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# Enhancement of Secondary Metabolites in a Medicinal Plant *Oxalis corniculata* L. by Whole Plant Elicitation Using Methyl Jasmonate

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## Abstract

Whole plant elicitation was done in field grown *Oxalis corniculata* for enhancing the accumulation of secondary metabolites using different concentrations of Methyl Jasmonate (MeJA). Shoot tissues were subjected to extraction process by three different solvents methanol, ethanol and water. Extracts were tested for the examination of total phenolic compound (TPC), total flavonoid content (TFC) and the DPPH activity. Untreated plants considered as control. The highest (47.06 ± 2.05 mg/g) amount of TPC of gallic acid equivalent was recorded in the shoot tissue extract prepared in water. In this case the concentration of MeJA was 500-750 ppm with a treatment period of 8-12 days. On the other hand, methanolic extract of shoot tissues gave 42.087 ± 2.49 mg/g TPC at a concentrations of 1000 ppm MeJA with a treatment period of 12 days. TPC level was increased about 4 folds when compared with the highest levels of TPC in the control 12.863 ± 0.52 mg/g. Highest level (95.32 ± 6.730) of Total Flavonoid Content (TFC) was found in ethanol and water extracts. Here the concentration of MeJA was 750-1000 ppm and the treatment period was 12 days. TFC levels also increased about 4 folds when compare to the highest levels (18.8 ± 2.75) of TFC in control. The DPPH activity of shoot tissue extracts in three different solvents had no statistical difference between treatments and control. These studies are helpful to conduct further experiments in field conditions for producing large amounts of TPC and TFC as raw materials.

## Introduction

Medicinal plants are excellent sources for secondary metabolites which are regularly used for the production of industrially important biochemicals such as pharmaceuticals, food additives and flavors. *Oxalis corniculata* is a small creeping woodsorrel that tends to grow well in moist climates, widely distributed worldwide and it is belongs to Oxalidaceae family (Groom et al. 2019, Saracila et al. 2022). It is also used in advanced

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practices in several approved Chinese patent medicines (Bharti et al. 2024). The extracts of *O.corniculata* were found to have several medicinal properties like antioxidant, anti-inflammatory, antimicrobial, hypoglycemic, and anticancer effects (Gao et al. 2022).

Secondary metabolites are biologically active compounds synthesized by plants that are not directly involved in primary growth and development but play important roles in plant defense, adaptation, and ecological interactions. These metabolites, including phenolics, flavonoids, alkaloids, terpenoids, and glycosides, are of considerable importance because of their medicinal, pharmaceutical, agricultural, and industrial applications.

Secondary metabolites contribute significantly to plant defense against pathogens, herbivores, and environmental stresses such as drought, salinity, ultraviolet radiation, and temperature fluctuations. Phenolic compounds and flavonoids, in particular, function as antioxidants that protect plant cells from oxidative damage by scavenging reactive oxygen species (ROS) generated during stress conditions (Taiz and Zeiger 2015). Moreover, these compounds participate in signaling pathways and stress adaptation mechanisms, enabling plants to survive under adverse environmental conditions (Akula and Ravishankar 2011).

From a medicinal perspective, secondary metabolites are valuable sources of therapeutic compounds. Phenolics and flavonoids exhibit strong antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities, making them important constituents in herbal medicines and pharmaceutical products (Pandey and Rizvi 2009). Many commercially important drugs, including vincristine, morphine, quinine, and paclitaxel, are derived from plant secondary metabolites, highlighting their economic and biomedical significance (Ramawat and Mérillon 2008).

The enhancement of secondary metabolite production through elicitation has emerged as an important strategy in plant biotechnology. Elicitors such as methyl jasmonate (MeJA) activate plant defense pathways and stimulate biosynthetic genes responsible for metabolite accumulation. MeJA-mediated elicitation has been reported to increase phenolic and flavonoid contents in several medicinal plants, thereby improving their pharmacological value and industrial utility (Wasternack and Hause 2013). Therefore, developing efficient elicitation approaches to enhance secondary metabolite production represents a promising avenue for sustainable utilization of medicinal plants and production of high-value phytochemicals. The present study involved in elicitation of secondary metabolites in field grown plants of *Oxalis corniculata* by whole plant elicitation using MeJA.

## Materials and Methods

Two months old field grown *Oxalis corniculata* at Dravidisn University, kuppam was subjected to different concentrations (250-1000 ppm) of MeJA (Fig. 1). The treatments were given at a time interval of 4, 8 and 12 days. After treatment total plants were

removed from the soil and washed with water. These plants were shade dried and separated them into shoot, leaves and roots. In this study 5g shade dried shoot tissue was used and 5 ml extract was prepared using different solvents like methanol, ethanol and water.

Total phenolic content (TPC) in field grown plants was quantified using modified Folin-Ciocalteu colorimetric techniques (Chutimanukul et al. 2022). A 500 µl of extracted solution was combined with 200 µl of 1 N Folin-Ciocalteu reagent. After 15 min of incubation at 25°C, 600 µl of 7.5% sodium carbonate ($Na_2CO_3$) was used to neutralize. After an hour of incubation at room temperature, the solution mix's absorbance was measured at 730 nm using a spectrophotometer (Elico SL-218, India). TPC was determined using the normal gallic acid range of 0-200 µg /ml (200, 180, 160, 140, 120, 100, 80, 60, 40, 20 and 0 µg /mL). The gallic acid solution was prepared by dissolving it in ethanol and fitting the calibration curves to calculate TPC concentration. The results are presented as milligram of gallic acid equivalent (mg of GAE) per gram dry weight of the sample.

Total flavonoid content was determined using a colorimetric approach similar to that published by Chutimanukul et al. 2022 with slight modifications. Mix 500 µl of extracted solution with 75 µl of 5% sodium nitrite ($NaNO_3$) in a 1.5 ml micro centrifuge tube. Centrifuge at 12,000 rpm for 2 min at 25°C. After 5 min at room temperature, add 75 µl of 10% aluminum chloride ($AlCl_3 \cdot 6H_2O$) and mix thoroughly using a vortex. The mixture was centrifuged as before and allowed to stand for 5 min. Finally, 500 µl of 1 M sodium hydroxide (NaOH) was added. The homogenate solution was centrifuged and incubated at 25°C for 15 min. The solution absorbance was measured at 515 nm using a spectrophotometer (Elico SL-218, India). The total flavonoid content was estimated using the standard curve of rutin dissolving in methanol in the concentrations from 0-100 µg/mL (100, 80, 60, 40, 20 and 0 µg) with concentration provided as mg of rutin equivalents per g dry weight of the sample.

The free radical scavenging activity of shade dried shoot tissue of *Oxalis corniculata* was investigated utilizing a slightly modified approach (Chutimanukul et al. 2022) with 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. The extracted solution of 500 µl was pipetted into 500 µl of 0.1 mM DPPH and carefully mixed. The mixture was then centrifuged at 12,000 rpm for 2 min before being stored at room temperature in the dark for 30 min. Following incubation, the solution's absorbance at 515 nm was measured using a spectrophotometer. Trolox was utilized as the reference antioxidant. The antioxidant activity was expressed as a percentage of DPPH scavenging using the equation (Abs control–Abs515)/Abs control) × 100.

All data were statistically analyzed using one-way analysis of variance (ANOVA) with IBM SPSS. Duncan's multiple range test (DMRT) was used to evaluate mean differences with a significance level of $P < 0.05$.

## Results and Discussion

Shoot extracts of elicited *O. corniculata* obtained from different concentrations of MeJA were analyzed for the enhancement of secondary metabolites accumulation particularly TPC and TFC followed by DPPH activity. Highest TPC levels 47.06 ± 2.05 (mg of gallic acid equivalent) are found with MeJA from 500-750 ppm with the treatment of 8-12 days for the shoot tissue of *O. corniculata*. Lowest levels of TPC $27.71^{d}$ ± 2.81 were found with water and ethanol treated for 12 days. The specific concentration range of MeJA 500-750 ppm treated for 8-12 days at field conditions is very suitable to produce high levels TPC in *O. corniculata* (Fig. 2). TPC levels were increased in several plants using abiotic elicitor such as MeJA, in marjoram very low concentration 100 ppm of MeJA has increased higher levels of TPC (Kandoudi and Németh-Zámboriné 2022**)**. But DPPH activity in *O. corniculata* is not proportionately increased with respective to increased levels of TPC.


Fig. 1 Elicitation of field grown plants of *Oxalis corniculata* by MeJA treatment: (A) partitioning of experimental field station for control and different concentrations of MeJA, (B) separation of leaves after treatment, and (c) separation of shoot tissue after treatment.

Total phenolic content (TPC) in plants can be significantly enhanced through methyl jasmonate (MeJA) elicitation. MeJA is an important signaling molecule involved in plant defense responses and secondary metabolite biosynthesis. It activates phenylpropanoid metabolic pathways by inducing the expression of key biosynthetic enzymes such as phenylalanine ammonia-lyase (PAL), chalcone synthase, and other stress-responsive genes, leading to increased synthesis and accumulation of phenolic compounds (Wasternack and Hause 2013). Elevated TPC following MeJA treatment has been reported in several medicinal and aromatic plants. For example, MeJA elicitation

increased phenolic accumulation in marjoram and other medicinal species by stimulating antioxidant defense mechanisms and phenolic biosynthetic pathways Kandoudi and Németh-Zámboriné (2022). Similarly, abiotic elicitation using MeJA enhanced phenolic production in numerous plant systems, indicating its effectiveness as a metabolic regulator for improving phytochemical content (Akula and Ravishankar 2011). Increased TPC is particularly important because phenolic compounds possess strong antioxidant, antimicrobial, anti-inflammatory, and pharmacological activities, thereby improving the medicinal and industrial value of plant materials (Pandey and Rizvi 2009). Therefore, MeJA-mediated elicitation represents an effective biotechnological strategy for increasing phenolic accumulation in medicinal plants.

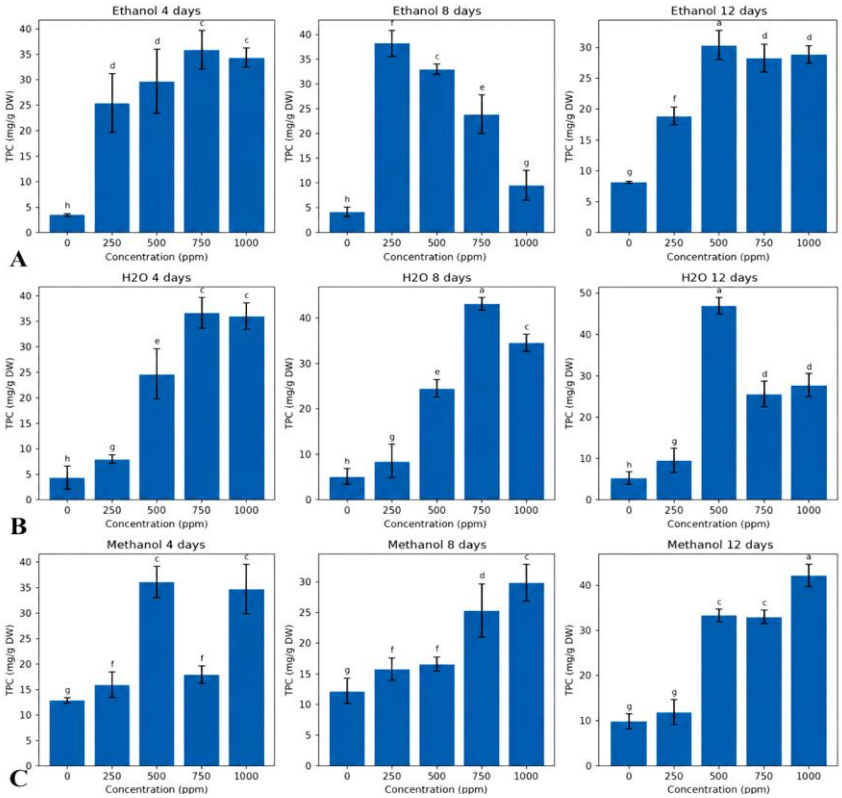


Fig. 2. Analysis of TPC levels (mg of gallic acid equivalent weight/ gram DW) in the shoot tissue of *O. corniculata*. Three different solvents methanol, ethanol and water were used for the extract preparation: (A) ethanol extracts obtained by the treatment of MeJA (500ppm) for 12 days produced 30.43 ± 2.4, at 250 ppm for 8 days 38.193 ± 2.66, for 4 days treatments at 1000 ppm gave 34.22 ± 1.88 (mg/g DW) TPC, (B) water extract obtained by the treatment of MeJA (250-500 ppm) for 8-12 days gave the highest level (47.06 ± 2.05), for 4 days at 750 and 1000 ppm gave 36.71 ± 3.04 mg/g DW) of TPC, and (C) Methanol extracts obtained by the treatment of 1000 ppm MeJA for 12 days gave 42.087 ± 2.49, for 8days at 1000 ppm 29.81±3.01, for 4 days at 500 ppm gave 36.07 ± 3.08 mg/g DW. Bars represent standard deviation, and values are expressed as mean ± SD (n = 3). Statistical analysis was performed using ANOVA followed by DMRT, with letters above bars indicating significant differences at $P < 0.05$.

Total flavonoid (TFC) has been analyzed in field grown plants of *O. corniculata* after treating with MeJA concentrations ranging from 0-1000 ppm with time intervals of 4, 8 and 12 days. Highest amount of TFC 95.32[a] ± 6.730 (mg of Rutin Equivalent/gDW) with ethanol and water extracts at concentrations 750-1000 ppm for 12 days treatment. Lowest amount of TFC found with extracts of methanol treatment for 8days and MeJA 750 ppm (Fig. 3).

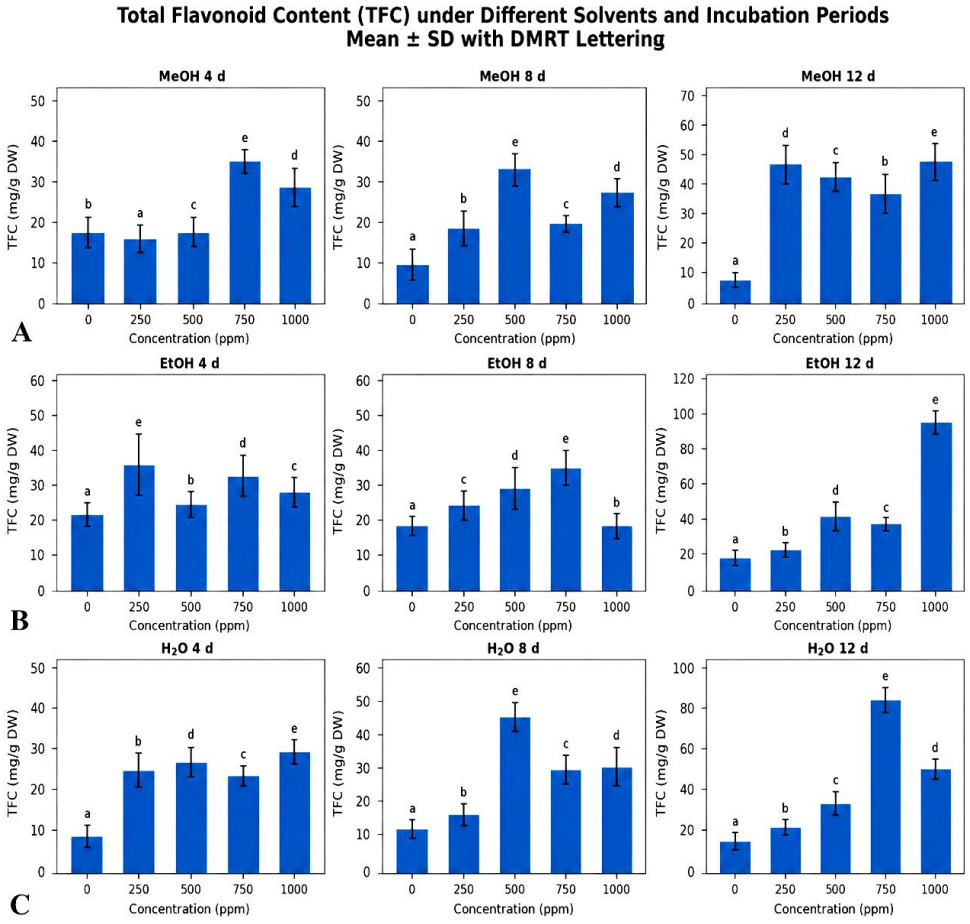


Fig. 3. Analysis of TFC levels (mg of rutin equivalent weight/ gram DW) in the shoot tissue of *O. corniculata*. Three different solvents methanol, ethanol and water were used for the preparation extracts: (A) methanol extracts obtained by the treatment of MeJA (1000 ppm) for 12 days produced 47.31 ± 6.87, 8days at 500 ppm 32.44 ± 4.47 and 4 days at 250 ppm 15.94 ± 3.08 (mg/g DW) TFC, (B ) ethanol extract obtained by the treatment of MeJA (1000 ppm) for 12 days gave the highest level (95.32 ± 6.73), for 8days at 750 ppm 33.83 ± 1.54, 4 days at 250 ppm 34.31 ± 7.41 mg/g DW) of TFC, and (C) water extracts obtained by the treatment of MeJA ( 750 ppm) for 12 days gave 85.01 ± 1.91, for 8 days at 1000 ppm 29.79 ± 3.63, for 4 days at 1000 ppm 28.75 ± 2.45 mg/g DW of TFC. Bars represent standard deviation, and values are expressed as mean ± SD (n = 3). Statistical analysis was performed using ANOVA followed by DMRT, with letters above bars indicating significant differences at $P < 0.05$.

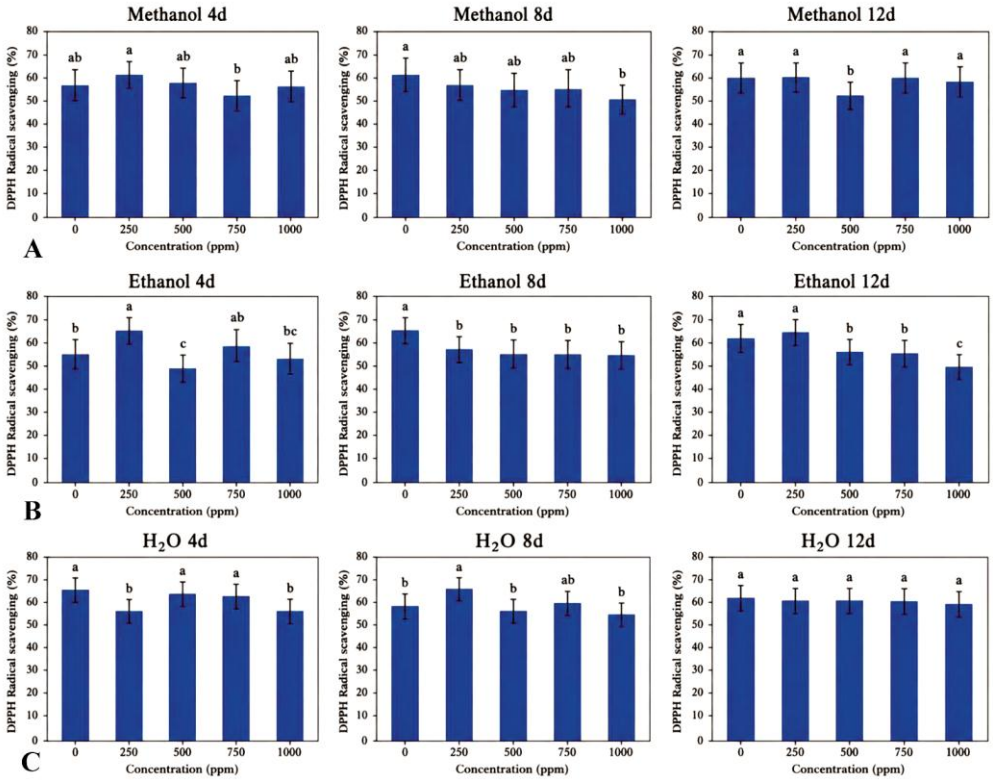


Fig. 4. Analysis of DPPH free radical scavenging activity (%) in the shoot tissue extracts of *O. corniculata*. Three different solvents methanol, ethanol and water were used for the preparation of extracts: (A) methanol extracts treated in the intervals of 4, 8 and 12 days. Both control and treated was shown to produce 64.21± 5.0, (B) plant extracts with ethanol and control was 60.04 ± 5.62, and (C) water extracts also did not show any statistical difference between treated samples and control. Bars represent standard deviation, and values are expressed as mean ± SD (n = 3). Statistical analysis was performed using ANOVA followed by DMRT, with letters above bars indicating significant differences at P <0.05.

Methyl jasmonate (MeJA) has been widely studied as an effective elicitor for enhancing total flavonoid content (TFC) in plants by activating defense-related signaling pathways and secondary metabolite biosynthesis. MeJA functions as a signaling molecule that regulates the phenylpropanoid pathway through induction of key enzymes such as phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and chalcone isomerase (CHI), which are directly involved in flavonoid biosynthesis (Wasternack and Hause 2013). Consequently, MeJA treatment promotes the accumulation of flavonoids in various medicinal and aromatic plants. Increased TFC following MeJA elicitation has been reported in several plant species, where enhanced flavonoid biosynthesis was associated with improved stress tolerance and antioxidant defense mechanisms (Gundlach et al. 1992, Akula and Ravishankar 2011). Similarly, MeJA-induced enhancement of flavonoid accumulation has been observed in medicinal plants such as marjoram (*Origanum majorana* L.) and other herbal species, demonstrating the

effectiveness of jasmonate signaling in regulating phytochemical production (Kandoudi and Németh-Zámboriné 2022). Flavonoids are important secondary metabolites possessing strong antioxidant, antimicrobial, anti-inflammatory, and pharmacological properties, thereby increasing the medicinal and industrial value of plant biomass (Pandey and Rizvi 2009). Therefore, MeJA-mediated elicitation represents a promising biotechnological strategy for increasing flavonoid accumulation and improving the phytochemical quality of medicinal plants.

DPPH free radical scavenging activity also analyzed in the field grown plants of *O.corniculata* by preparing plant extracts in three different solvents methanol, ethanol and water. The DPPH activity (%) remains same levels 60.04 ± 5.62 in both treated and control plants (Fig. 4).

Although methyl jasmonate (MeJA) treatment significantly increased total phenolic content (TPC) in plants, a proportional increase in DPPH radical scavenging activity is not always observed. This phenomenon suggests that antioxidant activity depends not only on the total quantity of phenolic compounds but also on their specific chemical composition, structural characteristics, and synergistic interactions with other antioxidant metabolites. MeJA activates phenylpropanoid metabolism and enhances the biosynthesis of phenolic compounds through induction of defense-related enzymes such as phenylalanine ammonia-lyase (PAL), thereby increasing TPC levels (Wasternack and Hause 2013). However, elevated phenolic accumulation does not necessarily translate into higher DPPH scavenging activity because different phenolic compounds possess variable antioxidant potentials depending on the number and position of hydroxyl groups and molecular structure (Rice-Evans et al. 1997). Similar observations have been reported in several medicinal plants where increased phenolic content following elicitor treatment did not correspond to a significant enhancement in DPPH activity, indicating that antioxidant responses are influenced by both qualitative and quantitative changes in metabolites (Akula and Ravishankar 2011). Moreover, antioxidant capacity may involve contributions from flavonoids, ascorbate, carotenoids, and enzymatic antioxidants, which may remain unchanged despite increased TPC (Prior et al. 2005). Therefore, the absence of a proportional increase in DPPH activity despite enhanced TPC suggests that MeJA-induced phenolic accumulation in plants may involve compounds with limited radical scavenging efficiency or altered antioxidant interactions.

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