



## PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF METHANOLIC STEM EXTRACT OF *INDIGOFERA ASPALATHOIDES VAHL*

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### ABSTRACT

The methanolic extract of *Indigofera Aspalathoides* (*Papilionaceae*) is reported to have great medicinal value. Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant based medicaments are the basis of many of the modern pharmaceuticals. We use today for our various ailments. Phytochemical screening including qualitative chemical examinations and quantitative analysis was carried out using HPTLC techniques. Identification, separation and quantification of chemical constituents were carried out on the methanolic extract of *Indigofera Aspalathoides*, using, chemical testing, TLC, and HPTLC techniques. Five major classes of secondary metabolites were detected. Alkaloids, Glycosides, Protein, Steroids and Phenolic compounds. These findings are useful in establishing a relationship between chemical composition of the methanolic extract and pharmacological activities of *Indigofera Aspalathoides* and also may assign a new potential role of these plants extract in human health care.

**Keywords:** *Indigofera Aspalathoides*, phytochemical, soxhlet extraction, Phytoconstituents.

### INTRODUCTION

Plant based drugs have been used worldwide in traditional medicines for treatment of various diseases. India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world<sup>1</sup>. Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way<sup>2</sup>. Quantitative and qualitative phytochemical screening of plant was done according to standard procedures. Qualitative analysis of some phytochemicals such as alkaloids, Glycosides, steroids, and phenolics were done by employing TLC technique<sup>3</sup>. Whereas quantitative chemical analysis of alkaloids, phenolics, glycosides and steroids, were done by HPTLC methods<sup>4,5</sup>. The plant is an erect much branched stiff under shrub and grows erect. Some are herbaceous and a few can become small trees up to 5-6m (16-20 ft) tall distributed geographically in tropical regions<sup>6</sup>. The whole plants are used in odematous tumors and ashes are used in preparation for dandruffs<sup>7,8</sup>. Studies with stem methanolic extract of *Indigofera Aspalathoides* indicate that it has antitumour, antiinflammation, antiviral, antimycobacterial, effects<sup>9</sup>. The plant is very specific for psoriasis, secondary syphilis and viral hepatitis<sup>10,11</sup>. Root is chewed as a remedy for tooth ache and aphthae.<sup>12</sup> The aqueous extract of *Indigofera Aspalathoides* treatment enhanced the recovery from 20- methyl cholanthrene-induced fibrosaeoma due to its antioxidants and antineoplastic properties.<sup>13</sup> An infusion of the plant is given in Indochina for the swelling of abdomen.<sup>14,15</sup>

### MATERIALS AND METHODS

The stems of *Indigofera Aspalathoides* for the present investigation were obtained from Tirunelveli District. The solvents used for extraction, testing, chromatography were all of LR grade and were used after distillation. Distilled under normal atmospheric pressure were employed for extraction of plant material and for phytochemical screening. Removal of solvents, wherever required was carried out by distillation or vacuum desiccators. The solutions and reagents were prepared using distilled water. The thin layer chromatography (TLC) was performed on glass plate coated with silica gel G, Preactivated at 110°C for 30 min. compounds were detected under UV light at 254 nm .

In the extraction methodology the stems were dried and powered. 500gms, of the drug was extracted with methanol solvent in a soxhlet extraction apparatus. The extraction was continued till a few drops of the last portion of the percolate did not leave any residue on drying. It took 22 hrs for complete exhaustion. Testing of the methanolic extract of *Indigofera Aspalathoids* for alkaloids, carbohydrates and glycosides, steroids, proteins and was carried out and the following results were obtained. The results of the foregoing experiment are summarized in Table 1. The TLC profile was developed using methanol; Chloroform: acetic acid: formic acid (90:5.0:2.5:2.5) for the methanolic fraction. Quantification of the extract was carried out using HPTLC techniques. HPTLC profile of the methanolic extract was developed in methanol: chloroform: acetic acid: formic acid (90:5.0:2.5:2.5) solvent system and the profile was obtained at different wavelengths using different



concentrations. The TLC pattern of the following extract is given in Fig. 1-3.

### HPTLC Profiles

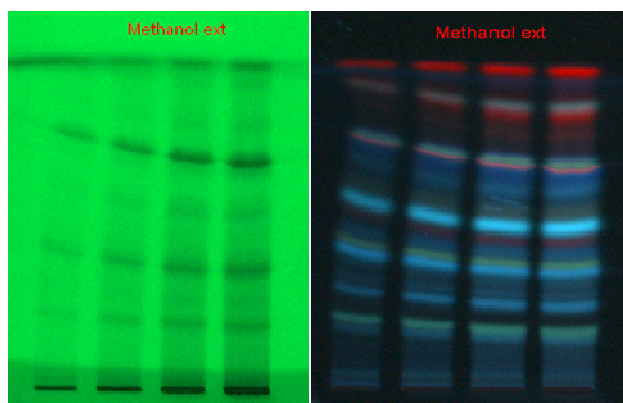
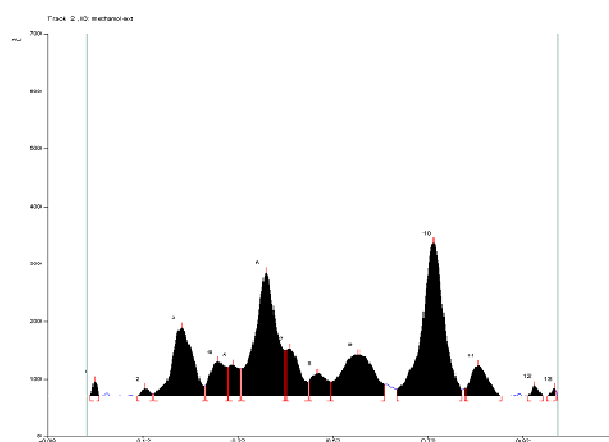


Figure 1: at 254 nm

Figure 2: at 366 nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	1.7	0.02	25.9	2.92	0.03	1.8	172.5	0.57	unknown
2	0.11	0.1	0.12	12.7	1.23	0.14	4.2	159.0	0.53	unknown
3	0.14	4.8	0.20	119.1	11.59	0.25	16.3	3754.8	12.42	unknown
4	0.25	17.0	0.28	59.0	5.74	0.30	50.6	1470.1	4.88	unknown
5	0.30	50.9	0.31	54.3	5.28	0.32	40.4	870.5	2.80	unknown
6	0.33	48.3	0.38	214.3	20.85	0.42	79.4	7090.0	23.45	unknown
7	0.42	79.8	0.43	81.8	7.96	0.47	25.0	1673.5	5.55	unknown
8	0.47	26.1	0.49	38.9	3.78	0.51	25.5	954.3	3.18	unknown
9	0.51	25.5	0.57	72.5	7.05	0.58	19.3	3563.8	11.79	unknown
10	0.65	12.0	0.73	265.8	25.88	0.79	10.6	8879.7	29.38	unknown
11	0.80	11.7	0.82	53.1	5.16	0.87	0.1	1373.9	4.59	unknown
12	0.93	0.2	0.94	17.4	1.99	0.96	0.4	179.3	0.59	unknown
13	0.97	0.0	0.99	13.0	1.27	0.99	10.5	90.1	0.30	unknown

Figure 3

## RESULTS AND DISCUSSION

Preliminary phytochemical testing of methanol extract shows the presence of various phytoconstituents such as, Glycosides, Alkaloids, carbohydrates, steroids, glycosides, proteins, and phenolic compound. TLC profile of the methanolic extracted developed using methanol: chloroform : acetic acid : formic acid (90:5.0:2.5:2.5) at different concentration 5UI, under UV 254nm and 10 UI under UV 366 nm and After derivation with VSR and 5 min heating and after derivation with VSR and 10min heating also shows the presence of three silent spots. The HPTLC chromatogram (Fig. 4-7) developed using methanol: chloroform: acetic acid : formic acid solvent system shows the presence of three peaks with maximum area under the curve indicating the possible quality of these three phytoconstituents in the methanolic stem extract.

## CONCLUSION

In conclusion it may stated that the approach given for standardization of any new herbal or medicinal plant includes chemical evaluation and comparison should be developed systematically for completion of database of newer plants. This shall help to obtain monograph of the future medicinally active plant. For developing analytical method pure active chemical constituent should be isolated in further study and identification on basis of reference standard shall be made. This also helps in setting in house standards of the medicinal plants used extensively by herbal manufacturers.

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