Hypericin accumulation in glands of *Hypericum perforatum* Linn.

Germplasm evaluation of medicinal plants invariably involves determination of active content as well as biomass yield. Often differential results are obtained with regard to the above two parameters. As these two parameters, i.e. active content percentage and biomass yield are quantitative in nature, this feature may not get reflected in the phenotypic characteristics of the strain under study. Isolating and multiplying strains often pose the problem of overlapping morphological features that makes it difficult to segregate useful strains from the rest. This not only hampers the development of better strains, but also involves laborious and cost-intensive techniques to determine the uniqueness of the strains under question. Morphological markers, wherever present, not only help in segregating useful strains, but can also serve as important breeding tools.

*Hypericum perforatum* L. (Figure 1a), belonging to the family Hypericaceae, is an important medicinal plant valued for its antidepressant activity. Commonly known as St. John’s Wort, its antidepressant activity is due to the active content hypericin present in its flowers and leaves. This species is distributed throughout USA, Australia and Europe. In India, it occurs in the sub-tropical regions of the Himalayas and also in Himachal Pradesh in shady and damp forests of Shimla and Kullu between 3000 and 8000 ft. in sporadic patches. The importance of the species as a phyto-pharmaceutical, especially for treatment of mild depression has significantly increased in the last couple of years. This is evidenced by the fact that market for St. John’s Wort has exceeded $210 million in the US alone and $570 million worldwide.

Flowers and leaves of *H. perforatum* are characterized by the presence of dark-coloured glands. These glands are present on the lamina margin (Figure 1b) as well as on the petal margins (Figure 1c), the number being more in the latter. One gland each is also present on each anther (Figure 1d). These glands are macroscopic and visible to the naked eye. During the course of our studies, it was noticed that these dark-coloured glands, when punctured, gave a red-coloured fluid (Figure 1e) which was found to contain hypericin (a red-coloured compound). Hypericin in the red-coloured fluid was detected with the help of thin layer chromatography.

These observations were confirmed by testing the glandular and non-glandular portions of the petals, with the former testing positive and the latter negative for the presence of hypericin (Figure 1f).

This indicates that the dark-coloured glands present on the petals, leaves and anthers of *H. perforatum* are the accumulation sites of hypericin. This is an important finding as the density and size of these

Figure 1. *Hypericum perforatum*. a. Flowering plant; b-d. Glands on lamina margin, petal margin and anthers respectively. e. Red-coloured drops (arrow) splashed out after pressing the glands on petals, and f. Ethanolic extract with (A) and without (B) red-coloured hypericin from glandular and non-glandular portion of petals respectively.
glands can serve as important morphological markers, indicating thereby the relative extent of hypericin present in a strain, without the need of chemical estimation. These dark-coloured glands can also serve as important breeding tools, as strains with more number of glands per petal/leaf can be isolated with consequent higher hypericin content. Strains developed on the basis of higher number and size of glands can be easily segregated from the rest, especially in any cultivation or breeding programme.

As is known in the literature and also confirmed by the authors, flowers of *H. perforatum* contain much higher hypericin content (approximately 1%) compared to leaves (approximately 0.2%). The higher hypericin content in flowers is probably due to the presence of much larger number of glands in petals compared to leaves.


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Identifying the dietary source of polyphagous *Helicoverpa armigera* (Hübner) using carbon isotope signatures

An animal with unlimited choice of food has an advantage over the one feeding on scarce resource, which is a logical benefit accruing to generalists. Evolutionarily, an insect that can feed on many host plants – polyphagous – enjoys better chance of survival in nature. On the other hand, monophagous insects have evolved superior abilities to search for the host plants of their preference. *Helicoverpa armigera* (Hübner), a highly dreaded pest of several agricultural crops, is highly polyphagous, feeding on more than 170 species of plants belonging to 41 families. However, the fitness of *H. armigera* population depends upon the host plant. This means though the insect is polyphagous, it may develop preference to a particular host, which however is not rigid. Thus, it is believed that *H. armigera* feeding on different hosts would develop into specific biotypes. Biotypes are more commonly distinguished by survival and development on a specific host or by developing feeding preference, oviposition.

There seems to be no equivocal agreement on the concept of the development of host biotypes in *H. armigera* populations. ‘Mark–release–recapture’ technique is widely used for demonstration of feeding behaviour of an insect. This technique involves marking the adult moths, releasing them in a particular crop patch, subsequently tracking and capturing of them on different hosts as an indication for their host acceptance. The marked moths from crop-A, if captured on crop-B might suggest host shift to crop-B. However, this settlement of the insect on crop-B could simply be an accidental landing of the insect. Further, mere landing of the moths on crop-B need not be followed by settlement and host utilization. On the other hand, observing for the oviposition of the marked moths on a different host plant could clearly indicate the development of biotypes. This would be impossible unless an accurate signature of the moths and their eggs is available.

In order to precisely understand the feeding behaviour of an insect, determining what the insect has actually eaten will be the best evidence for host utilization. Because of the similarity of the organic molecules of any host plant, this would initially seem like an impossibility. Stable isotope signatures of animal tissues can be used to study the trophic ecology, nutritional status and geographic origin of animals. This has led to an explosive burst of research in a new frontier in animal ecology. The carbon isotope composition of an animal has been shown to accurately reflect its diet and hence can provide useful leads to the identification of dietary sources.

During the photosynthetic conversion of carbon from inorganic to organic form, plants prefer the lighter isotope of