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STABILITY STUDY OF CRUDE EXTRACTS OF *MOULLAVA SPICATA* (DALZ.) NICOLSON BY HPLC

Chemistry

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ABSTRACT

Chromatographic technique such as HPLC is used for analysis of crude extracts of *m. spicata.* Extracts were evaluated for thermal stability under room temperature and at temperature (54 ±2°C) for accelerated stability studies. The samples were maintained under the conditions described for the period of 14 days. The accelerated storage stability study was carried out as per standard method of Collaborative International pesticides Analytical Council (CIPAC) method. As per the procedure samples were withdrawn everyday and analyzed by HPLC. Typical HPLC chromatograms of test samples indicated no change even after subjecting the *M. spicata* petroleum ether (MSPE), ethyl acetate (MSEA) and methanol (MSME) as a test material for the heat stress in all the three cases. It was seen that no changes in the chromatographic profile occurred during the period of analysis in all the cases. Thus the extracts showed good compatibility under stress conditions and can be stated to be stable. The focus of this paper is on the analytical methods, which include extraction and stability study of crude extracts.

KEYWORDS

Stability study, Crude extract, M. spicata, HPLC analysis.

INTRODUCTION:

Moullava spicata is monotypic genus of a robust woody climbing shrub growing 5 to 20 m long.. Important feature of this plant is that it is indigenous to western presidency[3]. It occurs abundantly in Maharashtra state, especially in Konkan region and on the Ghats near Mahabaleshwar[4]. *M. spicata* having various medicinal importance, various parts of the plants having many applications in different diseases[13]. Roots of the plant are used to cure pneumonia and in treatment of pulmonary tuberculosis[5,7,8,9]. Crude ethanol extract of this plant shows hypotensive activity and it effects on respiration and cardiovascular[15]. *M. spicata* possesses antiseptic properties[16]. It also heals diabetic wounds[6].The bark is having applications in skin diseases[6]. This plant also used to cure diorrhaea[6]. Preliminary phytochemical analysis, bioactivity study of crude extracts of this plant is studied[17,18]. Chemical investigation by GC MS analysis of this plant is also reported[14,19]. The stability testing is important to ensure the standard dose delivery of drug throughout its life and fulfil legal requirements concerning identity, purity, strength and quality of drug[10]. Stability testing of herbal products like any other pharmaceutical product is important to make herbal remedies, reliable medicines. The reasons for the determination of stability of pharmaceuticals are based on concern for public health. The WHO defines the stability of drugs and medicines as the ability of a pharmaceutical product to maintain its chemical, physical, microbiological and biopharmaceutical properties within special limits throughout the duration of the product usage[11]. Recently phytochemical profiling of *m. spicata* by HPTLC and GC MS was reported[12]. Recent attempt has been made to extract the *m. spicata* aerial part of plant material in various solvents with their increasing polarity and thermal stability study of crude extracts is done. The accelerated storage stability study was carried out as per standard method of Collaborative International pesticides Analytical Council (CIPAC) method[1]. A thorough survey of literature revealed that stability studies of crude extracts of *m. spicata* were not reported yet.

MATERIALS AND METHODS:

M. spicata plant material was collected from Dajipur, Radhanagari, Kolhapur and Authenticated at ARI, Pune. Dry powdered aerial part plant material (100 g) of *M. spicata* was extracted in petroleum ether, ethyl acetate and in methanol successively by using soxhlet apparatus. Extracts were labeled as MSPE, MSEA and MSME. All reagents and solvents were of analytical and HPLC grade. Ultrapure water obtained by a Milli-Q was used in all experiments. All other solvents and chemicals were of analytical grade. MSPE, MSEA and MSME were evaluated for thermal stability under room temperature and at temperature (54 ±2°C) for accelerated stability studies. The samples were maintained under the conditions described for the period of 14 days. For this purpose a samples of MSPE, MSEA and MSME were weighed (200 mg each) and packed in a glass vial (5 ml capacity). The glass vial was wrapped in aluminum foil to avoid exposure of light. Fifteen such vials were prepared and numbered serially. One of these vials (No. 1) of three samples i.e total three vials placed on laboratory bench at room temperature under natural climatic conditions at room temperature without direct light incident on it. Remaining fourteen vials (2 to 15) of all three samples were kept in the oven maintained at 54 ±2°C for accelerated stability studies. One vial of each extract from oven was withdrawn every day and analyzed by HPLC along with that kept at room temperature. Samples were analyzed at initial time (t_0) and every day up to 14 days after exposure (t_1 to t_{14}). 10 mg sample of MSPE, MSEA and MSME taken for analysis, it was dissolved in 10 ml acetonitrile. Samples were filtered through a 0.45µm filter. The analyses were carried out on Agilent 1100 HPLC system using column Lichro CART Purosphere STAR (4.6 X 250 mm, Rp 18e, particle size 5µm). The mobile phase consisted of 0.1% formic acid: acetonitrile (gradient). Both solvents were degassed and filtered through a 0.45µm filter pore size filter. Separations were done by gradient program as 0 min 35% B in A, after 6 min 45% B in A,15 min 50%B in A, 28 min 50% B in A, 30 min 35%B in A, followed by 3 minute re-equilibration time. The injection volume was 10µl and mobile phase flow rate was 1 ml /min. The analyses were carried out at 28°C, UV detection was done at 280 nm.

RESULTS AND DISCUSSION:

An accelerated study was used to check the stability study of the product by subjecting it to elevated temperatures. The accelerated storage stability study was carried out as per standard method of Collaborative International pesticides Analytical Council (CIPAC) method[1]. As per the procedure samples were withdrawn everyday and analyzed by HPLC. Typical HPLC chromatograms of test samples of MSPE, MSEA, MSME of the *M.spicata* at $t_{(0)}$ and $t_{(14)}$day is indicated in figures (a), (b) and © respectively.

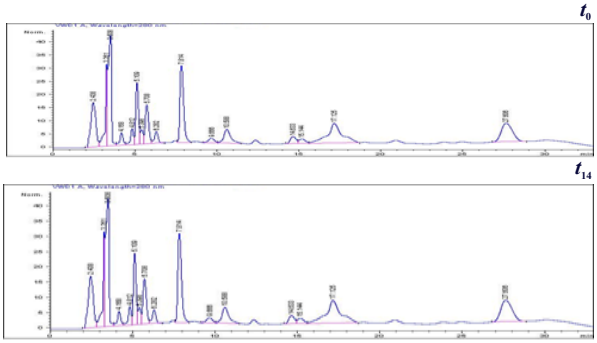

Fig. 1.1: Typical HPLC chromatograms of stress test samples of the M. spicata petroleum ether crude extract at room temperature at time zero (t0) and at accelerated temperature (54 ± 2°C) at day 14

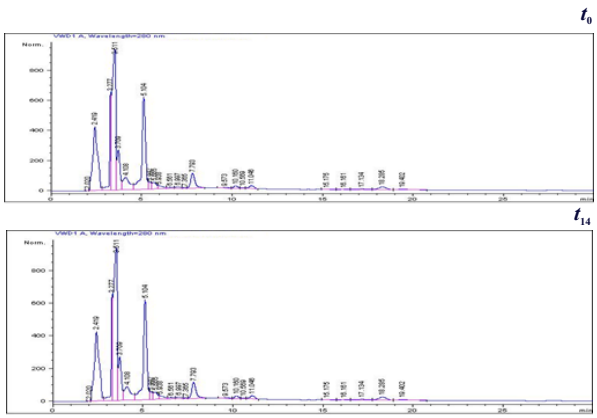

Fig. 1.2: Typical HPLC chromatograms of stress test samples of the M. spicata ethyl acetate crude extract at room temperature at time zero (t0) and at accelerated temperature (54 ± 2ºC) at day 14

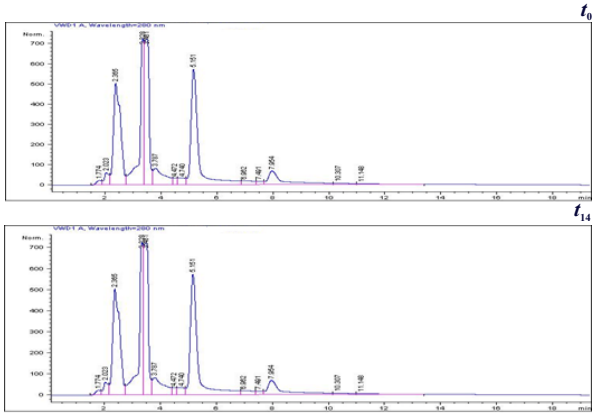

Fig. 1.3: Typical HPLC chromatograms of stress test samples of the M. spicata methanol crude extract at room temperature at time zero (t0) and at accelerated temperature (54 ± 2ºC) at day 14

It was noteworthy that the chromatograms indicated no change even after subjecting the M. spicata petroleum ether (MSPE), ethyl acetate (MSEA) and methanol (MSME) as a test material for the heat stress in all the three cases. It was seen that no changes in the chromatographic profile occurred during the period of analysis in all the cases.Thus the extract showed good compatibility under stress conditions and can be stated to be stable.

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