

# Effect of Foliar Application of Jasmonic Acid on Hypericin Content of St. John's Wort (*Hypericum perforatum* L.)

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## Abstract

St. John's wort (*Hypericum perforatum* L.) belongs to the family Hypericaceae, is one of important medicinal plants in the world. This herb has used in traditional folk medicine for the treatment of various ailments. Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MJ), is regarded as endogenous regulator that plays important roles in regulating stress responses, plant growth and development. A pot experiment was conducted to assess the effect of foliar application of JA on herbage yield, and hypericin content in the tetra-hydrofuran extract from the aerial parts of *H. perforatum*. Experimental treatments included (I) water foliar application (control), (II) water + acetone foliar application (as a solvent), (III-VI) 50, 100, 200, and 400  $\mu\text{L/L}$  JA. Statistical analysis indicated that there were no significant differences among treatments for total dry matter. The results indicated the different levels of the foliar application of JA do have highly significant impacts on hypericin content. The highest amount of hypericin obtained from foliar application 200  $\mu\text{L/L}$  JA with 0.8% (w/w) hypericin/extract in both harvesting times (before and after the flowering stages). Finally, foliar application JA could be used as a source for rapid and increased production of hypericin, as an important secondary metabolite in *H. perforatum* with a wide range of biologically active.

**Keywords:** Methyl jasmonate, tetra-hydrofuran extract, secondary metabolite, harvesting time.

## 1. Introduction

St. John's wort (*Hypericum perforatum* L.) belongs to the family Hypericaceae, is a medicinal herb that has for centuries been utilized in folk medicine for a range of purposes [1]. This herb is under intensive investigation for its wound-healing, antidepressant, diuretic, sedative, anti-inflammatory, antiviral, antibacterial, and antitumor effects and it has been used to treat neurological disorders and traumas [2-4]. *H. perforatum* contains a wide range of

biologically active secondary metabolites that belong to different chemical groups. Naphthodianthrones (hypericins), phloroglucinols (hyperforins), xanthenes, essential oil, phenolic acid, and flavonoids (flavonols, biflavones and procyanidins) have been identified in extracts of the plant [5,6]. Several pharmacological studies have confirmed that these compounds are the main contributors to the antidepressant activity of the species through various mechanisms of action [7]. The presence of red-pigmented naphthodianthrone derivatives is characteristic to several species of the genus *Hypericum* [8,9]. The naphthodianthrones detected in *H. perforatum*, include hypericin and its 2-methoxy derivative pseudohypericin as well as their precursor's protohypericin and protopseudohypericin. Hypericins are most abundantly present in petals, stamens of flowers, and leaves of *H. perforatum* [10]. Hypericins accumulate in the dark glands of *H. perforatum* [11]. The levels of hypericin and pseudohypericin in the *Hypericum* species can vary under different environmental parameters, but the precise environmental factors influencing the biosynthesis of naphthodianthrones and the partitioning with other secondary metabolisms are not well understood. It has been reported that naphthodianthrone production can be influenced by plant and environmental factors, including light intensity, nitrogen availability, temperature, seasons, and growing region. For example, an increased light intensity and decreased nitrogen supply resulted in a parallel increase of the number of leaf dark glands and hypericin content [12].

(-)-Jasmonic acid [(3*R*, 7*R*, 9*Z*)-jasmonic acid; (-)-(JA)] (Figure 1) and its methyl ester were first identified in the essential oils of jasmine (*Jasminum grandiflorum* L.), and rosemary (*Rosmarinus officinalis* L.) and their chemical structures were subsequently determined [13,14]. JA and its volatile methyl ester, MJ, collectively termed Jasmonates, are regarded as endogenous regulators that play important roles in regulating stress responses, plant growth and development [15]. Jasmonates are involved in many critical functions, including defense

against insects and pathogens by inducing phytoalexin production, protection from abiotic stresses, immunity, and plant growth and development, suggesting that they have critical roles in plant physiology [16].

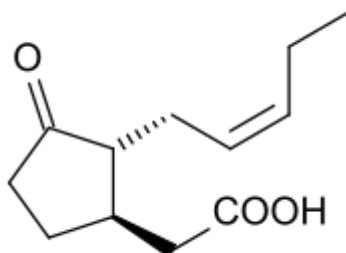


Figure 1. Structure of JA.

Few studies have been carried out to investigate the effect of foliar application of JA and/or MJ on the accumulation of secondary metabolites in medical plants, especially the *Hypericum* species. Therefore, the current study was done to find out the convenient rate of JA foliar application for optimum quality of *H. perforatum*.

## 2. Materials and Methods

### 2.1 Chemicals

Jasmonic acid (JA) was purchased from Sigma–Aldrich Co. (Steineheim, Germany). Acetone, ethanol, and tetrahydrofuran were bought from Merck Co. (Darmstadt, Germany).

### 2.2 Plant material and field site description

*H. perforatum* seeds collected from the Bakhtiari Zagros Mountains (latitude. 31° 20' to 31° 50'; longitude. 50° 50' to 51° 05'; 2700 to 3000 m above sea level), Chaharmahal va Bakhtiari province, Iran. Plant identity was confirmed by H.A. Shirmardi, and voucher specimen has been placed in the Herbarium of Research Center of Agriculture and Natural Resources, Chaharmahal va Bakhtiari, Iran (No. 1335). In spring 2011 in plastic greenhouse conditions, 25 seeds (0.001 g) of *H. perforatum* were sown in each plastic pot and after six weeks were thinned to four healthy seedlings per pot. The pots transferred to the field in Shahrekord (latitude 32° 20' N, longitude 50° 51' E, altitude 2061 m above sea level), southwestern Iran on May 2011. Plastic white pots with a top diameter of 20 cm and a depth of 25 cm were filled with clay loam soil. Type of study area climate by Emberger's climatology method is cold and semiarid (annual precipitation = 340 mm and average annual temperature = 12.2 °C). In this study, no inorganic fertilizer and systemic pesticide was used during the entire experiment, and weed control was done manually. For the first month (established phase), a watering level equivalent to 60-75% of soil field capacity was applied once a day. For the

subsequent months, the plots were irrigated when 55-60% of soil available water was depleted.

### 2.3 Treatments

Experimental treatments were arranged in a completely randomized design (CRD) with six replications. Experimental treatments, included (I) water foliar application (control), (II) water + acetone (10:1) foliar application (as a solvent), (III-IV) 50 (JA<sub>1</sub>), 100 (JA<sub>2</sub>), 200 (JA<sub>3</sub>), and 400 (JA<sub>4</sub>) µL/L JA.

Treatments were foliar-sprayed on four the plants per pot (200 ml of the solution per pot) in twice on July 17<sup>th</sup> at the vegetative stage of growth (93 days after sowing), and August 6<sup>th</sup> at the before blooming stage of growth (113 days after sowing). The aerial parts (leaves, and stem) of three pots in each treatment harvested before at the flowering or the early blooming stage of growth (127 days after sowing). In addition, the aerial parts (leaves, stem, and flower) of three pots in each treatment harvested at 10-50% flowering stages of growth (141 days after sowing).

### 2.4 Extract preparation

Air-dried and powdered the aerial parts of *H. perforatum* were macerated at room temperature with 500 ml of ethanol: water (80:20) for 48 h. The extractions continued two times and then were concentrated in a rotary evaporator under reduced pressure (Model Zirbus 302®, Italy). The extract samples were stored in universal bottles and refrigerated at 4 °C prior to use.

### 2.5 Extraction and quantification of hypericin

Extraction and hypericin determination were performed as described by British Pharmacopeia [17]. Test solution in a 100 ml flask; introduce 0.8 g of the powdered plant, 60 ml of a mixture of 20 volumes of water *R* and 80 volumes of tetrahydrofuran *R* and a magnetic stirrer. Boiled the mixture in a water-bath at 70 °C under a reflux condenser for 30 min. Centrifuged (2 min at 700 g) and decanted the supernatant into a 250 ml flask. The residue with 60 ml of a mixture of 20 volumes of water *R* and 80 volumes of tetrahydrofuran *R*. Heat again under a reflux consider for 30 min. Centrifuge (2 min at 700 g) and decanted the supernatant. Combined the extracts and evaporate to dryness. The residue with 15 ml of methanol *R* with the help of ultrasound and dilute to 25 ml with the same solvent. Centrifuge again, filter 10 ml through a syringe filter (0.2 µm). Discard the first 2 ml of the filtrate. Introduce 5.0 ml of the filtrate into a measuring flask and dilute to 25 ml with methanol *R*. Measure the absorbance at 590 nm of the test solution, by comparison with comparison liquid. Calculate the percentage content to total hypericinis, expressed as hypericin, using the flowing expression:

$$\frac{A \times 12}{m \times 870}$$

where, A= absorbance at 590 nm; m= mass of the drug to be examined in plant extract, in grams.

## 2.6 Analysis of data

The data was statistically analyzed using one-way ANOVA by the stat SPSS (17.0). Means of hypericin content compared by Duncan's multiple range test at  $p \leq 0.05$  level.

## 3. Results and Discussion

### 3.1 Effect of Light Variation on Leaf Growth of Lettuce

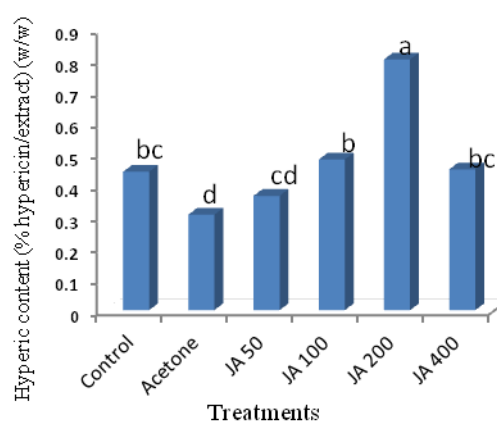
Statistical analysis indicated that there were no significant differences among treatments for dry matter, number of branches, and plant height of *H. perforatum* (Table 1). Generally, results of this study indicated that the foliar application of JA no significant influenced on growth and development of *H. perforatum*. Similarly, other researcher [18,19] also reported the foliar application of JA had no significant effect on herbage dry weight of *Thymus daenensis* Celak, and *Salvia officinalis* L. under greenhouse conditions.

Result of statistical analysis indicated that different levels of the foliar application of JA had significant impacts on hypericin content in the extract of *H. perforatum* harvested before and 50% flowering stages (Figure 2 and Figure 3). The highest hypericin contents in the tetrahydrofuran extracts of the aerial parts of *H. perforatum* was obtained from the foliar application of 200  $\mu\text{L/L}$  JA with 0.8% (w/w) hypericin/extract in both harvesting times (before and 50% flowering stages) (Figure 2 and Figure 3). Previous studies indicated that the foliar application of JA was increased some monoterpenes compounds (carvacrol, thymol, and  $\alpha$ -pinene) in the essential oils of *Salvia officinalis* [19], and *T. daenensis* [18]. The JA signaling pathway is generally regarded as an integral signal for biosynthesis of many plant secondary products [20]. Increasing evidence indicates that JA-induced changes in secondary metabolism constitute a ubiquitous plant defense response [20,21]. Cell cultures of *H. perforatum* treated with JA and incubated in the dark showed increased growth and hypericin production as compared with the cultures grown under light conditions [22]. JA and its derivatives are known to stimulate production of some secondary metabolites in medicinal and aromatic plants [23]. It is interesting to note the production of hypericin, a light-mediated insecticide [24], is stimulated upon JA treatment, since JA is known to be a key signaling compound activated upon insect feeding [25]. JA has also been implicated as the signal molecule responsible for increased synthesis of nicotine, an insecticidal compound produced by *Nicotiana sylvestris* upon leaf wounding [26].

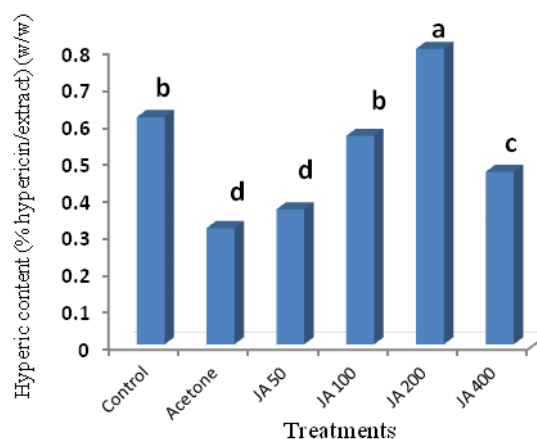
**Table 1.** Effect of foliar application of JA on growth characteristics of *H. perforatum*.

Foliar of JA	No. branches	Plant height (cm)	Herbage dry weight ( $\text{g pot}^{-1}$ )
Control	8.60 a*	21.72 a	19.60 a
Acetone (as a solvent)	7.60 a	19.34 a	18.76 a
JA 50 $\mu\text{L/L}$	8.90 a	19.22 a	18.48 a
JA 100 $\mu\text{L/L}$	7.40 a	24.55 a	17.36 a
JA 200 $\mu\text{L/L}$	8.90 a	18.07 a	16.78 a
JA 400 $\mu\text{L/L}$	9.1 a	23.03 a	21.03 a

ANOVA  $p > 0.05$   $p > 0.05$   $p > 0.05$   
 \*Means with common letters are not significant ( $p \leq 0.05$ ), according to Duncan's multiple range test.



**Figure 2.** Hypericin content in the extract from the aerial parts of *H. perforatum* harvested at before the flowering stage under JA foliar application ( $p \leq 0.01$ ). Means with common letters are not significant ( $p \leq 0.05$ ), according to Duncan's multiple range test.



**Figure 3.** Hypericin content in the extract from the aerial parts of *H. perforatum* harvested at 50% flowering stage under treatments foliar application ( $p \leq 0.01$ ). Means with common letters are not significant ( $p \leq 0.05$ ), according to Duncan's multiple range test.

The lowest hypericin contents in the extracts obtained from the foliar application water + acetone foliar application (as a solvent) with 0.3% (w/w)

hypericin/extract for both harvesting times. Similarly, Ghasemi Pirbalouti et al. [27] reported the foliar application of water + acetone (as a solvent) decreased contents of polyphenol, flavonoid, and carotenoid in the extract of *Calendula officinalis* L. cultivated in same conditions with the current study. The negative effect of foliar application of acetone might be attributed to toxic effect of acetone on metabolism and cell structure of the plant.

#### 4. Conclusion

Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors and other industrial materials. Hypericin is important secondary metabolites with a wide range of biologically active which extract from the *Hypericum* species. Results of current study indicated that JA is an effective elicitor for the production of hypericin in aerial parts of *H. perforatum*. JA could induce secondary metabolite production leading to naphthodianthrone biosynthesis. Increased hypericin content in the plants grown under the foliar application of JA (200 µL) may be attributed to stressed, which would activate the synthesis of secondary metabolites. The current study shows that the signaling compound JA, a new plant growth regulator, induces the production of hypericin. Finally, JA could be used as foliar application of hypericin production.

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