum*, which collected in fresh condition and from market stored at 30, 50, 75, 96 and 100% RH, They contained 8.30 and 8.19 % total protein. Minimum deterioration observed at 30, 50 % RH after 15 days of incubation, 8.30, 8.23 % and 8.19, 8.08 % but these values reduced to 7.88, 7.77% and 7.33, 7.22% after 90 days of storage period. Maximum reduction observed at 96 and 100% RH after 90 days of incubation in both samples (fresh and market); 7.61, 7.51% and 6.93, 6.83%, respectively (Table 1,2).

The fresh samples of *D. gangeticum* stored at various relative humidities and the deterioration of phenol contents also observed. The control and treated samples of this drug taken out after 15 days of storage and observed the reduction of phenol values. The control sample contained 14.46% total phenols. After 15 days of incubation total amounts of phenol increased to 14.40% but deterioration of phenol amounts continued. Deterioration of phenols amount started after 30 days of incubation in case of 30% RH, 14.37%, this amount gradually decreased to 14.01 % after 90 days of incubation. In other cases of RH 50, 75, 96 and 100% RH after 15 days of incubation, total amounts of phenol were 14.37, 14.37, 14.35, 14.40 % these values reduced to 13.99, 13.97, 13.79 and 13.62% after 90 days of incubation (Table 3). Market sample of this drug stored at same relative humidity and deterioration of total amount of phenols observed, at the first day13.20% recorded for total amount of phenols in this sample, more deterioration recorded for these samples as compared to fresh sample of this drug. After 90 days of incubation period showed more deterioration in total phenols amount under all tested RH, 11.49 (30 % RH), 11.39 (50 % RH), 11.24 (75 % RH), 11.18 (96 % RH) and 11.04 % (100% RH) (Table 4).

The fresh and market roots of *D. gangeticum* stored at various relative humidity and different incubation days and they showed different range of deterioration in total alkaloid amounts. The control genuine samples contained 4.06 % total alkaloid. Reduction of total alkaloids observed after 15 days of incubation under 96 % RH, 4.02% and under 100% RH reduced to 4 %. In case of 50 % RH, after 30 days total amount of alkaloids was observed 4.03% which reduced to 3.89% after 90 days of incubation, in case of 75, 96 and 100% RH deterioration more continued, while after 15 days to 90 days of storage period, total values of alkaloid from 4.06, 4.02, 4% deteriorated to 3.80, 3.67, 3.59%, respectively (Table 5).

In case of market sample of this drug more deterioration as compared to genuine sample observed. Control sample contained 4.016% total alkaloids. After 15 days of storage under 30, 50, 75, 96 and 100% RH observed 4.013, 4.013, 4.006, 3.99, 3.97% which deteriorated to 3.77, 3.73, 3.66, 3.58, 3.55% after 90 days of incubation (Table 6). Analysis of variance showed the effect of relative humidity and incubation days in reduction of total proteins, phenols and alkaloids content were significant at 5 % level of significance (P value <0.05).

More reduction in market samples as compared to fresh samples are showed. This may be due to unscientific methods of harvesting, collecting, handling and storage in unsuitable places, transporting and drying. These conditions promote the growth of fungi on storage herbal plants, also most of the storage fungi are cosmopolitan and their growth and sporulation are very fast and they proliferate on each suitable place with suitable temperature and

RH, therefore utilization and afterward deterioration of chemical constituents of drugs for their growth and developing of storage fungi increase. From this study also it is concluded that lower RH (30 and 50% RH) and short incubation period of drugs have less effectiveness on chemical constituents, this is may be due to less growth of fungi in these unfavorable conditions. During the course of interaction, microbes not only deteriorate their therapeutic value but it is considerably lost or reduced (Chourasia, 1995 and Roy *et al*.1988). So, the primary aim is to prevent deterioration of herbal drugs to maintain the quality. The medicinal properties of the plants vary with respect to different seasons. The age of the plant also decides its medicinal potency. Beside, the period of storage in sun or shade conditions also affects medicinal properties of the plants (Dubey *et al*. 2004). Information regarding suitable conditions for storage of medicinal drugs is not available, careless processing of medicinal drugs without considering these points is major reason for ineffectiveness of some traditional medicines. In many cases, chemical substances in plant medicine serve as the molecules of plant defense against microorganisms. However, several of these constituents possess medicinal properties (Mallikharjuna *et al*. 2007). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterol etc.

Table 1: Deterioration of proteins content (mg/100mg) in root of *Desmodium gangeticum* (Fresh samples) at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	8.30±0.013	8.31±0.001	8.31±0.10	8.31±0.013	8.31±0.12	8.31±0.013
15days	$8.31±0.010^{c}$	$8.30±0.008^{c}$	$8.23±0.036^{bc}$	$8.20±0.036^{ab}$	$8.23±0.008^{b}$	$8.18±0.052^{a}$
30days	$8.31±0.013^{e}$	$8.20±0.021^{d}$	$8.16±0.021^{cd}$	$8.12±0.050^{bc}$	$8.08±0.034^{ab}$	$8.05±0.013^{a}$
45 days	$8.31±0.053^{d}$	$8.12±0.041^{c}$	$8.09±0.028^{bc}$	$8.01±0.049^{ab}$	$8.05±0.080^{ab}$	$7.94±0.034^{a}$
60 days	$8.31±0.034^{c}$	$8.04±0.013^{b}$	$7.95±0.034^{ab}$	$7.91±0.027^{a}$	$7.90±0.077^{a}$	$7.88±0.073^{a}$
75 days	$8.31±0.068^{d}$	$7.94±0.021^{c}$	$7.90±0.013^{b}$	$7.79±0.013^{b}$	$7.76±0.060^{b}$	$7.63±0.021^{a}$
90 days	$8.31±0.034^{d}$	$7.88±0.057^{c}$	$7.77±0.068^{c}$	$7.65±0.076^{b}$	$7.61±0.073^{ab}$	$7.51±0.034^{a}$

Data 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 2: Deterioration of proteins content (mg/100mg) in root of *Desmodium gangeticum* (market samples) at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	8.19±0.086	8.19±0.086	8.19±0.086	8.19±0.086	8.19±0.086	8.19±0.086
15days	$8.19±0.08^{c}$	$8.19±0.08^{c}$	$8.08±0.073^{bc}$	$8.02±0.040^{b}$	$7.91±0.021^{a}$	$7.91±0.052^{a}$
30days	$8.19±0.073^{c}$	$8.08±0.073^{c}$	$7.88±0.027^{c}$	$7.76±0.027^{b}$	$7.59±0.059^{ab}$	$7.38±0.052^{a}$
45 days	$8.19±0.06^{c}$	$7.80±0.63^{c}$	$7.65±0.052^{bc}$	$7.61±0.063^{abc}$	$7.37±0.071^{ab}$	$7.18±0.063^{a}$
60 days	$8.19±0.086^{d}$	$7.50±0.73^{c}$	$7.43±0.036^{bc}$	$7.33±0.052^{abc}$	$7.20±0.27^{ab}$	$7.08±0.021^{a}$
75 days	$8.19±0.086^{c}$	$7.37±0.031^{c}$	$7.23±0.057^{bc}$	$7.11±0.021^{b}$	$7.05±0.021^{ab}$	$6.95±0.027^{a}$
90 days	8.19±0.089d	7.33±0.040c	7.22±0.021c	7.08±0.01b	6.93±0.021ab	6.83±0.057a

Data 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 total phenols content (mg/100mg) in root of *Desmodium gangeticum* (Fresh samples) at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	14.46±0.088	14.46±0.088	14.46±0.088	14.46±0.088	14.46±0.088	14.46±0.088
15days	$14.46±0.97^{b}$	$14.40±0.049^{a}$	$14.37±0.019^{a}$	$14.37±0.040^{a}$	$14.35±0.039^{a}$	$14.40±0.13^{a}$
30days	$14.46±0.80^{b}$	$14.37±0.070^{ab}$	$14.35±0.039^{ab}$	$14.37±0.037^{ab}$	$14.35±0.12^{ab}$	$14.21±0.07^{a}$
45 days	$14.44±0.006^{c}$	$14.37±0.10^{bc}$	$14.27±0.10^{ab}$	$14.21±0.070^{bc}$	$14.13±0.022^{b}$	$14.15±0.30^{a}$
60 days	$14.43±0.045^{c}$	$14.15±0.039^{ab}$	$14.13±0.039^{ab}$	$14.15±0.13^{ab}$	$14.11±0.069^{ab}$	$14.03±0.049^{a}$
75 days	$14.42±0.046^{b}$	$14.07±0.063^{a}$	$14.07±0.081^{a}$	$14.03±0.069^{a}$	$13.95±0.01^{a}$	$13.79±0.022^{a}$
90 days	$14.42±0.032^{d}$	$14.01±0.04^{c}$	$13.99±0.019^{c}$	$13.97±0.097^{bc}$	$13.79±0.052^{b}$	$13.62±0.11^{a}$

Data 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 *Desmodium gangeticum* (market samples) at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	13.20±0.022	13.20±0.022	13.20±0.022	13.20±0.022	13.20±0.022	13.20±0.022
15days	13.20±0.026^{d}	13.20±0.022^{c}	13.18±0.16^{b}	13.01±0.19^{ab}	12.85±0.17^{a}	12.79±0.11^{a}
30days	13.20±0.021^{d}	13.18±0.019^{c}	12.95±0.39^{bc}	12.61±0.010^{b}	12.06±0.23^{ab}	11.96±0.026^{a}
45 days	13.20±0.02^{d}	12.65±0.26^{c}	12.44±0.21^{b}	12.22±0.16^{b}	11.85±0.049^{a}	11.61±0.049^{a}
60 days	13.20±0.018^{d}	12.42±0.019^{c}	12.22±0.10^{b}	12.06±0.11^{b}	11.79±0.030^{ab}	11.55±0.09^{a}
75 days	13.20±0.02^{c}	11.83±1.1^{bc}	11.63±0.085^{b}	11.57±0.097a^{bc}	11.37±0.022^{a}	11.22±0.16^{a}
90 days	13.20±0.022^{c}	11.49±0.52^{b}	11.39±0.03^{b}	11.24±0.093^{b}	11.18±0.12^{a}	11.04±0.20^{a}

Data 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 *Desmodium gangeticum* (Fresh samples) at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	4.06±0.15	4.06±0.15	4.06±0.15	4.06±0.15	4.06±0.15	4.06±0.15
15days	4.06±0.10^{c}	4.06±0.19^{c}	4.06±0.49^{b}	4.06±0.40^{ab}	4.02±0.25^{a}	4±0.95^{a}
30days	4.06±0.10^{c}	4.06±0.49^{c}	4.03±0.60^{b}	4±0.26^{a}	3.96±0.32^{a}	3.87±0.29^{a}
45 days	4.060±0.62^{c}	4.03±0.39^{c}	4±0.11^{b}	3.94±0.62^{a}	3.84±0.82^{a}	3.80±0.81^{a}
60 days	4.06±0.15^{c}	4.01±1.15^{c}	3.97±1.86^{b}	3.91±1.13^{ab}	3.80±1.13^{a}	3.76±1.08^{a}
75 days	4.06±0.95^{d}	3.99±0.75^{c}	3.92±0.78^{b}	3.74±1.10^{ab}	3.74±1.17^{a}	3.69±1.16^{a}
90 days	4.06±0.15^{d}	3.93±0.45^{c}	3.89±0.94^{b}	3.80±0.47^{a}	3.67±0.61^{a}	3.59±0.69^{a}

Data 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 alkaloids content (mg/100mg) in root of *Desmodium gangeticum* (market samples) at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	4.016±0.11	4.016±0.11	4.016±0.11	4.016±0.11	4.016±0.11	4.016±0.11
15days	4.011±0.11^{b}	4.013±0.12^{b}	4.013±0.13^{a}	4.006±0.14^{a}	3.99±0.25^{a}	3.97±0.16^{a}
30days	4.006±0.10^{b}	4±0.49^{b}	3.99±0.10^{a}	3.95±0.13^{a}	3.90±0.10^{a}	3.91±0.078^{a}
45 days	4.99±0.095^{b}	3.98±0.11^{b}	3.94±0.11^{ab}	3.88±0.080^{a}	3.85±0.10^{a}	3.83±0.81^{a}
60 days	3.96±0.055^{c}	3.94±0.15^{b}	3.89±0.095^{ab}	3.86±0.085^{ab}	3.80±1.13^{a}	3.77±0.090^{a}
75 days	3.95±0.043^{c}	3.86±0.75^{b}	3.81±0.78^{b}	3.76±0.075^{ab}	3.70±0.091^{ab}	3.63±0.11^{a}
90 days	3.94±0.045^{d}	3.77±0.068^{cd}	3.73±0.062^{c}	3.66±0.56^{bc}	3.58±0.052^{ab}	3.55±0.064^{a}

Data 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

CONCLUSION

The result of this investigation indicates that relative humidities 75, 96 and 100% RH show the significant reduction in total proteins, phenols and alkaloids amount. Increased storage period (45 and 60 days of incubation) also is effective on biodeterioration of these chemical constituents. More reduction in market samples as compared to fresh samples are showed.

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