



## Research Article

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### QUALITATIVE AND QUANTITATIVE ESTIMATION OF A POLYHERBAL COMPOUND DASHANG LEPA

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**ABSTRACT**

In Ayurvedic system of medicine mainly polyherbal compounds are used for the treatment of various infections. Dashang lepa is one of the polyherbal compound which contain mixture of ten indigenous drugs. It is used to treat inflammatory swellings like as cellulitis by external application. As several study show qualitative and quantitative estimation of individual drug but none of the estimation involves combined study of Dashang lepa. Qualitative phytochemical, thin layer chromatography and quantitative loss on drying, ash value, pH value analysis were studied for polyherbal drug Dashang lepa. Methanolic extraction was carried out by hot percolation method through soxhlet apparatus. Phytochemical study was performed using various standard methods and TLC analysis was done with four type of solvent system. Determination of moisture content (loss on drying), measurement of ash value and pH value were also carried out as per standard guidelines of Ayurvedic Pharmacopoeia of India. The phytochemical analysis showed the presence of alkaloid, saponin, tannins, terpenoids, steroids, glycosides, phenols and flavonoids. TLC analysis shows six spots using chloroform: ethyl acetate: methanol (6: 3: 1) and toluene: chloroform: methanol (6: 3:1) two different solvent system. Loss on drying, ash value and pH were obtained 9 %, 8 % and 5.5 respectively.

**Keywords:** Dashang lepa, phytochemical, TLC, ash value, pH value, methanolic extract.

**INTRODUCTION**

India has a long tradition of using drugs derived from plants in Ayurvedic system of medicine. The medicinal plants are potent source of many secondary metabolites like alkaloids, glycosides, steroids, flavonoids, tannins, terpenoids and phenolic compounds. In Ayurvedic system of medicine mainly polyherbal compounds are used for treatment of various infections. A polyherbal compound named Dashang Lepa is a mixture of ten indigenous drugs in the powdered form which is mentioned in different Ayurvedic literature<sup>1-5</sup>. It is used with lukewarm cow's pure butter (ghrita) as external application over inflammatory swelling such as cellulitis. Dashang lepa contains Shirisha [*Albizia lebeck*], Madhuyashti [*Glycyrrhiza glabra*], Tagara [*Valeriana wallichii*], Raktachandnam [*Pterocarpus santalinus*], Ela [*Elettaria cardamomum*], Jatamansi [*Nardostachys jatamansi*], Haridra [*Curcuma longa*], Daruharidra [*Berberis aristata*], Kushta [*Saussurea lappa*] and Hrivra [*Pavonia odorata*]. As all these plants are able to synthesize a multitude of organic molecules / phytochemicals, referred to as "secondary metabolites"<sup>6,7</sup>. These molecules play variety of role in the life span of plants, ranging from structural ones to protection. In this polyherbal compound *Albizia lebeck* has anti-inflammatory<sup>8</sup>, antioxidant properties<sup>9</sup>, anti allergic activity<sup>10</sup> and analgesic activity<sup>11</sup>. *Glycyrrhiza glabra* has anti-inflammatory and antimicrobial activity<sup>12</sup>, wound and ulcer healing

properties<sup>13</sup>. *Pterocarpus santalinus* contains anti-inflammatory properties<sup>14</sup>. *Elettaria cardamomum* has anti-inflammatory, analgesic and antioxidant properties<sup>15</sup>. *Curcuma longa* has anti-inflammatory<sup>16</sup>, anticarcinogenic<sup>17</sup> and antimicrobial properties<sup>18</sup>. Although several research work have been reported the qualitative and quantitative estimation of individual drug of this compound but none of them has done estimation for combined formulation. Therefore this study was carried to evaluate preliminary phytochemical estimation, TLC, loss on drying (moisture content), ash value and pH value. This is the first publication ever regarding Dashang lepa.

**MATERIAL AND METHOD****Plant material and preparation of extract**

Some plants of Dashang lepa were collected from periphery of Varanasi, India and some were obtained from local market. All ten plants were taken in equal amount and fine powder was made. A hundred gram of powder was subjected to methanolic extraction by hot percolation method through Soxhlet apparatus. Thereafter extract was filtered through whatmann filter paper no. 42 (125 mm) and then through cotton wool. Then extract was concentrated using rotary evaporator with hot water bath at 40°C and dried extract was put to the process of standardization. The percentage yield of the extract was determined and found to be 18 % (18 g)<sup>19</sup>. This extract of

Dashang lepa was used for Thin Layer Chromatography (TLC), phytochemical study and measurement of loss on drying value, ash value and pH value. These were done according to standard guideline of Ayurvedic Pharmacopoeia of India.

### Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was used to separate the different part of Dashang lepa extract into different spots on the chromatoplate. The chromatograms developed on the microscope slide, were dried and observed visually for the different parts of powdered extract components.

The Retention factor ( $R_f$ ) was calculated using the following equation:

$$R_f = \frac{\text{Distance move by the substance (cm)}}{\text{Distance move by the solvent (cm)}}$$

Observation in different solvent and results of Dashang lepa are shown in Table 1.

### Preliminary phytochemical screening of the Dashang lepa

The concentrated alcoholic extract of Dashang lepa was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures<sup>20,21</sup>. The powder extract was assayed for the presence of secondary metabolites as alkaloids, glycosides, flavonoids, tannins, phenolics compounds,

carbohydrates, saponins, phytosterol and triterpenes. Results are shown in Table 2.

### Estimation of Ash value

1 g of the powder was incinerated in a silica crucible at a temperature not exceeding 450°C until free from carbon, cool and weigh. Then exhaust the charred mass with hot water, and the residue was collected on an ash less filter paper, incinerate the residue and filter paper and filtrate was added and evaporated to dryness and ignite at a temperature on at about 450°C. The percentage of total ash was calculated with reference to air dried drug.

### Determination of Moisture Content (Loss on Drying)

10 g of the powder was taken in a tarred evaporating dish, dried at 105°C for 5 h and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 percent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

## RESULTS AND DISCUSSION

As the result reveals maximum number of six spots were detected in two solvent systems as Chloroform: Ethyl acetate: Methanol (6: 3: 1) and Toluene: Chloroform: Methanol (6: 3:1).

Table 1: TLC Result of alcoholic extract of Dashang lepa on silica gel- g plate and visualized by LB spray

S. N	Solvent system	Number of spots	Distance of spot (cm)	Solvent front (cm)	$R_f$ value
1.	Chloroform : Ethyl acetate : Methanol (4: 5: 1)	5	1.8, 3.5, 4.3, 12.1, 12.9	13	0.13, 0.27, 0.33, 0.93, 0.99
2.	Chloroform : Ethyl acetate : Methanol (6: 3: 1)	6	2.5, 3.4, 5.1, 7.2, 12.1, 12.9	13.1	0.19, 0.26, 0.39, 0.55, 0.92, 0.98
3.	Toluene : Chloroform : Methanol ( 6 : 3 :1)	6	5.1, 6.0, 7.2, 10, 14.1, 15.1	15.3	0.33, 0.39, 0.46, 0.64, 0.91,0.98
4.	Toluene : Ethyl acetate : Methanol (6 :3 :1)	5	1.8, 3.0, 3.5, 11.5, 12.5,	14.2	0.13, 0.21, 0.25, 0.81, 0.88,



Toluene: Chloroform: Methanol (6: 3:1)

Figure 1: Thin Layer Chromatography (TLC) result of methanolic extract of Dashang lepa on silica gel- g plate

Different phytochemicals like alkaloids, glycosides, flavonoids, tannins, phenolics compounds, carbohydrates, saponins, phytosterol and triterpenes were determined by their respected tests. The results revealed presence of following phytochemicals in Table 2. Flavonoids show anti-inflammatory activities by modulation of the activities of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase, lipoxygenase) and nitric oxide synthase<sup>22</sup>, antioxidant activity by their

mechanism of action are through scavenging or chelating process<sup>23,24</sup> and anti-microbial properties<sup>25</sup>. Plant derived terpenoids can inhibit signalling via the NF-kB system and hence possess beneficial therapeutic effects against inflammatory diseases and cancer<sup>26</sup>. Topical use of drugs containing tannin shows analgesic properties by decreasing synthesis of prostaglandins<sup>27</sup>, anti-inflammatory<sup>28</sup> and antimicrobial properties<sup>29</sup>. Phytosterol ( $\beta$ -Sitosterol) possess anti inflammatory property and also improves immune system by increasing blood T-cells<sup>30</sup>.

### Total loss on drying

Total loss on drying was calculated of 10 g of powdered Dashang lepa as per method described earlier. Weight of porcelain disc was 45.820 g. Total weight of disc and powder of Dashang lepa (10 g) were 45.820 + 10 = 55.820 g. Total weight after drying on 105°C for 5 hours was found 54.860 g. After 1, 2, 3 hour total weight was 54.848, 54.830, 54.830 g respectively. Loss on drying was 55.820- 54.830 = 0.09 g that is 90 mg and as result % loss on drying was 9 %.

Table 2: Phytochemical analysis of methanolic extract of Dashang lepa

S. N	Constituents	Tests	Extract
1.	Alkaloids	Mayer's reagent	+
2.	Saponin	Foam test	+
3.	Tannin	Extract + 5 % Fecl3	+
4.	Terpenoids	Salkowski test	+
5.	Steroid	Liebermann-Burchard reaction	+
6.	Glycosides	Keller-Kiliani test	+
7.	Phenolics Compound	Extract + 5 % Fecl3	+
8.	Flavonoids	Residue + Lead acetate solution	+
9.	Reducing sugar	Benedict's reagent	+

**Ash value**

Ash value was calculated as per method described earlier as:

Weight of silica crucible = 16.830 g

Total weight of crucible + powder of Dashang lepa (2 g) = 16.830 + 2 = 18.830 g

Net wet after keeping on 450°C until free from carbon = 16.990 g

Ash value = 8 %

**pH (1 % aqueous solution) : 5.5**

Table 3: Physical properties of Dashang lepa

Loss on drying	9 %
Total Ash value	8 %
pH value	5.5

**CONCLUSION**

As many of the secondary metabolite show anti-inflammatory and analgesic properties by the various mechanism of action, so the drug Dashang lepa can be proved as topical anti-inflammatory medicine. The further research work on this highly effective drug is in process.

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