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EFFECT OF DRYING ON PROTEIN PROFILE OF *MURRAYA KOENIGII* LEAVES

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Abstract: The present study was aimed at exploring the effect of drying on the protein profile of *Murraya koenigii* (curry leaves) leaves. *M. koenigii* leaves were subjected for protein profile study in two forms: i. Fresh leaves and ii. Dried leaves. Aqueous extract of the experimental samples were subjected for protein profile study by the standard SDS-PAGE procedure. The polypeptide bands in the gel were analysed against a clear white background. The numbers of bands falling within each molecular weight (kDa) range were accounted for assessment of general protein profile. There were a total of 19 prominent bands in fresh *M. koenigii* leaves, of which 14 (73.7%) were above 36 kDa. In the dried samples there were only 13 prominent bands with an overall reduction in 31.6 per cent of detectable proteins. Majority of proteins were lost in the range of 36-53 kDA (50.0 %) and 118-211 kDA (66.7 %). Drying of *M. koenigii* leaves led to loss of several proteins and administration of fresh herbs should be preferred rather than dried and processed products for better results.

Keywords: *Murraya koenigii* – fresh and dried leaves – protein profile.

Introduction

Plants are a rich source of secondary metabolites with interesting biological activities and therapeutic properties. With the increasing recognition of herbal medicine as an alternative form of health care and with the emerging 'one health concept', commercialization of herbal products is also rapidly flourishing. In the process of preparation of commercial products, herbs are subjected for bulk procurement, drying, processing and preservation, during which time they may lose their valuable components. Hence, as a proof of validation, the screening of medicinal plants for active compounds has become very significant.

Murraya koenigii, popularly known as curry leaves, is a plant found throughout tropical and subtropical East Asia from India to China. Because of its multifaceted medicinal properties as well as the changing demographics nationwide, it has created a ready market and greater

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demand for this spice. Satheshkumar and Punniamurthy (2009 and 2010) studied the effect of fresh *M. koenigii* leaves and found that it has the potential to augment the ovarian function in terms of follicular development in cattle. Based on these studies, it could be assumed that fresh *M. koenigii* leaves have certain proteins related to follicle stimulating properties. On the other hand, administration of *M. koenigii* in the dried powdered form did not yield similar results (unpublished data). It is hypothesized that protein profile of the herb could be altered by the process of drying resulting in reduced response. Hence the present research was designed with the objectives to study the effect of drying process on protein profile of *M. koenigii* leaves.

Materials and methods

Leaves of *M. koenigii* were collected afresh and utilized for the study. The leaves were subjected for protein profile study in two forms: Group 1: Fresh leaves: Leaves were subjected for the experiment within 2-3 hrs of collection and Group 2: Dried leaves: The leaves were collected and shade dried for 3-4 days and powdered.

Aqueous extraction of these leaves samples was carried out as described by Parekh *et al*. (2005) with slight modification. Ten grams of leaves was added to distilled water and heated for 4 h at slow heat (60–70^{o}C). Every 2 h it was filtered through Whatman filter No.1 and the extract was centrifuged at 5000 g for 15 min. The supernatant of the extract was collected and concentrated by boiling to make the final volume to one-fifth of the original volume and left undisturbed in a sterile environment.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE gel process was carried out according to the modified method of Laemmli (1970). SDS-PAGE gels were prepared as discontinuous buffer system with a 12% separating gel and a 5% stacking gel. Aliquots of 5 µ1 of samples viz., aqueous extracts of fresh and dried *M. koenigii* were diluted with an equal volume of sample buffer and boiled for 4 min before being loaded onto each lane. Molecular-weight standards (Broad range: 6 – 211 kDa; Bio-Rad) were also routinely loaded. Electrophoresis was performed at a constant intensity of 50 mA / gel. At the end of migration, gels were stained with Coomassie blue stain overnight at room temperature and destained by repeated rinsing in gel de-staining solution.

Analysis of polypeptides

The polypeptide bands in the gel were analysed against a clear white light. The numbers of prominent bands falling within each molecular weight (kDa) range were counted for assessment of general protein profile. The differences in the number of polypeptide bands of

fresh and dried herb samples were calculated as percentage and compared to arrive at the effect of drying the *M. koenigii* leaves.

Results

The results of SDS–PAGE analysis of fresh and dried samples of *M. koenigii* leaves were presented in Table 1 and Fig. 1. Perusal of the data revealed that, there were a total of 19 prominent bands in fresh *M. koenigii* leaves, of which 14 (73.7%) were above 36 kDa. In the dried samples there were only 13 prominent bands with an overall reduction in 31.6 per cent of detectable proteins. Eventhough, the low molecular weight proteins seemed to be unaffected, the number of bands representing heavy molecular weight proteins (above 36 kDa) were reduced in the dried samples when compared with the fresh samples. Majority of proteins were lost in the range of 36-53 kDA (50.0 %) and 118-211 kDA (66.7 %).

Discussion

Perusal of prominent polypeptide bands in aqueous extracts of fresh and dried *M. koenigii* leaves revealed 31.6 per cent reduction of proteins in the latter samples. Similarly, Messman *et al.* (1994) found that drying forage (on a laboratory bench) for five days resulted in a 25 to 30% loss in electrophoretically identified proteins. Nijole *et al*. (2012) also recorded high concentration of protein (1.18 mg/g) in protein fractions isolated from fresh *Urtica dioica* herb than their dry extract (0.40 mg/g). The finding indicated that the process of drying causes loss of proteins in the herb studied.

Eventhough low molecular weight proteins were not much affected, heavy proteins with molecular weight above 36 kDa were significantly lost in the process of drying. A ligand blotting experiment by Komatsu et al. (1996) proved the presence of EGF binding proteins (35-50 kDa) capable of binding to EGF and suggested the possibility of EGF-like regulation in plants. It has been well established that EGFs and IGFs function as modulators of gonadotrophin and play an important role in folliculogenesis in many mammalian species (Armstrong and Webb, 1997; Fujinaga *et al.,* 1992). Fresh *M. koenigii* leaves have stimulatory effect on follicular development (Satheshkumar *et al.,* 2009 and 2010) than dried leaves. The loss of the therapeutic potential in the dried herb could be attributed to the loss of essential proteins.

Table 1: Distribution of polypeptide profile in sediments of Fresh and Dried *M. koenigii* samples

S.No.	Molecular weight range (kDa)	No. of bands		Loss of protein (%)
		Fresh	Dried	
1	**118 - 211**	3	1	66.7
2	**78 - 118**	6	4	33.3
3	**53 - 78**	3	2	33.3
4	**36 - 53**	2	1	50.0
5	**28 - 36**	1	1	--
6	**17 - 28**	3	3	--
7	**6 - 17**	1	1	--
Total		**19**	**13**	**31.6**

Conclusion

The study of protein profile in *M. koenigii* leaves highlighted the protein file of fresh and dried samples. It could be concluded that drying of *M. koenigii* leaves led to loss of several proteins. Hence administration of fresh herbs should be preferred rather than dried and processed products for better results.

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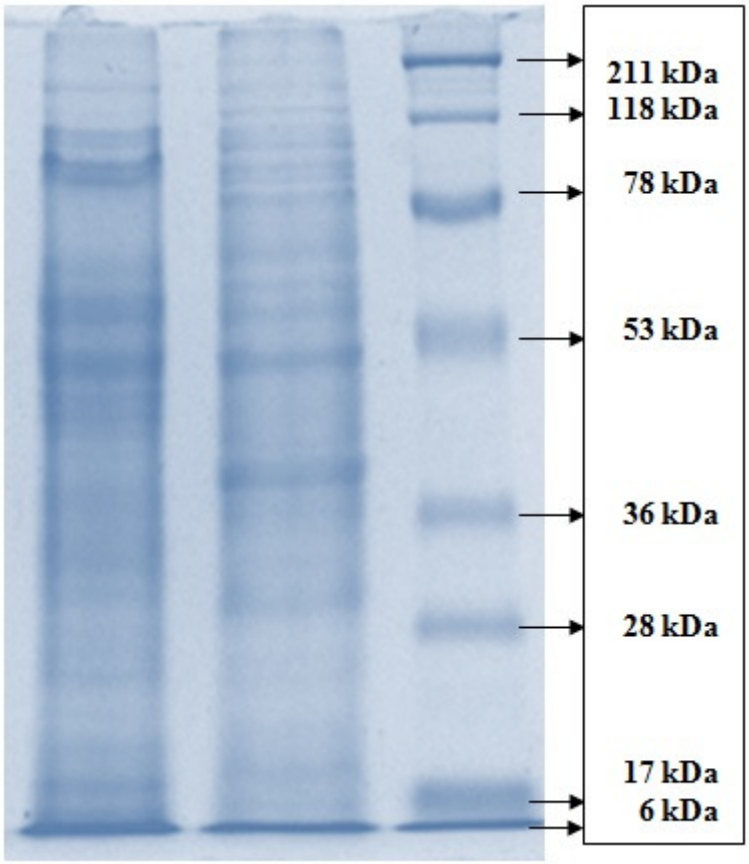
Fig.1: SDS-PAGE of extract of Fresh and Dried *M. koenigii* samples



Lane 1 : Dried *M.koenigii* - sediment
Lane 2 : Fresh *M.koenigii* - sediment
Lane 3 : Protein marker (Broad range)