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NOTE

EFFECT OF TIME OF HARVEST ON HYPERICIN AND ESSENTIAL OIL CONTENT OF *HYPERICUM PERFORATUM* L.

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ABSTRACT

The aerial parts of St. John's wort (*Hypericum perforatum* L.) contain active substances that are used in antidepressive and wound healing drugs. The main purpose of this study was to determine the effect of harvest time on herb yield, hypericin and essential oil content of *H. perforatum*. The herb of *H. perforatum* in full-bloom stage contained higher amount of hypericin (329 $\mu g\ g^{-1}$ in dry weight) than pre-bloom and fruit set stages (22 $\mu g\ g^{-1}$ and 148 $\mu g\ g^{-1}$, respectively). Essential oil content at full-bloom stage was higher (0.35 ml $100g^{-1}$ dry weight) than pre-bloom and fruit set stages (0.12 and 0.18 ml $100g^{-1}$, respectively). The suitable time for harvesting of *H. perforatum* is, therefore, full-bloom stage to maximize hypericin and essential oil levels.

Key words: *Hypericum perforatum*, Hypericin, Essential oil, Harvest time.

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اثرزمان برداشت بر مقدار هیپریسین و اسانس گل راعی

(Hypericum perforatum L.)

رضا امید بیگی و مجید عزیزی

به ترتیب دانشیار و دانشجوی دوره دکترای گروه باغبانی دانشکده کشاورزی دانشگاه تربیت مدرس، تهران، جمهوری اسلامی ایران.

چکیده

بخش هوایی گل راعی حاوی مواد موثر ارزشمندی است که از آن ها در تهیه داروهای ضد افسردگی و درمان سوختگی استفاده می شود. هدف از انجام این تحقیق، تعیین تاثیر زمان برداشت گل راعی بر عملکر د بخش هوایی، میزان هیپریسین و اسانس موجود در بخش هوایی این گیاه بود. بیشترین مقدار هیپریسین (۳۲۹ میکروگرم در گرم وزن خشک) در مرحله گلدهی کامل در مقایسه با مقدار آن در مرحله قبل از گلدهی و تشکیل میوه (به ترتیب۲۲ و ۱۴۸ میکرو گرم در گرم وزن خشک) در بخش هوایی گیاه ساخته و ذخیره شد. گیاه همچنین، در مرحله گلدهی کامل حاوی بیشترین مقدار اسانس (۰/۳۵ میلی لیتر در یکصد گرم وزن خشک) در مقایسه با قبل از گلدهی و تشکیل میوه (به ترتیب ۰/۱۲ و ۰/۱۸ میلی لیتر در یکصد گرم وزن خشک) بود. بنابراین، زمان مناسب برای برداشت بخش هوایی این گیاه به منظور کسب حداکثر هیپریسین و اسانس، مرحله گلدهی کامل است.

INTRODUCTION

St. John's wort (*Hypericum perforatum* L.) is an important medicinal plant that has been used since ancient times due to production of a wide range of secondary metabolites with significant pharmaceutical activity in wound healing and treating depression (2, 4, 5, 6, 15, 18). Hypericin, a polycyclic aromatic dione (Fig. 1) is an important secondary metabolite. It shows antiviral and antiretroviral activities (2, 3, 6, 9). The core cells of multicellular glands seem to be a possible site of hypericin production. These glands are found in flowers (petal, sepal and stamen), leaves and stems. Essential oil is accumulated in translucent spheroidal cavities.Translucent dots are scattered throughout the leaf lamina and extend from abaxial to adaxial epidermis (6).

St. John's wort belongs to the *Clusiaceae* family and occurs naturally in Asia minor, northern Iran, northern Africa and Europe apart from arctic regions (11). It is an erect perennial herb with branched stems near the top and simple, opposite, sessile, or subsessile leaves (6). Bright yellow flowers 13-30 mm in diameter are arranged in broad corymbs. The flowers have five petals, a five lobed calyx, many stamens and a three-celled ovary (6). Because hypericin content changes during ontogeny, we sought correlation between hypericin content and harvest time. There are a few publications on hypericin content at different stages of flower development but none relating to mechanical harvesting (10, 13). This investigation describes the determination of hypericin at different harvest times for *H. perforatum* under the growth conditions prevailing in Iran.

MATERIALS AND METHODS

Field Experiment

Seeds of *Hypericum perforatum*, cultivar 'Topas' were washed overnight, air-dried and sown on March 15, in an outdoor bed to 5 mm depth and irrigated regularly. After 10 d, the seeds germinated. Seedling growth was slow. After 6 mon when the height of seedlings was 25 cm they were

transplanted into a sandy loam soil in the field (8, 13, 15). The statistical design used was a randomized complete block design with three replicates in each treatment. Each plot was 160×125 cm. The seedlings were planted in rows 40 cm apart and seedlings spaced at 25 cm. Hoeing and mechanical weeding were done regularly. Irrigation was regular during vegetation period. Growth rate of the seedlings in the first year was very low. In spring and after satisfying the chilling requirement, stem initiation occurred in the second year. Plants were harvested at three different stages: before flowering, at full-bloom and in fruit set stage (green capsule). Plant material was dried in the dark at 30±5° C before analysis.

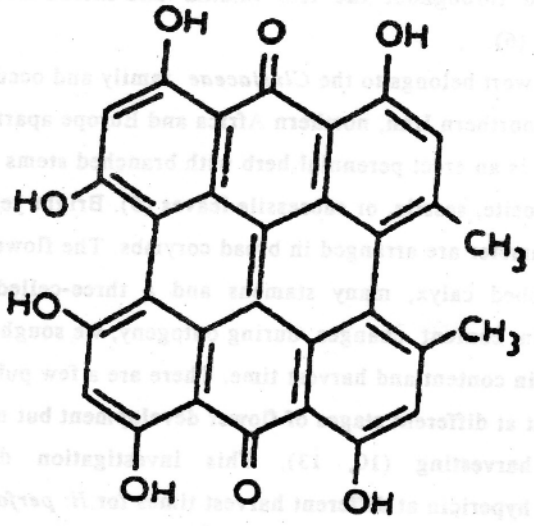

Fig. 1. Structural formula of hypericin.

Site Situation

This trial was carried out at the experimental farm of College of Agriculture, Tarbiat Modarres University, Paykan Shahr situated near Tehran. Chemical and physical analyses of the soil and weather conditions were: pH 7.9, CEC 13.8 meq 100 g^{-1} , EC 0.9 dS m^{-1}, organic mater 0.92 %, total N 0.05%, available P, 4.2 mg kg^{-1}, available K, 305 mg kg^{-1}. Elevation

1215 m above sea level, latitude 35°, 43′ north, lowest temperature, -7.6° C, mean year precipitation 242.7 mm, average humidity 42% and climatic category semiarid.

Hypericin Analysis

One g of the air-dried and homogenized aerial parts of *H. perforatum* was extracted in a Soxhlet with chloroform (200 ml) until chlorophyll ceased extracting (4 h). During this period the temperature of the water bath was 70° C. After removal of the chloroform the thimble was reextracted with acetone (200 ml) until the red pigment, hypericin, ceased extracting (6 hr). The water bath temperature was 65 °C. The filtrate was evaporated in a rotary evaporator at water bath temperature of 35 °C. The hypericin concentrate was then made up to 25 ml in methanol.

The mixture was stirred and filtered. A 5-ml aliquot of the mixture was diluted with 20 ml of methanol. Methanol extract was then analyzed by a spectrophotometer (Unicam 8700 series U.V. Visible) at 590 nm (Fig. 2)(16). The hypericin contents were calculated as follows (1):

$$H = \frac{A.\ h.\ 1000}{B \times 718 \times (100 - a)}$$

where :

H : Hypericin content, $\mu g\ g^{-1}$.

A : Absorbance at 590 nm.

h : Dilution parameter, 125.

B : Diameter of cuvette (1cm).

718 : Specific absorbance of hypericin.

a : Plant moisture content of dried sample.

Essential Oil Isolation

The air-dried aerial parts of the plant were water-distilled in all-glass Clevenger apparatus according to the methods recommended by the Hungarian pharmacopoeia (7).

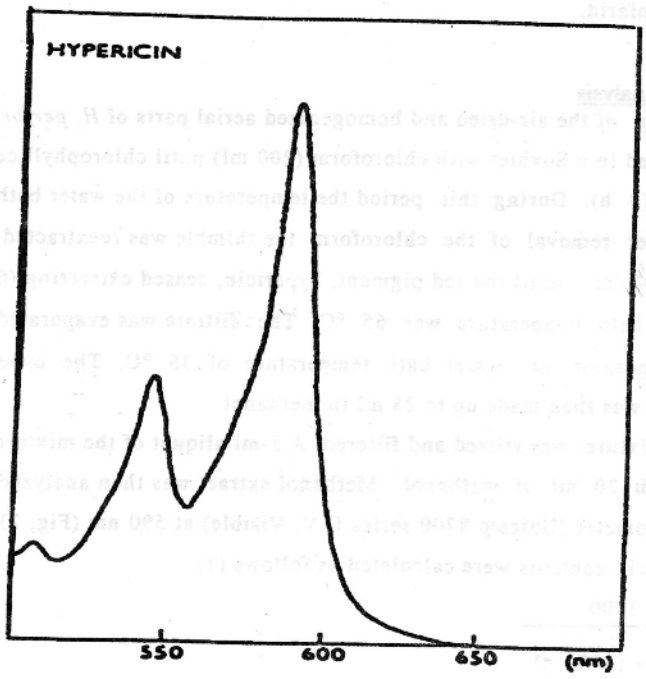

Fig. 2. Absorption spectrum of hypericin.

RESULTS AND DISCUSSION

Herb Yield

Harvest time had a significant effect on the fresh and dry herb yield of St. John's wort. As shown in Fig.3, the highest fresh and dry herb yields (18.07 and 4.69 t ha^{-1}, respectively) were obtained from plots harvested at fruit set stage and the lowest (7.50 and 2.00 t ha^{-1}, respectively) produced from plots harvested before flowering. These results are in agreement with the results of Pluhar and Zelnik (13).

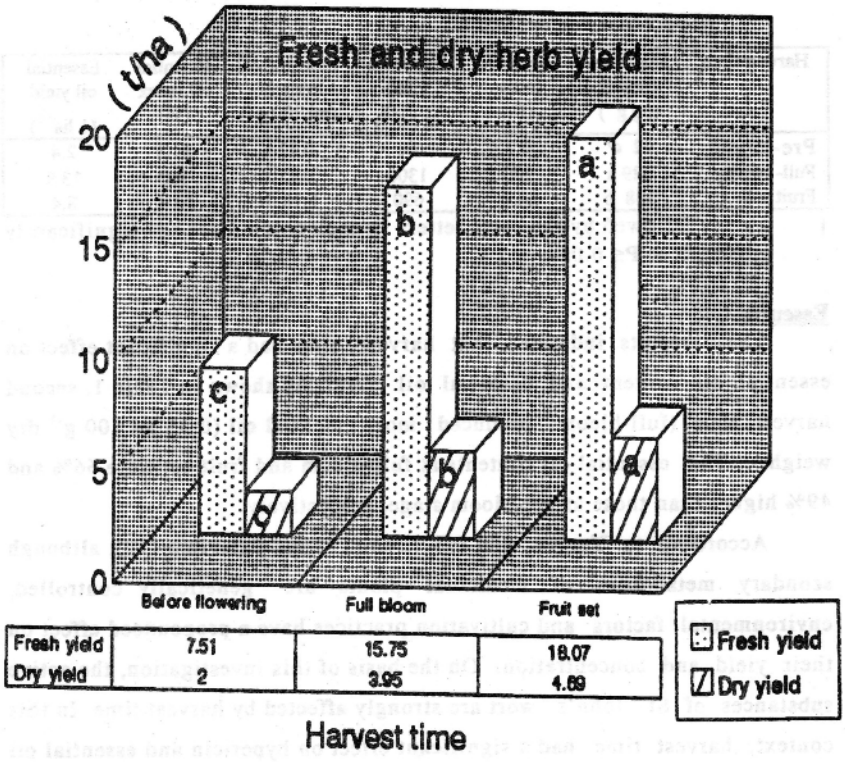

Fig. 3. Fresh and dry herb yield of St. John's wort at three different harvest times.

Hypericin

The hypericin content of St. John's wort was significantly affected by harvest time (Table 1). The highest hypericin content (329 μg g^{-1}) accumulated in the plants harvested in full-bloom and the lowest (22 μg g^{-1}) obtained from plants harvested pre-bloom. The hypericin content at fruit set stage was intermediate (148 μg g^{-1}). Hypericin content at full-bloom was 93 % higher than at pre-bloom stage. The results are similar to those of Pluhar and Zelnik (13). The highest hypericin yield (1300 g ha^{-1})was obtained from plants harvested at full-bloom rather than at fruit set (690 g ha^{-1}) or at pre-bloom (44 g ha^{-1}) (Table 1).

Table 1: Effect of harvest time on hypericin and essential oil content of *Hypericum perforatum* L.

Harvest time	Hypericin content ($\mu g\ g^{-1}$)	Hypericin index (%)	Hypericin yield ($g\ ha^{-1}$)	Essential oil content ($ml\ 100\ g^{-1}$)	Essential oil index (%)	Essential oil yield ($l\ ha^{-1}$)
Pre-bloom	22 c†	6.7	44	0.12 c	34.3	2.4
Full- bloom	329 a	100	1300	0.35 a	100	13.8
Fruit set	148 b	44.8	690	0.18 b	51.4	8.4

† Means followed by the same letters in each column are not significantly different at P≤ 0.01.

Essential Oil

These results indicated that harvest time had a significant effect on essential oil content and essential oil yield. As shown in Table 1, second harvest time (full-bloom) produced more essential oil (0.35 ml 100 g^{-1} dry weight). The essential oil contents at full-bloom and fruit set were 66% and 49% higher than those at pre-bloom stage, respectively.

According to Yanive and Palevitch (17) and Palevitch (12), although scondary metabolites of medicinal plants are genetically controlled, environmental factors and cultivation practices have a pronounced effect on their yield and concentration. On the basis of this investigation, the active substances of St. John's wort are strongly affected by harvest time. In this context, harvest time had a significant effect on hypericin and essential oil content of *H. perforatum*. Similarly, according to results of Repcak and Martonfi (14), petals and stamens are the most important flower parts that accumulate hypericin. Before flowering these parts are absent. On the other hand, during fruit set these flower parts may be detached and shriveled thus the hypericin content is decreased considerably. It can be concluded that full-bloom stage is the most suitable time for harvesting of St. John's wort to produce maximum hypericin and essential oil yield.

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