Research Article

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DETERMINATION OF NATURAL COMPOUNDS IN DASHMOOL EXTRACTS BY THIN LAYER CHROMATOGRAPHY AND HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Mittal Sandhya[1]*, Rao Nidhi[1], Sudhanshu[1], Menghani Ekta[2]
[1]Suresh Gyan Vihar University, Jaipur, India
[2]Mahatma Gandhi Institute of Applied Sciences, JECRC University, Jaipur, India

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***Corresponding author**
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ABSTRACT
The determination of the natural compounds lupeol, beta sitosterol and stigmasterol in plant extracts using HPLC Shimadzu Model LC2010 A_{HT} Auto Sampler (UV –VIS Detector) is reported. The methods were applied to the analysis of lupeol, beta sitosterol and stigmasterol in petroleum ether extract originating from Dashmool. The result of TLC of Dashmool petroleum ether extract showed the presence of β- sitosterol, stigmasterol and lupeol in Dashmool plant extract at different Rf. HPLC profile of petroleum ether extract of Dashmool have characteristics peaks at retention time 2.888, 2.971, 3.135, 3.442, 4.018, 4.220, 4.406, 4.885, 5.388, 5.657, 6.083 (Stigmasterol), 7.848, 9.137, 10.012, 17.656 (Lupeol), 18.138 (β-Sitosterol) and 23.096. These peaks showed that there are different compounds and characteristic fingerprints of drug to judge in a herbal formulation.
Keywords: Lupeol, β-Sitosterol and Stigmasterol, high-performance liquid chromatography.

INTRODUCTION

Dashmool is a combination of ten ayurvedic herbs; *Aegle marmelos* L., *Oroxylum indicum* (Linn) Vent, *Gmelina arborea* L., *Stereospermum suaveolens* L., *Premna integrifolla* L., *Desmodium gangeticum* (L.) DC., *Uraria lagopoides* L., *Solanum indicum* L., *Solanum xanthocarpum* SCHRAC&WENDLE *and Tribulus terrestris* L. which is used in pacifying vata dosha. The herbs are used in making certain therapeutic massage oils, and are also used in the form of tea to treat certain conditions. In the Ayurvedic system of medicine it is used as analgesic, antiarthritic, against cough and rheumatism etc[1].

Considering the fact that Dashmool is easily available and more useful, some herbal industries tend to use Dashmool for different purpose. So it is critical to build up a appropriate and consistent classification system to corroborate the value of extracts and herbal drugs. Separation and detection of diverse constituents in plants have been always convoluted. While conventional research mostly focuses on fortitude of the active components, fingerprinting can offer characterization of a complex system with a degree of quantitative reliability, so it has gained increasing interest for quality control systems over the past years [2]. Chromatography methods including TLC and HPLC techniques are mainly used for fingerprinting[3-5]. TLC is a common quick and cost-efficient method used for fingerprinting plant extracts. Moreover, several samples can be chromatographed concurrently on a single plate and complex instruments are not necessary[4-6]. In this examination, TLC and HPLC chromatograms of essential oil and extract of Dashmool were prepared and their patterns were compared with each other to specify the similarities and differences between them.

MATERIALS AND METHODS

Collection: Plant sample was collected with the help of various tribes living in tribal pockets of Mount Abu, arid zone of Rajasthan, in the month of Feb, 2010. This plant was used by these tribes in their daily lives to cure various ailments.

Identification: This sample was authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence (funded by DST), MGIAS, Jaipur (Rajasthan) with herbarium voucher number *Oroxylum indicum* Linn Vent (MP0089), *Gmelina arborea* L. (MP0055), *Stereospermum suaveolens* L. (MP00156), *Premna integrifolla* L. (MP00102), *Desmodium gangeticum* (L.) DC. (MP0032), *Uraria lagopoides* L. (MP00178), *Solanum indicum* L. (MP00139), *Solanum xanthocarpum* Schrac & Wendle (MP00148) and *Tribulus terrestris* L. (MP00165), *Aegle marmelos* L. (MP0020)

Preparation of test extracts: Crushed powder of species was soxhlet extracted. Later the homogenate was filtered and the residue was re-extracted twice for complete exhaustion and extract was cooled individually. Filtrate was concentrated to dryness in vitro and re dissolved in respective solvent which was stored at 4°C in a refrigerator, until screened for TLC and HPLC profiling.

Thin layer chromatography (TLC)

Extraction procedure

For TLC profile of selected species, dried and powdered (100 gm) test samples was soxhelt extracted in petroleum ether for 6 hours. These extract were filtered, evaporated to dryness and weighed. Extract (10 mg) was dissolved to make a concentration of 1mg/ml used for further studies.

TLC plates

Extract was applied on silica gel G Thin Layer Chromatography (TLC) coated plates (Merck: 20x20 cm; with thickness 0.2-0.3mm) which were activated at 100°C for 30 minutes and brought to room temperature, just before use. Extract of species was applied 1cm above the

edge of the chromatographic plates along with the reference compounds and developed in air-tight chamber already saturated with 200 ml of solvent system[7].

TLC solvent system

Extract of test sample was subjected to different solvent systems for identification of any significant bio molecules. After having used different solvent systems, on the basis of better resolution of spots for generating "Thin Layer Chromatography (TLC) fingerprints" for chemical libraries of the test drugs, following solvent system were used in the present study- Acetone : Hexane (1:3) for Petroleum ether extract.

TLC spraying reagents

During the work of present studies, different visualizing reagents i.e. 10% sulphuric acid (10 ml conc. Sulphuric acid dissolved in 100ml absolute alcohol), I_2 vapour (Saturated iodine chamber) and Dragendorff reagent were used.

Qualitative TLC

Thin glass plates were coated (0.2-0.3 mm) with silica gel G (30 g/60 ml distilled water) and dried at room temperature. The coated plates were activated in an oven at 100°C for 30 minutes and cooled. The plates were then placed in developing tanks having 150 ml of an organic solvent mixture of Acetone: Hexane (1:3) for Petroleum ether extracts. The lid of the developing tanks was sealed with vaccum grease. The plates were removed after making the solvent front and were air-dried. The dried plates were sprayed with 10% sulphuric acid (10 ml concentrated Sulphuric acid dissolved in 100ml absolute alcohol) and further addition of 28 g (KI) and alkaloid. Positive spot (R_f value) was calculated.

Preparative TLC

Silica gel G thick layer plates were activated at 100° C for 30 min. The petroleum ether extract and the reference compound β- sytosterol[8], Stigmasterol and Lupeol were applied separately as a streak 1 cm above the edges of the plates and developed in an organic solvent mixture of Acetone: Hexane (1:3). A portion of the plate containing the applied standard reference and the extract was visualized under 10 % sulphuric acids (10 ml conc. Sulphuric acid dissolved in 100ml absolute alcohol) and also exposed to I_2 vapour for 10 min. The spots coinciding to the reference compounds were marked and compared with that of standard reference compound.

HPLC Analysis

The HPLC analysis was performed using a Shimadzu Model LC2010 A_{HT} Auto Sampler (UV –VIS Detector) set at 254nm, column Hypersil BDS C18 (250 x 4.6 mm; 5 Micron), flow rate: 1ml/min, injection volume 20µl in methanol (HPLC grade).

Standard Preparation

One mg of standard compound that is lupeol, stigmasterol, β- sitosterol were isolated from TLC fingerprinting of plant and authenticated by spectral analysis. They were dissolved in methanol and volume of which was raised to 1ml and used.

Table 1: R_f values of Dashmool Pet.ether extract with standard

Pet. ether	Plant Extract	β- Sitosterol	Stigmasterol	Lupeol
R_f(A)	.72	-	-	.72
R_f(B)	.62	-	.62	-
R_f(C)	.60	.60	-	-

Table 2: HPLC retention time and area of Lupeol, β- Sitosterol, Stigmasterol pure compound

Peak	Retention Time	Area
1.	2.833	357078
2.	6.714	765257
3.	17.656	965560
4.	18.136	970432

Table 3: HPLC retention time and area of Dashmool petroleum ether extract

Peak	Retention Time	Area	Height	Area%	Height%	K'
1.	2.888	348101	76298	3.267	7.995	0.000
2.	2.971	662293	68659	6.216	7.195	0.029
3.	3.135	240598	54072	2.258	5.666	0.085
4.	3.442	4556016	299958	42.758	31.433	0.192
5.	4.018	28142	4315	0.264	0.452	0.390
6.	4.220	474367	67956	4.452	7.121	0.461
7.	4.406	3826856	348190	35.915	36.487	0.525
8.	4.885	21577	3192	0.203	0.334	0.691
9.	5.388	31955	2576	0.300	0.270	0.866
10.	5.657	99463	10712	0.933	1.123	0.959
11.	6.083	1667	1205	0.156	0.126	1.106
12.	7.848	15617	1372	0.147	0.144	1.717
13.	9.137	21400	1388	0.201	0.145	2.164
14.	10.012	72482	5592	0.680	0.586	2.466
15.	17.656	21210	976	0.199	0.102	3.681
16.	18.138	27163	1334	0.255	0.140	6.097
17.	23.096	191459	6478	1.797	0.679	6.996

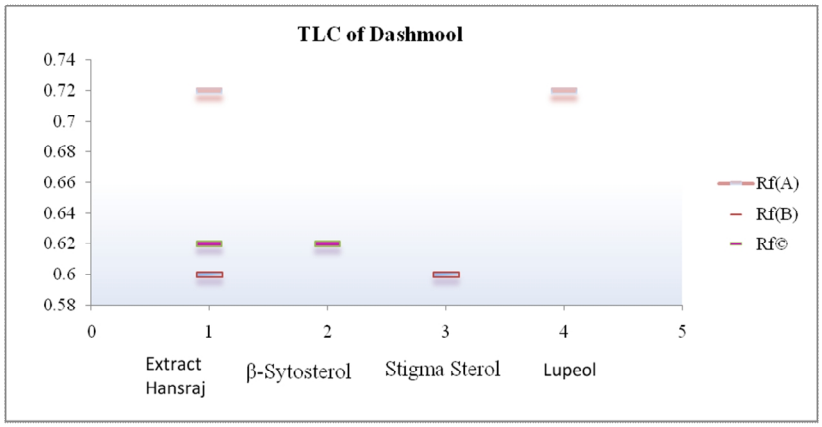

Figure 1: Presence of β- Sitosterol, Stigmasterol and Lupeol in plant extract

Figure 2: TLC plate of Dashmool Pet.ether extract

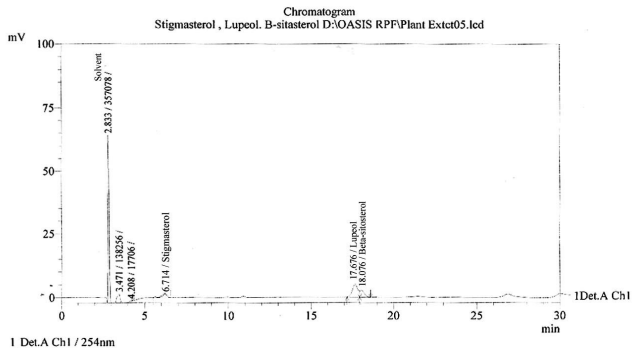

Figure 3: HPLC chromatograms of Lupeol, β- Sitosterol and Stigmasterol pure compound

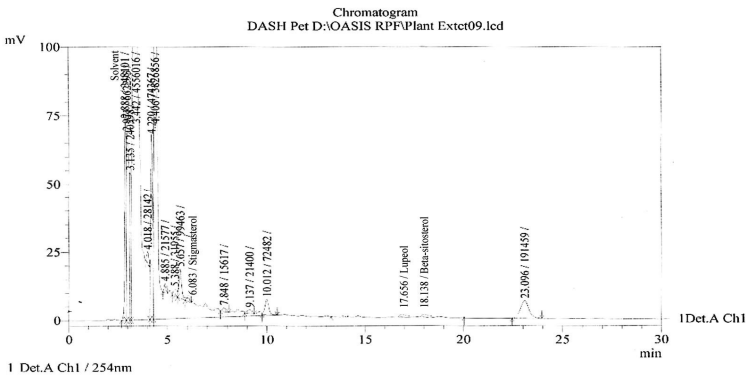

Figure 4: HPLC chromatograms of Dashmool petroleum ether extract.

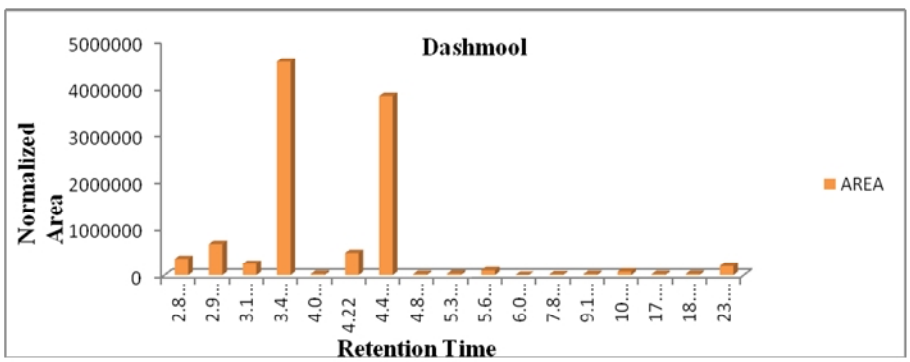

Figure 5: Normalized fingerprints of alcohol soluble Dashmool extract

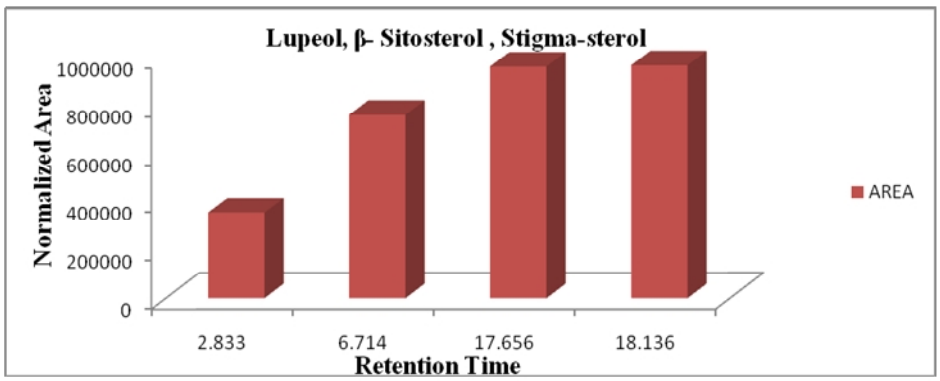

Figure 6: Normalized fingerprints of alcohol soluble Lupeol, β- Sitosterol and Stigmasterol

Quantification of natural compounds in Dashmool by HPLC

Pet. ether extract (piperine-rich fraction) of Dashmool was weighed and dissolved in methanol. 20 µl of concentration of Dashmool was injected onto HPLC and the peak which appeared at the same retention time as that of standard was recorded. This value was used to calculate the amount of standard in the extract by using the linear equation obtained from the composite standard curve. In the present work, various calculations were achieved by Height/ Area method. This method utilizes the fact that the area of a peak is a function of its height and standard deviation. To determine efficiency, values for peak height and area are used in a different formula: $N = 2\pi (ht_r)^2/A^2$

(Where h =peak height; t_r= retention time; N= number of theoretical plates; A=area). A computer is usually necessary to use this method in order to calculate the area and height.

RESULTS AND DISCUSSION

Dashmool Petroleum ether extract when run in solvent system Acetone: Hexane (1:3) with standard showed 3 spots by visible / naked eye at R_f .72 (Purple), R_f .62(Purple), Rf .60 (Pink) by spraying with 10% H_2SO_4. Graph showing the result of TLC of Dashmool Pet.ether extract. It showed the presence of β- Sitosterol, Stigmasterol and Lupeol in Dashmool plant extract.

+In the present study, HPLC was performed for Lupeol, β-Sitosterol and Stigmasterol run in methanol under 254 nm, the time recorded at 18.138, 6.714 and 17.656, which showed that as the column size increases it affects on retention time (column size α rt). It also affects the peak sharpness.

HPLC profile of petroleum ether extract of Dashmool have characteristics peaks at retention time 2.888, 2.971, 3.135, 3.442, 4.018, 4.220, 4.406, 4.885, 5.388, 5.657, 6.083 (Stigmasterol), 7.848, 9.137, 10.012, 17.656 (Lupeol), 18.138 (β-Sitosterol), 23.096. These peaks showed that there are different compounds and characteristic fingerprints for each drug to judge in an herbal formulation. There normalized fingerprints are principal markers that can check the purity/impurity of drug at very low concentration.

CONCLUSION

In present investigations attempts were made to isolate various pure bioactives from Dashmool and it is noteworthy that beta sitosterol, stigmasterol and lupeol were isolated which are potentials source as anti HIV agents. Thus, these plants have potentials role in future as drug or therapeutic targets.

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REFERENCES

1. Anonymous. Preparation of kwath and dashmulakwath. Bangladesh national formulary of ayurvedic medicine. (Approved by the Govt. of Bangladesh vide Ministry of Health and Family Welfare, Memo No. Health – 1/Unani – 2/89/(Part – I) 1992; 116: 20-32.
2. Committee of National Pharmacopoeia. In: Pharmacopoeia of PR China. Press of Chemical Industry, Beijing. Thymus monograph, 2000; (2): 99-100.
3. Wang LC, Cao YH, Xing XP and Ye JN. Fingerprint studies of Radix Scutellariae by capillary Electrophoresis and High Performance Liquid Chromatography. Chromatographia. 2005; 62 (5/6): 283 - 288. http://dx.doi.org/10.1365/s10337-005-0624-6
4. Birk CD, Provens G, Gosman G, Reginatto F and Schenkel EP. TLC Fingerprint of Flavonoids and Saponins from Passiflora species. J. Liquid Chromatography & Related Technologies. 2005; 28 (14): 2285 - 2291. http://dx.doi.org/10.1081/JLC-200064212
5. Obradovic M, Krajsek SS, Dormastia M and Kreft S. A New Method for the Authentication of Plant Samples by Analyzing Fingerprint Chromatograms. Phytochem. Analysis 2007; 18: 123 - 32. http://dx.doi.org/10.1002/pca.960 PMid:17439013
6. Tyrpien K. Analysis of Chosen Organic Tobacco Smoke Components and Their Metabolites by Planar Chromatography. Polish J. of Environ. Stud. 2006; 15 (4): 609 - 14.
7. Harborne JB. "Phytochemical Methods", London, Chapman and Hill 1973.
8. Heftmann E (ED). Chromatography: Laboratory Handbook of Chromatographic and Electrophoretic Methods. Van Nostrand-Reinhold Company, New York 1974; 610-636.

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