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# INFLUENCE OF SOIL ELEMENTS ON PHOTOSYNTHESIS AND SECONDARY METABOLITES IN SELECTED MEDICINAL PLANTS

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**ABSTRACT:** The influence of mineral nutrients on photosynthesis and production of secondary metabolites was studied and analyzed in the plants *Alstonia scholaris*, *Tabebuia argentea* and *Jacquinia barbasco* growing in Botanical Garden of Acharya Nagarjuna University, Guntur during 2015 and 2016. Of all the plants maximum mineral nutrients uptake was observed in *T. argentea*. The rate of photosynthesis ($A_{max}$), rate of transpiration ($E$) and stomatal conductance ($g_s$) were found to be more in *T. argentea* i.e 7.09 µmoles $m^{-2}s^{-1}$ $CO_2$, 2.95 mmol $m^{-2}s^{-1}$, and 0.07 mmol $CO_2$ $m^{-2}s^{-1}$ respectively. Results also indicated that an optimum accumulation of mineral nutrients increased the rate of photosynthesis and secondary metabolite production in *T. argentea*.

**KEYWORDS:** photosynthesis, soil nutrients, phytochemicals, secondary metabolites.

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## 1.INTRODUCTION

Photosynthesis is a key physiological process influences all other cellular activities by providing ATP requirements. Adenosine tri phosphate produced during phosphorylation reactions not only utilized in sugars production but also involved in synthesis of so many metabolic intermediates and precursors for various secondary metabolites [1,2,3]. But the rate of photosynthesis depends on so many factors. Availability of minerals is one of such factors regulating the photosynthetic process [4]. These mineral nutrients may include both macro and micro elements/nutrients such as N, P, K, Zn, Fe, Mn, Mg, Cuand S. Of these mineral nutrients some are metal ions. These metal

ions may bound to proteins and make them ready for the transport of molecular species [5,6]. Copper is an important constituent of plastocyanin (Pc) and Cu/Zn superoxide dis mutase. Former acts as an electron carrier between PS I and PS II in photosynthetic reactions [7] and latter acts as a protection agent during oxidative stress [8,9]. In leaves 'Fe' present in Ferretin forms and mineralized with phosphate [10]. These Ferretins may be potential 'Fe' donors in buildup of chloroplast. On the other side 'Fe' also plays an important role in formation of PS I, PS II, Cyt b 559 [11] and Ferridoxin. Previous literature reported that in order to catalyze the water oxidation process with in the OEC complex plants require 'Mn' to form the 'Mn' cluster in PS II which is located in the donor side [12,13,14]. Photophosphorylation in chloroplasts membranes may be accomplished by the reversal of ATPases activated by $Ca^{+2}$. Thus reduction in 'Ca' results in disturbances in energy conversions in chloroplasts [15,16]. The catalization of the rapid inter-conversion of $CO_2$ and $H_2O$ to $HCO^{-3}$ during photosynthetic 'C' fixation is promoted by Zn containing metalloenzyme β-carbonic anhydrase (β-CA) which is located in the stroma of chloroplast [17]. The coordinated action of both nitrogen and phosphorus influence Ribulose 1,5 – bisposphate carboxylase oxygenase (Rubisco) the key enzyme in photosynthetic fixation [18,19]. As the phospholipids are important constituents of membranes, decreased availability of phosphorus may disturb the membrane integrity of cellular organelles [16,20]. Potassium plays a significant role in '$CO_2$' assimilation, where it is involved in stomatal regulation, ATP synthesis and enzyme activation [21]. However non stomatal regulation of 'K' deficiencies like reduced chlorophyll content, inhibition of PS II activity and '$^{-}$e' transport were also observed [22]. Magnesium (Mg) involved in grana stacking and LHC II was found to participate in the cation mediated formation of grana [23]. Magnesium also catalyzes many enzyme reactions involving phosphate transfer and ATP metabolism [24]. Chloroplasts contain protein rich in sulfur. The decrease in chlorophyll content might result in 'S' deficiency which further results in disruption of chloroplast structure [25]. Sulfur is an important component of Fe-S cluster which are co-factors of proteins that perform electrons transfer, redox and nonredox catalysis [26]. The opening and closure of stomata is mediated by the fluxes of potassium and accompanying anions such as malate and chloride [27]. Chloride is necessary for the water- splitting reaction in PS II. Besides 'Mn' chloride plays a fundamental role in the water-splitting system of PS II [28]. On the other side 'Cl' acts as a bridging ligand for stabilization of the oxidized state of manganese [29]. As discussed, the impact of nutrient availability on assimilatory process was well established in most of the medicinal plants. On the other side it is well evident that effective photosynthetic processes will enhance the production of primary and secondary metabolites in so many plants including medicinal plants [30,31,32]. The available literature confirmed that there is a strong correlation between nutrients, photosynthesis and secondary metabolite production. Plants such as *Alstonia scholaris, Tabebuia argentia* and *Jacquinia barbasco* are the traditional medicinal plants and the

extracts obtained from these plants were used to treat many ailments such as fever, malaria [33], tumors [34], asthma [35], antidiabetic [36] and antioxidant [37]; antitumor activity, antimetastatic activity anti-microbial and antifungal activity [38,39], antiviral activity [40], anti-inflammatory [41], antiparasitic activity [42], leishmanicidal activity [43]and molluscicidal activity [44]; antifungal, antibacterial, antioxidant activity [45] respectively. But their photosynthetic efficiency in response to nutrient availability was not explored till now. By studying the photosynthetic capacity of these plants we can further understand the ability of these plants in producing secondary metabolites.

## 2. MATERIALS AND METHODS

The present study was conducted in the Botanical Garden of Acharya Nagarjuna University (latitude 16°22'31.16" N and longitude 80°31'042.9" E). The soil is red loamy and the average rainfall is around 831 mm with a monthly average temperature of 33.6 °C (midsummer) and 24.1 °C (midwinter). Three traditional medicinal plants *Alstonia scholaris* (Devil tree), *Tabebuia argentea* (Silver trumpet tree) *and Jacquinia barbasco* (torchwood tree) of the same age group growing in Botanical Garden of Acharya Nagarjuna University are selected for this study and the data was taken on the following parameters. Photosynthetic rate (µmoles $m^{-2}s^{-1}$ $CO_2$), rate of transpiration (mmol $m^{-2}s^{-1}$) and stomatal conductance (mmol $CO_2$ $m^{-2}s^{-1}$) were measured with a portable open-path gas exchange system with $CO_2$ control (LC pro portable IRGA (ADC, UK). Measurements were undertaken using the standard 20 × 30 mm chamber equipped with blue-red light emitting diodes mounted on the top of the chamber with a photosynthetic photon flux density (PPFD) of 1600 µmol $m^{-2}$ $s^{-1}$ and $CO_2$ of 370 ppm. After enclosure in the chamber, the leaves were left to equilibrate until a constant $CO_2$ flux was observed (up to 5 min). Measurements were made between 09:00 and 11:00 h Indian standard time (IST). Chlorophyll and carotenoid contents were determined by using dimethyl sulfoxide (DMSO) method [46]. Chlorophyll 'a' and chlorophyll 'b' were measured at 645 nm and 663 nm. Carotenoids were estimated at 480 nm and 510 nm using UV-VIS Spectrophotometer (Elico *SL 159*). Saturation extracts of soils were analyzed for electrical conductivity using an electrical conductivity meter. Soil pH was determined on air-dried samples using a 1: 2.5 soil to 1 M KCl ratio. Micro and macro nutrients such as $K^+$, $Zn^{2+}$, $Cu^{2+}$, $Mn^{+2}$, S, Fe, $N_2$ and $Mg^{2+}$ were determined by atomic absorption (Varian Spectra AA-10, Mulgrave, Australia). Phosphorus was determined by the molybdenum blue procedure [47] and a spectrophotometer (Beckman DU 640, Fullerton, USA). Leaf protein content was determined according microkzeldal method [48].

**Qualitative Phytochemical Screening:** Shade dried plant material was extracted with methanol and preliminary phytochemical screening was done by using the standard tests.

**Alkaloid Test (Dragendroff's Test):** A 2 ml of plant extract was acidified with few drops of dilute hydrochloric acid. To this acidic medium, 1 ml of Dragendroff's reagent (Potassium

bismuth iodide) was added. An orange or reddish brown precipitate produced indicates the presence of alkaloids.

**Anthocyanidins:** To the plant extract an equal volume of methanolic HCl was added. Appearance of red or purple color indicated the presence of anthocyanidins.

**Flavonoid Test (Shinoda Test):** The presence of flavonoids was confirmed by treating the alcoholic plant extract with few fragments of magnesium ribbon and hydrochloric acid. The reaction mixture develops pink colour indicating the presence of flavonoids.

**Saponin Test (Foam Test):** One millilitre of each extract was shaken with 10 ml of distilled water and it was agitated in a graduated cylinder for 10 min. The formation of persistent honey-comb like froth indicated the presence of saponins.

**Quinone Test:** A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

**Tannin Test (Lead Acetate Test):** To 2 ml of each extract few drops of 10% Lead acetate was added. The appearance of white precipitate indicated the presence of tannins.

**Terpenoids and Steroids:** To the test tube containing a mixture of methanolic HCl and acetic anhydride, 50% $H_2SO_4$ was added. Change in color, from green to blue-green indicated the presence of terpenoids and steroids.

**Phenol Test:** To 2 ml of extract 0.5 ml of $FeCl_3$ (w/v) solution was added, formation of an intense colour indicated the presence of phenols.

**Coumarin:** To the methanolic extract, a few drops of alcoholic sodium hydroxide was added. Formation of yellow color indicated the presence of coumarins.

**Test for Glycosides:** Methanolic extract was mixed with a little anthrone on a watch glass. Few drops of conc. $H_2SO_4$ was added and warmed gently over water bath. The presence of glycosides was identified by dark green color formation.

**Resins:** Plant extracts were treated with acetone. To this, small amount of water was added and shaken. The appearance of turbidity indicates the presence of resins.

**Quantitative determination of phytochemicals**

**Total phenols**

Total phenolic content in the plant methanolic extracts was determined using spectrophotometric method [49]. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$ aqueous solution. The samples were thereafter incubated in a thermostat at 45 ºC for 45 minutes. The absorbance was determined using spectrophotometer at wave length 765 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GA/g of extract).

**Crude saponins**

To the 20 g of extracted methanolic sample 100 ml of 20 % aqueous ethanol was added. The solution was heated for 4 h with constant stirring at 55°. Solution was filtered and was extracted with 200 ml 20 % ethanol. After that both extracts were mixed and solvent was evaporated till 40 ml volume of extract remains. The concentrate was extracted with 20 ml of diethyl ether in separating funnel. The aqueous layer was recovered while the ether layer was discarded. The aqueous extracts were purified by adding 60 ml n-butanol. Further it was washed with twice 10 ml of 5 % aqueous NaCl. The solution was dried and the saponin content was calculated as percentage [50,51].

**Tannins**

Amount of tannins present in the methanolic leaf extracts were determined by Folin-Ciocalteu method [52]. About 0.1 ml of the sample extract was added to a 10 ml volumetric flask containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml by adding distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. Absorbance was measured against the blank at 700 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

**Alkaloids**

The plant extract (1 mg) was dissolved in dimethyl sulfoxide (DMSO), 1 ml of 2 N HCl was added and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 μg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Vis spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract [53,51].

**Steroids**

The steroids present in the plant sample was estimated [54]. A 1ml of Methanolic extract of steroid solution was transferred into 10 ml volumetric flasks. Sulfuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20 °C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

**Coumarins**

Coumarin content was estimated following the standard methods [55,56]. To 500 μl of plant extract, 2 ml distilled water and 500 μl of lead acetate (5%, w/v) solution were added in a test

tube. After shaking thoroughly, 7 ml of distilled water was added and mixing well, 2 ml of this solution was taken in another test tube and 8 ml of 0.1 M (v/v) hydrochloric acid solution was added. The solution was kept for 30 minutes at room temperature and absorbance was recorded at 320 nm using UV-Visible spectrophotometer. The total coumarin content was expressed as mg of coumarin equivalents per gm of sample extract (mg CE/g).

**Cardiac glycosides**

Cardiac glycosides were determined according Muhammad and Abubakar [57]. To the 8 ml of plant extract 60 ml of $H_2O$ and 8 ml of 12.5% lead acetate were added, mixed and filtered. From this 50 ml of the filtrate was transferred into another 100 ml flask and 8 ml of 47% $Na_2HPO_4$ were added to precipitate excess $Pb^{2+}$ ion. This was mixed and completed to volume with water. The mixture was filtered twice through same filter paper to remove excess lead phosphate. Now 10 ml of purified filtrate was transferred into clean Erlyn – Meyer flask and treated with 10ml Baljet reagent. A blank titration was carriedout using 10ml distilled water and 10ml Baljet reagent. This was allowed to stand for one hour for complete colour development. The colour intensity was measured colorimetrically at 495 nm.

**Calculation**

$$\% \text{ Total glycosides} = \frac{A \times 100}{77}$$

**Where A = absorbance**

## 3. RESULTS AND DISCUSSION

### Results

Reduced mineral nutrients may affect the photosynthesis and in turn secondary metabolite production. To identify the effect of soil elements on photosynthesis and secondary metabolite production the present study was conducted.

### Gas exchange parameters

In present study the mean photosynthetic rate was ranged between 2.35 to 7.09. Of the three plants studied highest maximum photosynthetic rate ($A_{max}$) 7.09 µmoles $m^{-2}s^{-1}$ $CO_2$ was recorded by *T. argentea*, followed by *A. scholaris* and *J. barbasco* (Fig. 1). The mean stomatal conductance ($g_s$) and transpiration rate ($E$) was ranged from 0.024 to 0.076 m mol $CO_2$ $m^{-2}s^{-1}$ and 1.26 to 2.95 mmol $m^{-2}s^{-1}$ respectively. Coupled with maximum $A_{max}$ high $g_s$ and $E$ was observed in *T. argentea* i.e 0.076 m mol $CO_2$ $m^{-2}s^{-1}$ and 2.95 mmol $m^{-2}s^{-1}$ (Fig. 1).

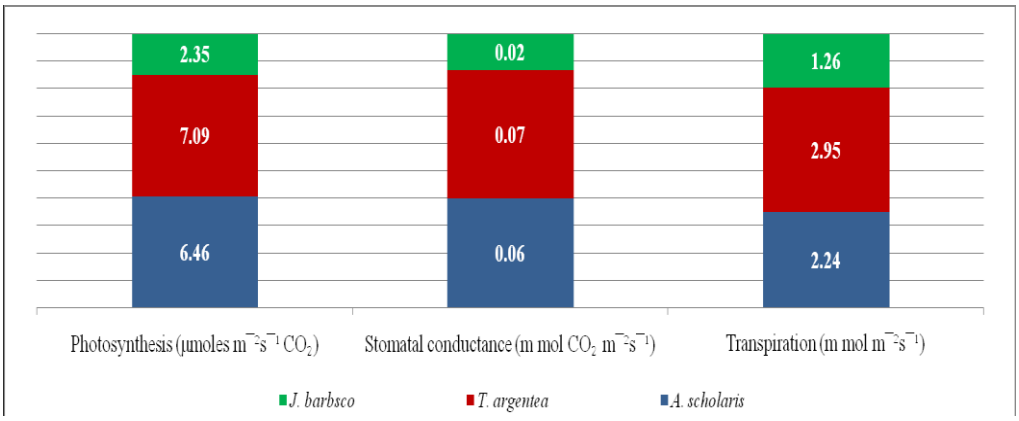


**Figure 1. Variation in Gas exchange parameters**

**Leaf chlorophyll**

Leaf chlorophyll characters such as Chl 'a', Chl 'b' and total chlrophyll content were estimated (Fig. 2). Maximum chl 'a' (1.25 mg/g) and Chl 'b' (0.94 mg/g) along with total chlorophyll content was observed in *T. argentea* (1.95 mg/g). Coupled with chlorophyll content the maximum carotenoid content (0.90 mg/g) was observed in *T. argentea*.

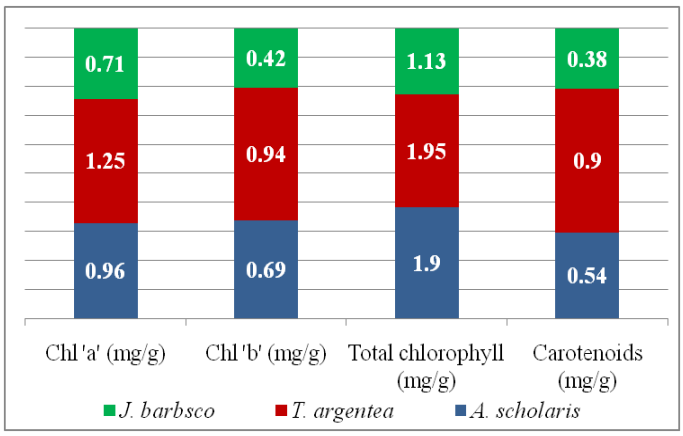


**Figure 2. Leaf chlorophyll characters of the studied plants**

**Leaf protein**

Proteins would play major role in metabolic activities such as photosynthesis and production of secondary metabolites. In present study high leaf protein content (0.019 µg/mg) (Fig. 3) was reported in *T. argentea* followed by *A. scholaris* (0.018 µg/mg) and *J. barbasco* (0.016 µg/mg).

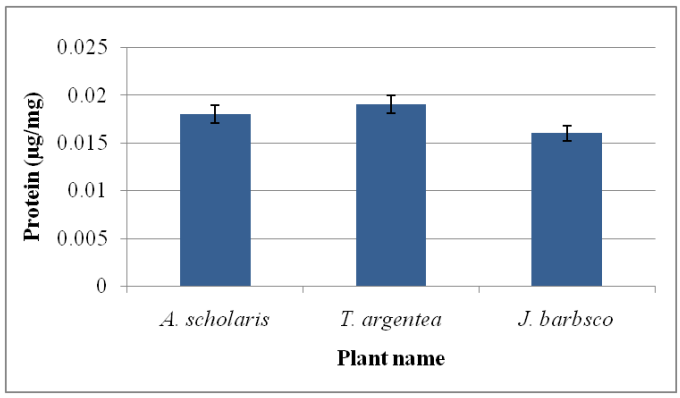


**Figure 3. Total protein content of the leaves**

**Qualitative phytochemical screening**

The present study revealed that the methanolic leaf extracts of *T. argentea*, *A. scholaris* and *J. barbasco* confirmed the existence of different phytochemicals (Table 1). Coumarins, phenols, saponins, tannins, steroids and alkaloids are present in all the three methanolic leaf extracts. But the existence of coumarins and glycosides are present only *T. argentea* and *J. barbasco*.

**Table 1. Phytochemical screening of methanolic leaf extracts**

| **Phytochemical** | ***A. Scholaris*** | ***T. Argentea*** | ***J. barbasco*** |
|---|---|---|---|
| Anthocyanidins | - | - | - |
| Phenols | + | + | + |
| Coumarins | - | + | + |
| Quinones | + | + | + |
| Glycosides | - | + | + |
| Saponins | + | + | + |
| Tannins | + | + | + |
| Flavonoids | - | - | - |
| Terpenoids/Steroids | + | + | + |
| Resins | + | + | + |
| Alkaloids | + | + | + |

**Quantitative determination of phytochemicals**

Total secondary metabolites content were determined by UV spectrophotometric method. The present investigation shows that significant variation in the contents like alkaloids, saponins, phenols, tannins, cardiac glycosides, coumarins, and steroids. The alkaloid content was examined in plant extracts and expressed in terms of atropine equivalent as mg of AE/g of extract ($y=0.0002x+0.002$, $R^2=0.988$) shown in Fig. 4. The total alkaloid content ranging from 28.10±2.13 to 48.14±1.4 % w/w. The highest alkaloid concentration 48.14±1.4 % w/w was recorded in *T. argentea* (Table 2).

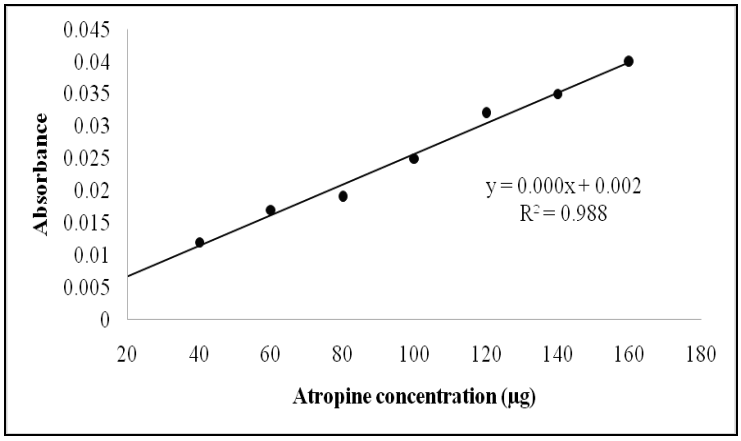


**Figure 4. Standard curve of Atropine for alkaloids**

Total saponin content ranging between 1.82±1.85 to 2.56±0.49 % w/w. Of all the plants saponin content was found to be more (2.56±0.49 % w/w) in *T. argentea* (Table 2). Phenolic compounds are essential for the growth and reproduction of plants, and plays prominent role in plant defense mechanism. Using the standard plot of gallic acid (y=0.0289x+0.0311, $R^2$=0.9936) (Fig. 5), the phenolic contents of leaf methanolic extract of *T. argentea*, *A. scholaris* and *J. barbasco* were estimated. The phenolic content was ranging from 0.81±1.21 to 2.89±0.45 mg GA/g sample and in present study maximum phenol content was recorded in *T. argentea* (Table 2).

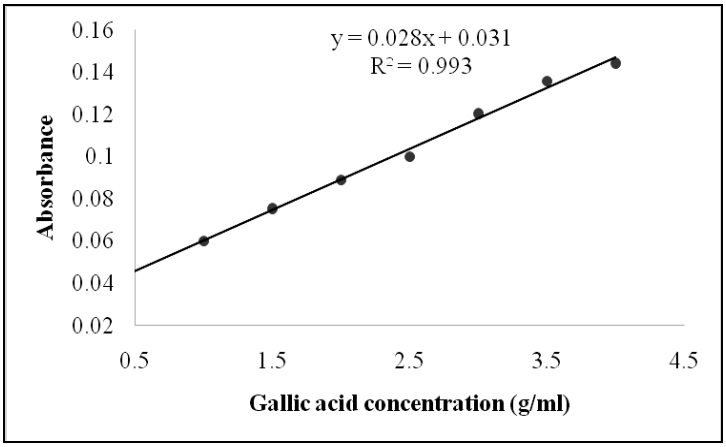


**Figure 5. Standard curve of Gallic acid for phenols**

To determine the amount of tannins present in the methanolic leaf extracts a standard plot was drawn by using tannic acid as standard (0.0005x+0.0487, $R^2$=0.9947) shown in Fig. 6. In present study total tannin content was varied between 2.14±2.15 to 7.99±0.84 %w/w. The total tannin content was found to be more in *T. argentea* (7.99± % w/w) (Table 2).

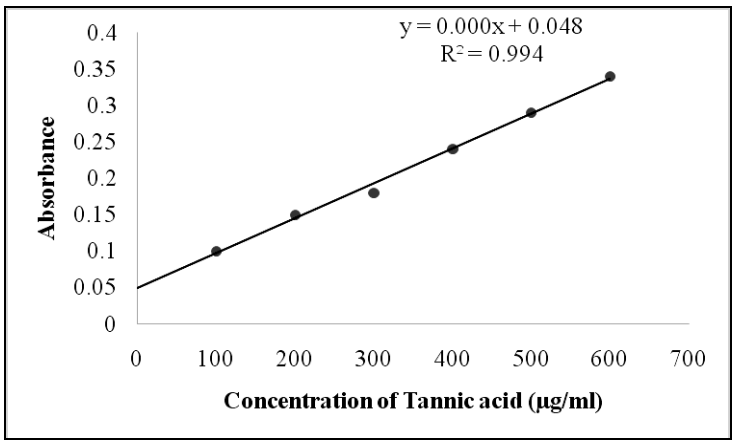


**Figure 6. Standard curve of Tannic acid for tannins**

Cardiac glycoside and coumarins were not found in *A. scholaris*. The maximum cardiac glycosides and coumarins content 2.10±0.48 g/100g and 17.32±0.82 mg/g was reported in *T. argentea* respectively (Table 2). Coumarin content was estimated using coumarin equivalents as standard (CE mg/g) (y=13.9x-0.392, $R^2$=0.966) presented in Fig. 7.

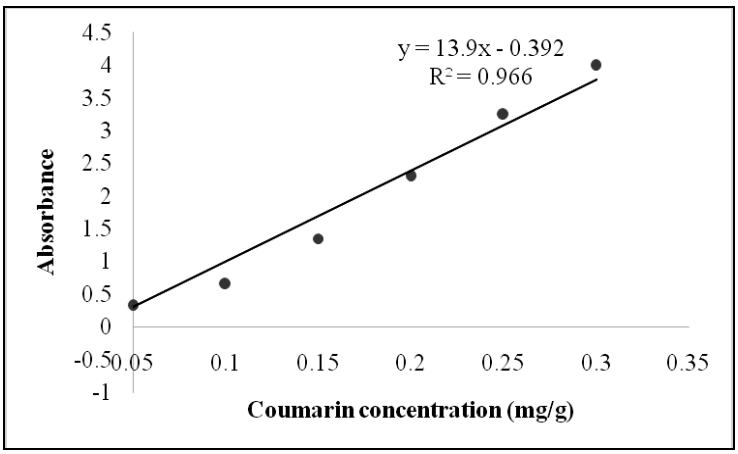


**Figure 7. Standard curve of coumarin for coumarins**

The steroids content was ranging from 76.36 µg to 118.12 µg and the maximum steroids content was observed in *T. argentea* (Table 2). A standard graph was plotted for the steroids by using cycloartenol as standard (y=3.74+0.048, $R^2$=0.996) and expressed in µg/mg (Fig. 8).

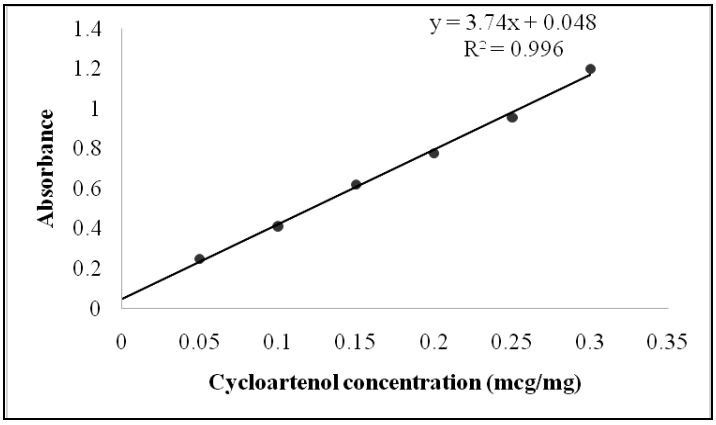


**Figure 8. Standard curve of Cycloartenol for steroids**

**Table 2. Quantitative estimation of methanolic leaf extracts**

| **Secondary metabolite** | ***A. scholaris*** | ***T. argentea*** | ***J. barbasco*** |
|---|---|---|---|
| Alkaloids (% w/w) | 36.28 | 48.14 | 28.10 |
| Saponins (% w/w) | 2.10 | 2.56 | 1.82 |
| Phenols (mg/g) | 1.72 | 2.89 | 0.81 |
| Tannins (% w/w) | 5.41 | 7.99 | 2.14 |
| Cardiac glycosides (g/100g) | - | 2.10 | 1.16 |
| Coumarins (mg/g) | - | 17.32 | 11.15 |
| Steroids (µg/mg) | 95.21 | 118.12 | 76.36 |

**Elemental analysis**

Availability different micro and macro nutrients of soils of Botanical Garden, Acharya Nagarjuna University and experimental plant leaves were studied and were presented in Fig. 9. Data presented in Fig. 9 represents that nitrogen content ranged from 8.10 µg/mg to 10.95 µg/mg. The maximum nitrogen content was recorded with *T. argentea* (10.95 µg/mg, followed by *A. scholaris*

(8.86 µg/mg) and *J. barbasco* (8.10 µg/mg). The values of phosphorus accumulated in the studied plants ranged from 1.89 µg/mg to 3.20 µg/mg and it is clear that the leaf samples of *T. argentea* (3.20 µg/mg). The potassium content of the plants leaves ranged from 3.82 µg/mg to 5.25 µg/mg and the maximum potassium content was observed in *T. argentea* (3.82 µg/mg). As seen from the data presented in Fig. 9, the studied plants varied significantly in accumulating zinc and it was ranged from 0.59 µg/mg to 1.10 µg/mg. Of the three plants studied highest accumulation of zinc was reported in *T. argentea* (1.10 µg/mg). The accumulation of the iron content in the studied plants ranged from 5.81 µg/ to 8.92 µg/mg. Highest iron accumulation was recorded in *T. argentea* followed by *A. scholaris* (6.98 µg/mg) and *J. barbasco* (5.81 µg/mg). Significant amount of manganese was reported in the studied plants and it was ranged between 1.76 µg/mg to 3.92 µg/mg. Maximum accumulation of manganese was reported in *T. Argentea* (3.92 µg/mg). The copper content ranged from 1.21 µg/mg to 2.18 µg/mg (Fig.2). The values of copper content in *T. argentea* (2.18 µg/mg), *A. scholaris* (1.81 µg/mg) and *J. barbasco* (1.21 µg/mg). The sulfur contents recorded leaf samples of the studied plants was ranged from 1.80 µg/mg to 3.60 µg/mg. The highest sulfur content was observed in leaf samples of *T. argentea* (3.60 µg/mg). It is clear from the Figure 2 that the magnesium content of plants was ranged from 1.42 µg/mg to 2.74 µg/mg. And the least magnesium content was reported with leaves of *J. barbasco* (1.42 µg/mg).

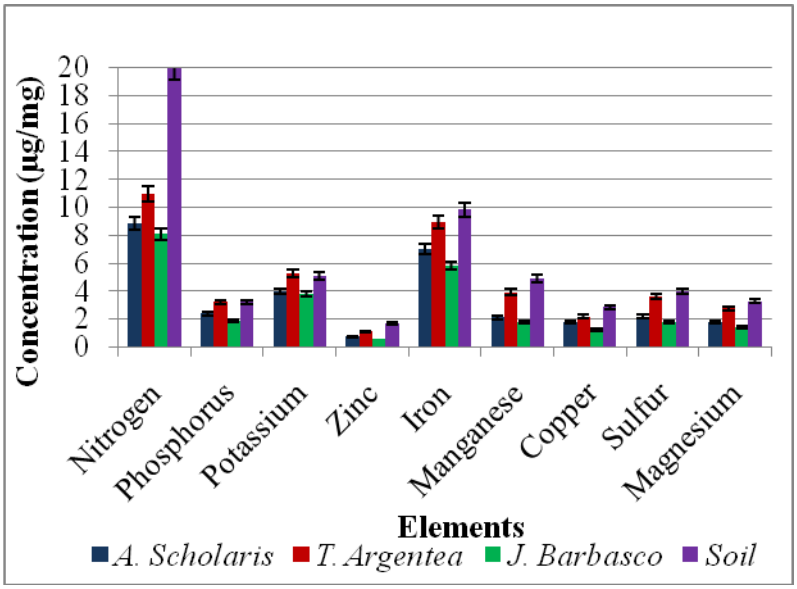


**Figure 9. Nutrient availability in studied plants and corresponding soil**

## DISCUSSION

The present study was conducted to identify the effect of available soil mineral elements on photosynthesis coupled with secondary metabolites accumulation. Plants exhibit a remarkable ability to sense environmental factors such as soil mineral elements. Ribulose 1,5 – bisposphate carboxylase oxygenase (Rubisco) is the key enzyme in photosynthetic fixation process and its production is entirely depends upon available $N_2$ [23]. In present study highest $A_{max}$ (7.09 µmoles $m^{-2}s^{-1}$ $CO_2$) along with $g_s$ (0.07 mmol $CO_2$ $m^{-2}s^{-1}$) and $E$ (2.95 m mol $CO_2$ $m^{-2}s^{-1}$) was observed in

*T. Argentea* in which high leaf $N_2$ content was present (Fig. 9). Adequate accumulation of $N_2$ on the other hand tend to produce more production of alkaloids in *T. argentea* (Table 2), which is previously reported in basil [58]. Moreover limitations in Mg, P and Fe also influence the synthesis of alkaloids [59]. In present study the accumulation of these nutrients found to be low in leaves of *J. barbasco* and *A. scholaris* and this might be the reason for the decreased production of alkaloids (Table 2). Quantification of chlorophyll is considered as an important parameter to verify the concentration of pigments involved in light absorption and energy transfer during the phytochemical process of photosynthesis. Of the three trees high chlorophyll content was observed in *T. Argentea* (Fig. 2) which shows a direct relationship between photosynthetic rate and chlorophyll content. In turn chlorophyll production depends on Mg and S, where Mg deficiency has been shown to induce an impairment of the linear photosynthetic electron transport [60] later inhibit the chlorophyll synthesis by causing a shortage of the proteins required for chlorophyll formation [61]. By maintaining optimum uptake of Mg and S the plant *T. argentea* showed high $A_{max}$. Coupled with Mg and S, Fe and Zn also had a significant effect on *Chl*'a' and *Chl*'b' by reducing chlorophyll biosynthesis and poor development of chloroplast [52]. The carotenoid content of the leaf was very much reduced by the deficiency of Fe followed by Mg and Zn deficiency. A similar influence of Fe-deficiency on carotenoid content was previously reported [63,64]. On the other side the Zn-deficiency had caused inhibition in both PS I and PS II activation [13]. In present study *T. argentea* showed maximum *Chl* 'a' content and carotenoid content by maintaining adequate amounts of Zn and Fe. Copper plays a prominent in role structural composition of different electron carriers which involves in photosynthesis and respiration [65,66]. By maintaining the adequate amounts of 'Cu' *T. argentea*is is able to manage the electron carriers such as plastocyanin and cytochrome oxidase and thereby maximum photosynthesis. Required concentrations of Zn, Cu, Fe reported to be play a prominent role in bio-synthesis of phenols in present study (Table 2). This is previously reported in vetiver [67]. The adequate leaf potassium concentration makes the plants to have a steady net $CO_2$ assimilation rate ($A_{max}$) and in turn leaf chlorophyll concentration [68]. Coupled with 'Fe' and 'Zn', the availability of 'Mn' also plays a prominent role in photosynthesis. In present study higher leaf 'Mn' concentration (Table 2) was observed in *T. Argentea* and its concentration is directly proportional to photosynthesis. In order to catalyze the water oxidation process with in the OEC complex plants require 'Mn' to form the 'Mn' cluster in PS II which is located in the donor side [12]. On the other side the formation of Mn cluster requires 'Mn' ions along with $Ca^{+2}$ ion [69]. Besides 'Mn' chloride plays a fundamental role in the water-splitting system of PS II [70]. Optimal availability of Mn along with Fe increased the flavanoids concentration in present study especially in *T. argentea* (Table 2). This is in agreement with previous reports [71,72]. Potassium impacts photosynthesis through both stomatal and non-stomatal aspects of photosynthesis. At the stomatal

level, regulation of conductance is closely impacted by the leaf $K^+$ concentration through the reversible flux of $K^+$ ions into and out of the stomatal guard cells [73]. The influx of $K^+$ into the guard cell lowers the osmotic potential of the cell causing water to flow into the cell. In response to this inward flow of water, the cell elongates and thereby forces the stomata open. This situation makes the leaves to have a high efficiency to $CO_2$, and there by more $CO_2$ fixation in *T. Argentea*. Coupled with 'K', zinc plays a prominent role in transpiration process [74]. On the other side the accumulation of required potassium levels in plants tend to increase the bio-synthesis of phenols and flavonoids in present study especially in *T. argentea* (Fig. 2). This is may be due to the enhancement of total non-structural carbohydrate [75]. This might due to role of 'K' in stimulating and trans-location activity of both photosynthesis and carbohydrates to plant parts [76].

## 4. CONCLUSION

The present study confirms that the availability of mineral nutrients may influence the photosynthesis and there by secondary metabolite production, which is clearly seen in *T. argentea*. By proper accumulation of soil nutrients *T. argentea* maintained optimum photosynthesis and thus high secondary metabolite concentration when compared to *A. scholaris* and *J. barbasco* grown in same soil with similar physico-chemical properties.

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## CONFLICT OF INTEREST

Authors do not have any conflict of interest.

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