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Distribution of psoralen in different organs of *Psoralea corylifolia* L.

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Abstract
Psoralea corylifolia L. is an important medicinal plant widely used to treat various ailments and also from ancient times it is a part of Ayurveda and in Chinese medicines. Psoralen a linear furanocoumarin widely distributed in this plant which has anticancer activity. The present study carried out to evaluate the distribution of psoralen in various orangs of *P. corylifolia.* Organs such as seed, seed coat, endosperm, cotyledons, leaf, shoot and root of *P. corylifolia* L. used for the quantification of psoralen. Psoralen was quantified by high performance liquid chromatography (HPLC). Higher content of psoralen were recorded in whole seed than other organs used in the study.

Keywords: *Psoralea corylifolia* Linn, Psoralen, High performance liquid chromatography, Seed, distribution

Introduction
Psoralea corylifolia Linn is an medicinal herb, extract of this plant known to possess antitumour [1], immunomodulatory [2], anti-inflammatory, and antiproliferative activities [3], anti-microbial [4], cytotoxic activities [5], DNA polymerase inhibitory, DNA topoisomerase II inhibitory [6]. And anti-HIV activities [7]. Psoralen an important linear furanocoumarin found to be inhibiting human tumour cancer cell lines [8] which is widely distributed in this plant.
In recent times, as increasing the awareness about side effects of allopathic drugs had made the pharmaceutical industries turn towards herbal medicines. *P. corylifolia* has an extensive demand in pharmaceutical industries owing to various pharmacological properties. However, whole plant has been used to prepare the drugs and do not deal with the identification of active principles that are responsible to treat particular diseases. Quantification of such active principles from various plant parts is valuable for the standardization of herbs and formulations thereof. It is important to know the distribution of these compounds in order to choose the right plant parts and to obtain the right resource for extraction. In the present study, a detailed investigation of the anticancer compound psoralen distribution in various parts of *P. corylifolia* was carried out using HPLC. In particular, the concentration of psoralen content in various parts such as seed, seed coat, endosperm, cotyledon, leaves, shoot and roots were determined and compared.

Materials and Methods
Plant Material
Different organs such as seed, seed coat, endosperm, cotyledons, leaf, stem and roots were excised from the mature plants of *Psoralea corylifolia*, were dried, powdered and extracted using Soxhlet apparatus.

Extraction of Psoralen
Accurately weighed portion of plant material were defatted with petroleum ether (40-60º C) was performed in a Soxhlet apparatus for about 15h, after which same material was continued with methanol again for 15h. The obtained extracts were concentrated using vacuum evaporator and concentrated extracts were subjected for quantification of psoralen.

HPLC analysis
Psoralen was quantified by HPLC analysis using Agilent 1260 infinity HPLC unit equipped with diode array detector (DAD), C18 column at 20 ˚C, methanol and water (50:50) used as mobile phase with flow rate of 0.8 mL/min and psoralen was detected at 254 nm. Obtained HPLC peaks were correlated with psoralen standard (Sigma-Aldrich USA) prepared in HPLC grade methanol.

Statistical analysis

All the results were expressed as mean ± SEM and data were analyzed by one way ANOVA using Tukey-Kramer (HSD) principle range test at $P \leq 0.05$ [9]. For each sample triplicates were carried and the graphs were drawn using MS-excel programme.

Results and discussion

Psoralen content was evaluated in different organs of *P. corylifolia*. The chromatogram of psoralen in *P. corylifolia* seeds was shown in Fig 1 with a retention time of 10.4 min. It was observed that the amount of psoralen significantly different from one organ to other organs of *P. corylifolia* L., (Fig. 2). Maximum content of psoralen was obtained in whole seed (2.62 mg.g^{-1} DW), followed by the seed coat, leaves, cotyledons, shoot and endosperm (1.7 mg.g^{-1} DW, 1 mg.g^{-1} DW, 0.933 mg.g^{-1}, 0.345 mg.g^{-1} DW, 0.31 mg.g^{-1} DW, respectively), whereas root accumulated the lowest concentration of psoralen (0.269 mg.g^{-1} DW). The content of psoralen was 1.54, 2.62, 0.35, 7.59 and 8.45 times higher in seed than that in seed coat, leaves, cotyledons, shoot, endosperm and root respectively. The above results showed that the highest amount of psoralen was present in whole seed (with seed coat and endosperm intact) which was significantly different, and the seed coat had higher content (1.7 mg.g^{-1} DW) than endosperm (0.31mg.g^{-1} DW). Thus the seed coat contained 5.48 fold higher than that of endosperm. These values proved that the higher concentrations of psoralen were accumulated at the seed coat, compared to the other parts of the plant (excluding whole seed).

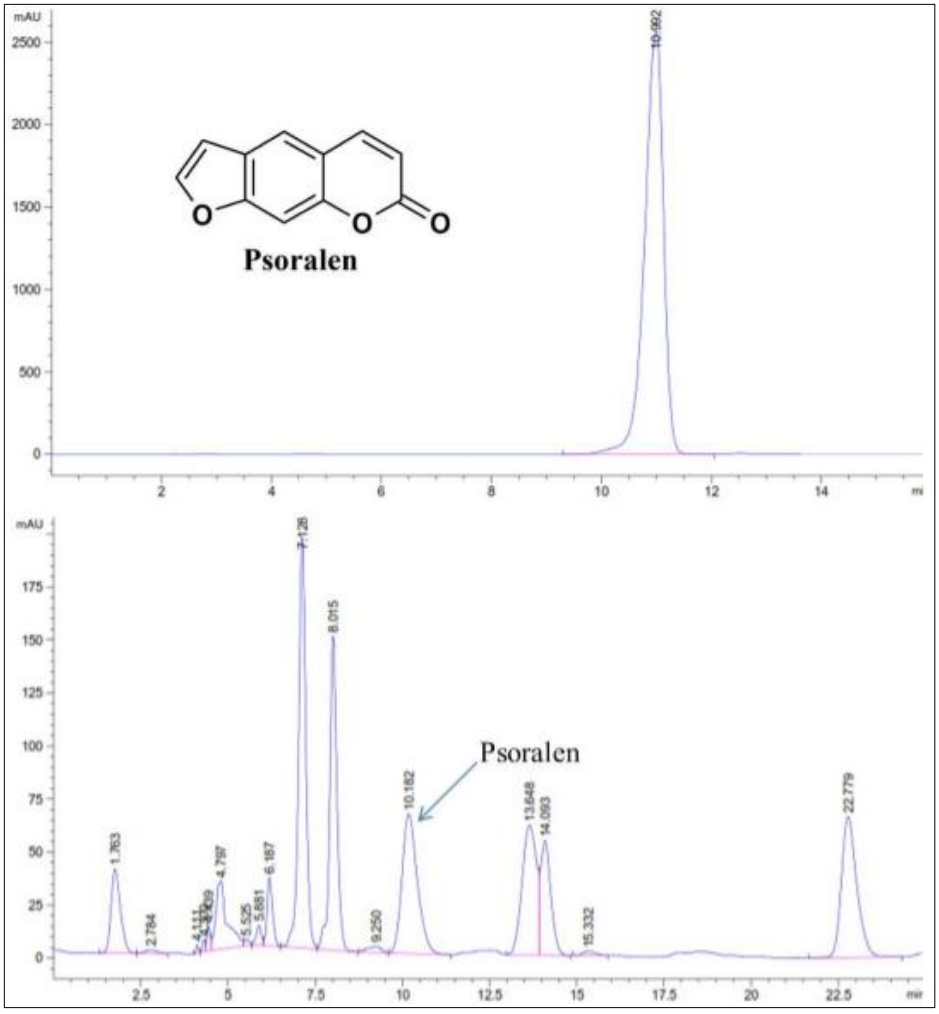

Fig. 1 HPLC chromatograms showing a) Psoralen standard with b) chromatogram showing the presence of highest levels of psoralen in seed

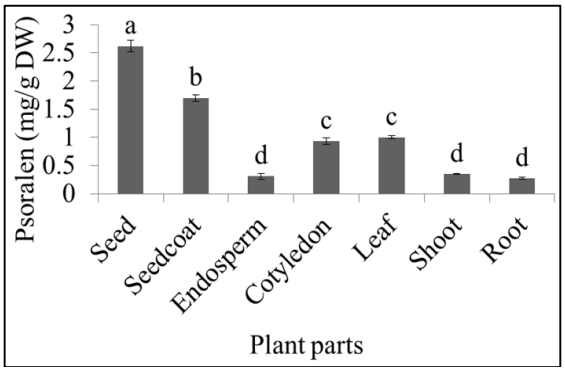

Fig.2 Distribution of psoralen in different organs of *Psoralea corylifolia* L., (Values are mean ± SE of five replicates)

The study showed that distribution of psoralen varied in different parts of the plant. The content of psoralen is higher in whole seeds than the other parts of the plant, and within the seed higher amounts are present in seed coat. Similar results were reported in distribution of backside A in *Bacopa monnieri* [10]. Distribution of Gymnema acid in *Gymnema sylvestre* [11]. And distribution of anticancer drug camptothecin in *Nothapodytes foetida* [12]. However, in the present study psoralen also found in aerial parts of the plant such as leaves (1 mg.g^{-1} DW), cotyledons (0.933 mg.g^{-1}), shoots (0.345 mg.g^{-1} DW) and roots 0.269 (mg.g^{-1} DW) respectively, which are the renewable source for the production of psoralen using plant cell cultures. However from the results of the present study, it could be noticed that,

the content of psoralen gradually decreased from aerial parts to the sub-aerial parts *i.e.*, from leaves to the roots. The results of the present study provide the valuable information pertaining to distribution and accumulation of psoralen in different parts/organs of the *P. corylifolia*. Further, results of the present study also provide the scientific evidence for the rational development and utilization of the psoralen resources.

Conclusion

In conclusion the content of psoralen in whole seed was higher than that in any other organs. The present study also proved that the areal parts are also a good resource for the production of psoralen. Thereby, especially the leaves are worthy to be the new resource for the production/extraction of psoralen. The leaves and cotyledons of *P. corylifolia* can be used as raw material for improvement of psoralen production using plant cell cultures.

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