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# Bioinoculants influence accumulation of phytochemicals in *Oroxylum indicum* (L.) Benth. ex Kurz seedlings

**Chandrima Debi and Vipin Parkash**

**Abstract**
The seedlings of *Oroxylum indicum* were inoculated with plant growth promoting microbes (PGPMs) mainly, *Pseudomonas putida, Trichoderma harzianum* and *Glomus mosseae* both alone and consortium. The preliminary qualitative and quantitative phytochemical analysis of the root extract of the inoculated seedlings of *O. indicum* showed the presence of carbohydrates, protein, saponins, tannins, alkaloids, phenols, flavonoids, terpenoids, and glycosides. The tannin concentration was found maximum (0.0500±0.037 mg/g) in $T_M$ (mycorrhizal treatment), while it was minimum (0.0341±0.321 mg/g) in non-inoculated/control treatment. Maximum (0.993±0.006 mg/g) chlorophyll content was present in $T_{MB}$ (Mycorrhizal + Bacterial treatment) and minimum (0.639±0.01 mg/g) in control treatment. The total alkaloid content was maximum (0.769±0.031) in $T_{MB}$ (Mycorrhizal + Bacterial treatment) while it was minimum (0.115±0.055) in control treatment. The total protein content was maximum (960.65±0.065 mg/g) in $T_{MBF}$ (Mycorrhizal + Bacterial + Fungal treatment) while it was minimum (299.04±0.0943 mg/g) in control treatment. The total phenolic content (TPC) was maximum (29.271± 0.388 mg/g in GAE) in $T_B$ (Bacterial treatment) while TPC was minimum (3.1±1.01 mg/g in GAE) in Control treatment. It can be concluded that the bioinoculants whether alone or in consortial form influences the phytochemicals in *O. indicum* but the consortial form has significant influence than alone treatments.

**Keywords:** Pseudomonas putida, Trichoderma harzianum and Glomus mosseae, bioinoculants, phytochemicals

## Introduction
*Oroxylum indicum* is a medicinally important forest tree species *Oroxylum indicum* is an active ingredient of well-known Ayurvedic formulations like *Amartarista, Awalwha, Brahma rasayana, Bruhatpancha, Chyawanaprasha, Dantyadarishta, Dasamoola, Dhanwantaram ghrita, Mulyadi Kwath, Narayana taila and Shyonaka patpak* [1, 2]. The main ingredients of Dashamula are procured from the roots of five herbaceous and five tree species, *Shyonaka* being one of them. This species also constitutes one of the ingredients in *Chyawanprasha* [3]. It is estimated that 70–80% of people worldwide rely chiefly on traditional, largely herbal medicine to meet their primary healthcare needs. Since time immemorial, medicinal plants have been used in virtually all cultures as a source of medicine [4, 5]. The production, consumption and international trade in medicinal plants and phytomedicine (herbal medicine), have grown and are expected to grow further in the future. To satisfy growing market demands, surveys are being conducted to unearth new plant sources of herbal remedies and medicines and at the same time develop new strategies for better yield and quality. This can be achieved through different methods including micro propagation [6]. It may help in conserving many valuable tree species in the process and may open new vistas in the forest biotechnology. The plant is used in many ayurvedic preparations widely used by people for health care. The existence of *O. indicum* in natural population is highly threatened and has been categorized as vulnerable by the government of India [7].This tree has been harvested so heavily for medicinal purposes that its survival is in jeopardy. Problems related with its natural propagation and indiscriminate exploitation for medicinal purpose has pushed *O. indicum* to the list of endangered plant species of India [8].
Phytochemicals are the natural bioactive compounds found in plants. These phytochemicals work with nutrients and fibers to form an integrated part of defence system against various diseases and stress conditions [9]. The most important of these bioactive constituents of plants

Are alkaloids, tannins, flavonoids, steroid, terpenoid, carbohydrate and phenolic compounds [10]. Several parts of *O. indicum* contain alkaloids and flavonoids [11, 12] of medicinal value. *In vitro* regenerated plantlets have been reported to produce higher yields of active compounds [13-15]. The plant is reported to possess antiinflammatory, diuretic, anti-arthritic, antifungal, and antibacterial activities [16]. The plant is also used in Asian folk medicine for the treatment of abdominal tumors [17].

Numerous studies have shown that AMF can directly or indirectly influence the secondary metabolism of plants, causing changes in secondary metabolite levels [18, 19]. For example, AMF can induce the synthesis of caffeic acid and rosmarinic acid in the medicinal plant sweet basil (*Ocimum basilicum*) [20]. Lu *et al*. [21] reported that the inoculation of yams with AMF can expedite plant growth and increase the content of secondary metabolites in tubers. Some bacteria provide plants with growth promoting substances and play major role in phosphate solubilizing ability [22]. Phosphate solubilizing microorganisms are another sort of bio-fertilizers which have the ability to solubilize organic and inorganic phosphorus compounds by producing organic acid or phosphatase enzyme [23]. Mixed inoculation with diazotrophic bacteria and arbuscular-mycorrhizal fungi creates synergistic interactions that may result in a significant increase in growth, in the phosphorus content in plants, enhanced mycorrhizal infection and an enhancement in the uptake of mineral nutrients such as phosphorus, nitrogen, zinc, copper, and iron [24-33].

The literature review revealed that bioinoculation studies has been mainly carried on various fields including the influence of bioinoculants on growth and mycorrhizal occurrence in the rhizosphere, plant growth stage, fertiliser management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in wheat, impact of endomycorrhizal fungi and other bioinoculants on growth enhancement. Role of bioinoculant increasing in growth, flavour content and yield, effect of bioinoculation on rice varieties of India, role of arbuscular mycorrhizae fungi and multibioinoculants in cotton plant growth, response of bioinoculants on growth, yield and fiber quality of cotton under irrigation, effect of bioinoculants on biomass productivity in agroforestry systems. But there is no report on the influence of bioinoculation on the accumulation of phytochemicals in *O. indicum*. The positive effect on the production of pharmacologically active compounds in medicinal plants through mycorrhization would mean a higher benefit and at the same time would contribute to a more sustainable practice of conservation of plant species [34]. Research towards mass multiplication, conservation and higher production of the active compound under *in vitro* culture conditions is essential [35]. On this aspect an experiment was conducted to study the influence of bioinoculation on the biotization [36] and accumulation of some phytochemicals in the seedlings of *O. indicum*. The seedlings were inoculated with Plant Growth Promoting Microbes (PGPMs) mainly, *Pseudomonas putida, Trichoderma harzianum* and *Glomus mosseae* both alone and in consortium. The inoculated seedlings were further analysed for the qualitative as well as quantitative assessment of accumulated phytochemicals.

## Material and methods

An experiment was set up in the nursery of Rain Forest Research Institute, Jorhat to study the inoculation effect. For this purpose seeds from different seed sources were analysed for seed germination ability and seedlings were raised [37]. The experiment was designed in Randomized Block Design (RBD), where three replications of each treatment were taken. Different treatments like single and combined/ synergistic/influential of plant growth promoting microbes (PGPMs) mainly, *Pseudomonas putida, Trichoderma harzianum* and *Glomus mosseae* were applied for the present investigation. In control sets, no bioinoculant (inoculum) was added. The seedlings treated with bacteria ($T_B$), fungus ($T_F$) and mycorrhiza ($T_M$), dual consortium of bacteria and fungus ($T_{BF}$), bacteria and mycorrhiza ($T_{BM}$), mycorrhiza and fungus ($T_{MF}$), and mixed consortium of bacteria, mycorrhiza and fungus ($T_{MBF}$) were harvested after 270 days of inoculation. The roots were sun dried and then powdered. 3 gram of the powdered plant sample was macerated with 50 ml solvent for 3 days at room temperature (25 °C). Solvents used for extraction were distilled water and ethanol 70%. Extracts were filtered using Whatman filter. Aqueous as well as ethanolic extracts were evaporated to dryness. The extract was used for the qualitative and quantitative analysis of the phytochemicals.

### Phytochemical analysis

### Test for Alkaloids

The extract of the plant sample was stirred with 5 ml of 1% HCl on water bath. The solution was filtered and the filtrate was treated with Mayer's and Hager's reagent.

### Mayer's Test

The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract [38].

### Hager's Test

Hager's reagent was added to 2-3 ml of the filtrate. Yellow colour indicated the presence of alkaloids.

### Test for tannins

A small quantity of the extract was boiled with 5 ml of 45% solution ethanol for 5 minutes. The mixture was cooled and filtered. The filtrates were used for the following test:

### Lead Sub Acetate Test

1ml of the different filtrate was added with three drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins.

### Ferric Chloride Test

1ml each of filtrate is diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black colour indicates the presence of Tannins.

### Test for flavonoids

The presence of flavonoids in the plant sample was determined by following tests [39, 40]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2SO_4$. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% Aluminium chloride solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of

ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

**Test for terpenoids (Salkowski test)**

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated $H_2S0_4$ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

**Test for glycosides (Keller-Killani test)**
Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. Add 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates the presence of glycosides.

**Test for Saponins**
0.5g of the powdered sample was mixed with 5 ml of distilled water and was introduced into a tube, the mixture was vigorously shaken for 2min., and formation of froth indicated the presence of Saponins [38].

**Test for phenols**
5 ml of the plant extract was dissolved in 5 ml of distilled. Few drops of neutral 5% ferric chloride solution were added to the solution. A dark green colour indicated the presence of phenolic compounds

**Test for Carbohydrates**
5 ml of both aqueous and ethanolic extract was treated with conc. $H_2SO_4$ and then with Molisch's reagent. The appearance of pink or violet colour indicates the presence of Carbohydrates. The plant extract was boiled with Fehling's and Benedict's solution. Formation of brick red precipitate in Fehling's and Benedict's solution is a positive test for the reducing sugars respectively.

**Test for proteins (Xanthoproteic test)**
1ml of the aqueous as well as alcoholic extract was treated with 1ml of $H_2SO_4$ (conc.). The appearance of white precipitate indicates the presence of proteins.

**Quantitative analysis of the phytochemicals**
**Determination of Total Alkaloid**
1g of the plant sample was weighed into a 250 ml beaker and 40 ml of 10% acetic acid in ethanol was added and covered. It was allowed to stand for 4 h. After filtration the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was over. The entire solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide. It was again filtered. The residue collected is the alkaloid, which was dried and weighed [40].

**Determination of Total Phenolic Content in the plant extracts**
The total phenol content of the plant sample was determined by the help of Folin-Ciocalteu reagent method [41]. An aliquot (100 µl) of extract was mixed with 250 µl of Folin-Ciocalteu reagent and it was allowed to stand at room temperature for 5 min. 1.5 ml of 20% Sodium bicarbonate was added to the mixture and incubated at room temperature for 120 min. The absorbance was measured at 765 nm using a spectrophotometer. A standard curve was plotted using different concentrations of gallic acid and the amount of total phenolics was calculated as gallic acid equivalents in mg/ g of dried extract. The concentration of phenolics was determined (mg/ml) from the calibration curve and the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract) from the equation.
T=C X V/M
Where, T= Total Phenolic Content (mg/g) of extract as GAE,
C= Concentration of GA established from the calibration curve in (mg/ml),
V= Volume of the extract solution in ml
M= weight of the extract in g.

**Determination of Total flavonoid content**
The total flavonoid content of the seedlings of *O. indicum* was determined by the help of Aluminium chloride method [42]. Quercetin was used as standard and flavonoid contents were measured in Quercetin equivalent.1ml of standard or extract solution (0.1, 0.5, 1, 2.5,5 mg/ml) was taken into 10ml volumetric flask, containing 5 ml of distilled water and 0.3ml of 5% $NaNO_2$. 0.3ml 10% $AlCl_3$ was added to the mixture after 5min. At the 6th min 2ml of 1M NaOH was added consecutively and the volume made up to 10ml with distilled water. The test solution was briskly shaken. After 10 min. of incubation, the absorbance was recorded at 510nm using UV-Visible spectrophotometer.

**Determination of Total tannin content**
1 g of the plant sample was mixed with 10 ml distilled water on a shaker for 1 hr. 5 ml of filtrate was pipette out into a tube and mixed with 0.1 M $FeCl_3$ (3ml) in 0.1 N HCl and 0.008 M potassium ferrocyanide. Absorbance was measured at 605 nm within 10 min. Tannic acid (100 ppm) was used as standard [43].

**Determination of Total Protein content**
The total protein content was determined by Lowry's method [44]. 500 mg of plant sample was mixed in 10 ml of distilled water and centrifuged for 10 min at 3000 rpm. The supernatant was collected and made up to 1 ml with distilled water. The reagents were prepared of reagents by taking 2 % Sodium Carbonate in 0.1N Sodium Hydroxide (Reagent A) and Reagent B was prepared by taking 0.5 % Copper Sulphate in 1% potassium sodium tartrate. 3 ml of Reagent A was mixed with 1 ml of Reagent B and 0.02 ml of Folin-Ciocalteau Reagent was added. The mixture was incubated for 30 mins. at room temperature. Readings were taken at 600 nm in triplicates. Bovine serum albumin was taken as standard.

**Estimation of Total Polysaccharide Content in *O. indicum***
Dissolve about 1mg of the powdered root sample in 10ml distilled in water. Take 1ml for sugar analysis to estimate the polysaccharide content in *O. indicum*, add 1ml of 5% phenol to the 1ml of sample solution, and then add 5ml of conc.$H_2SO_4$ and measure the absorbance after 10 minutes at 488nm against blank [45].The experiment was carried out in triplicate [46].

**Determination of Total Chlorophyll Content**
The total Chlorophyll content in the leaves of *O. indicum* was determined using [47] method. 1 g of the plant leaf sample was homogenized with 20 ml of 80% Acetone. The extract was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and readings were taken in triplicates at 645 nm and

663 nm. The total Chlorophyll content was calculated using the following formula:

Total Chlorophyll= 20.2 (A645) + 8.02 (A663) × V/ (1000×W)

All the data of the study were statistically analysed for ANOVA test using MS Excel 2007.

## Results and Discussion

The preliminary phytochemical analysis of the root extract of the seedlings of *Oroxylum indicum* revealed the presence of carbohydrates, protein, saponins, Tannins, alkaloids, phenols, flavonoids, terpenoids, glycosides as shown in Table 1.

**Table 1:** Qualitative analysis of root extract of *Oroxylum indicum*

| Phytochemicals | Presence/Absence | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Alcoholic extract | | | | | | | | Aqueous Extract | | | | | | | |
| | $T_c$ | $T_M$ | $T_F$ | $T_B$ | $T_{MB}$ | $T_{BF}$ | $T_{MF}$ | $T_{MBF}$ | $T_c$ | $T_M$ | $T_F$ | $T_B$ | $T_{MB}$ | $T_{BF}$ | $T_{MF}$ | $T_{MBF}$ |
| Alkaloids | ++ | ++ | + | ++ | + | ++ | ++ | ++ | + | + | + | + | + | + | + | + |
| Phenols | + | ++ | ++ | + | + | + | ++ | ++ | - | - | + | - | - | - | + | + |
| Flavonoids | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carbohydrates | + | ++ | ++ | ++ | + | ++ | + | ++ | - | - | + | - | - | - | + | + |
| Terpenoids | - | ++ | ++ | - | ++ | ++ | + | ++ | - | + | + | - | + | + | + | + |
| Glycosides | - | - | - | + | + | - | + | ++ | - | - | - | - | - | - | + | + |
| Saponins | + | + | + | + | + | + | + | + | - | - | + | - | + | - | + | + |
| Tannins | ++ | + | ++ | ++ | ++ | + | ++ | ++ | - | + | + | + | + | + | + | + |
| Proteins | ++ | + | ++ | ++ | + | + | ++ | + | + | + | ++ | + | ++ | + | ++ | ++ |

$T_c$= Control treatment, $T_M$ = Mycorrhiza treatment (*Glomus mosseae*), $T_B$ = Bacteria treatment (*Pseudomonas* sp.), $T_F$= Fungi treatment (*Trichoderma harzianum*), ± = SEM (Standard error of mean) + - low; ++ - high.

**Table 2:** Total Tannin, Chlorophyll and Alkaloid, Total phenol content (TPC) and Total flavonoid content (TFC), Total Protein and Total Polysaccharide content in the seedlings of *O. indicum* after final stage of inoculation.

| Treatment | Tannin concentration (mg/ g) | Alkaloid concentration (mg/g) | Phenol content mg/g GAE | Flavonoid conc.(alc.) (mg/g) | Protein conc. (mg/g) | Polysaccharide conc. (mg/g) | Chlorophyll Content (mg/g) |
|---|---|---|---|---|---|---|---|
| $T_c$ | 0.0341±0.321 | 0.115±0.055 | 21.12±6.515 | 0.334±0.003 | 299.04±0.0943 | 3.688±0.309 | 0.639±0.01 |
| $T_M$ | 0.0500±0.037 | 0.371±0.017 | 32.24±0.384 | 0.429±0.002 | 415.20±0.076 | 4.554±0.177 | 0.978±0.01 |
| $T_F$ | 0.0422±0.445 | 0.127±0.056 | 22.030±0.547 | 0.374±0.002 | 927.32±0.220 | 5±0.411 | 0.790±0.005 |
| $T_B$ | 0.0493±0.149 | 0.543±0.044 | 29.271±0.388 | 0.344±0.003 | 627.82±0.081 | 5.467±0.239 | 0.776±0.005 |
| $T_{MF}$ | 0.0496±0.078 | 0.124±0.069 | 25.919±0.337 | 0.365±0.0028 | 799.54±0.109 | 9.452±0.445 | 0.857±0.005 |
| $T_{BF}$ | 0.0472±0.059 | 0.655±0.062 | 23.055±0.673 | 0.370±0.0034 | 733.88±0.049 | 9.885±0.044 | 0.927±0.006 |
| $T_{MB}$ | 0.0498±0.044 | 0.769±0.031 | 26.024±0.293 | 0.348±0.0027 | 642.47±0.061 | 5.993±0.316 | 0.993±0.006 |
| $T_{MBF}$ | 0.0493±0.048 | 0.649±0.258 | 25.654±0.433 | 0.352±0.0013 | 960.65±0.065 | 7.373±0.185 | 0.957±0.005 |

**ANOVA summary**

| *Source of Variation* | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 3086086.928 | 6 | 514347.8213 | 66.86186059 | 6.83708E-22 | 2.290431783 |
| Within Groups | 376941.9968 | 49 | 7692.693813 | | | |

$T_c$= Control treatment, $T_M$ = Mycorrhiza treatment (*Glomus mosseae*), $T_B$ = Bacteria treatment (*Pseudomonas* sp.), $T_F$= Fungi treatment (*Trichoderma harzianum*), ± = SEM (Standard error of mean)

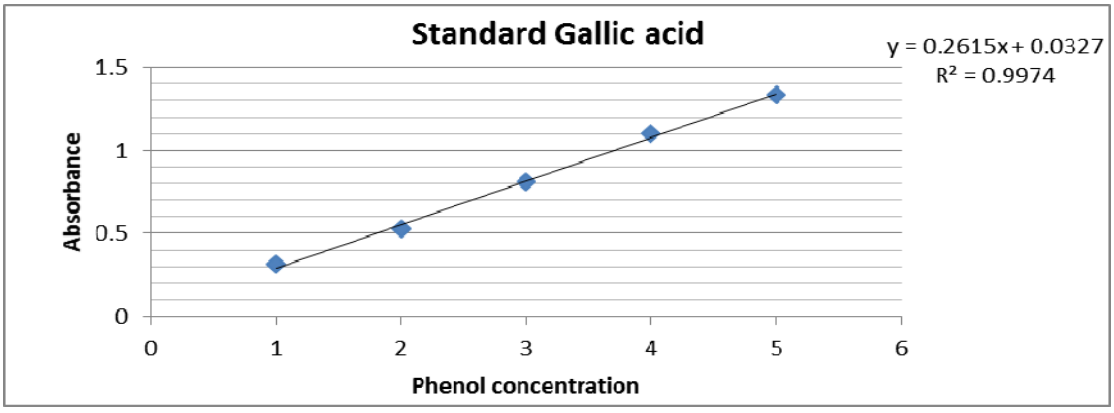


**Fig 1:** Standard Curve of Gallic acid

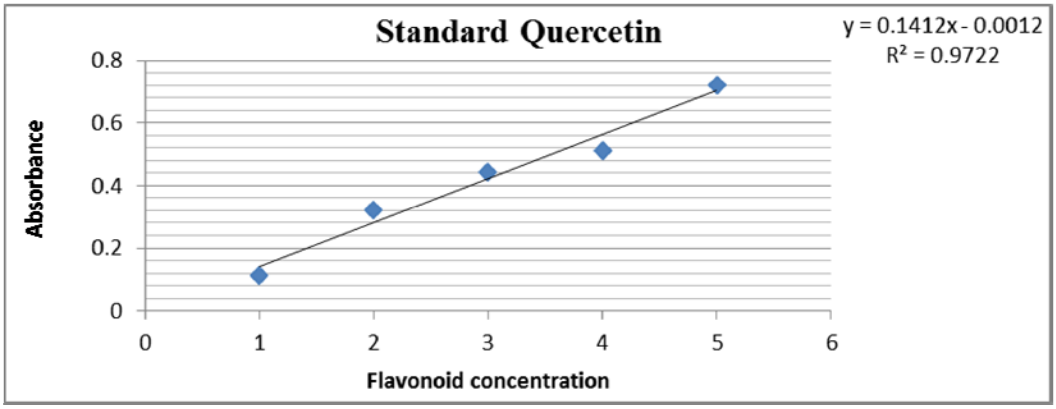


**Fig 2:** Standard Curve of Quercetin

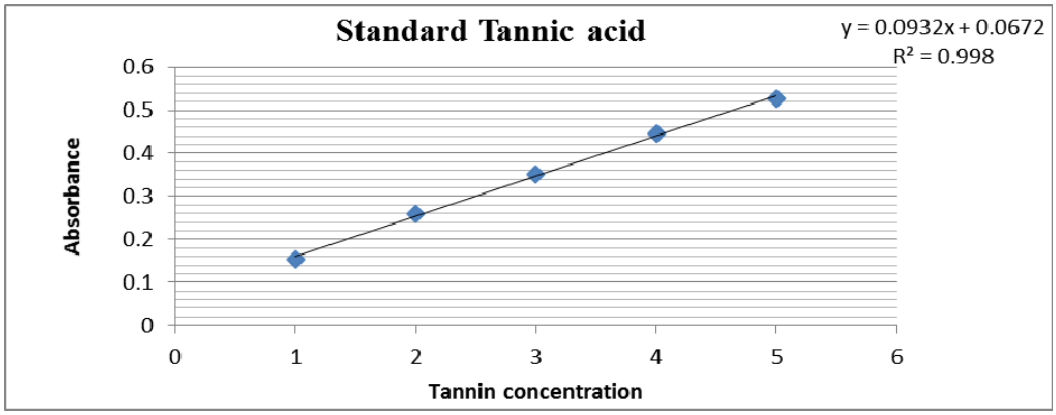


**Fig 3:** Standard Curve of Tannic acid

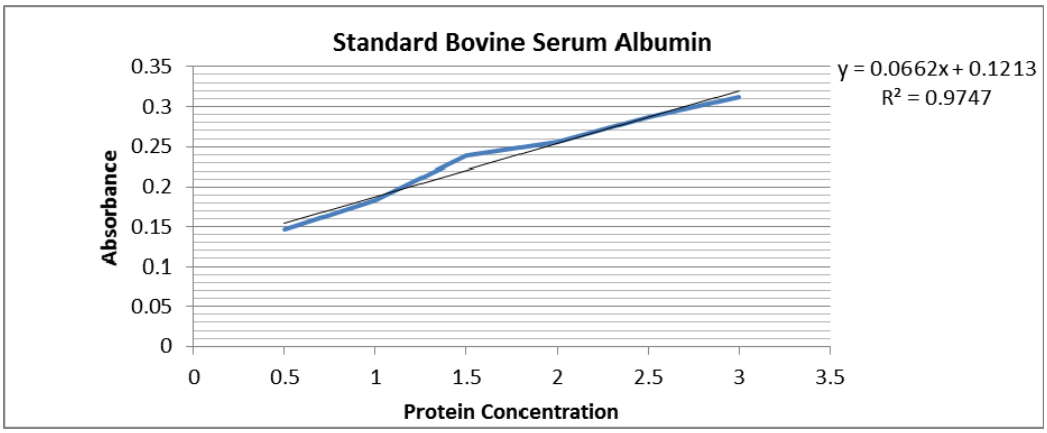


**Fig 4:** Standard Curve of Bovine serum albumin

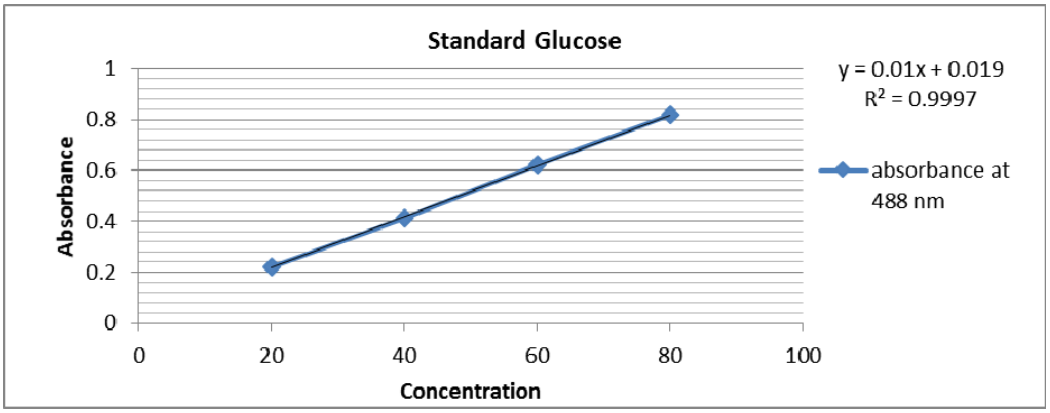


**Fig 5:** Standard Curve of Glucose

The results were more pronounced in ethanolic extracts as compared to aqueous extract. The ethanolic root extract showed pronounced presence of alkaloids as compared to aqueous in all treatments including control. The presence of phenols was more pronounced in ethanolic extract as compared to aqueous extract. The phenols were absent in aqueous root extract of control, $T_M$, $T_B$, $T_{MB}$, $T_{BF}$ treatments. The flavonoids were present along all treatments in both ethanolic as well as aqueous extracts, but the presence was more prominent in ethanolic extracts. Presence of carbohydrates was pronounced in ethanolic extracts in all the treatments, while aqueous extract of $T_F$, $T_{MF}$, $T_{MBF}$ only showed traces of carbohydrates. Similarly, the qualitative analysis of terpenoids showed the pronounced presence in ethanolic extract as compared to aqueous extract. In ethanolic root extracts terpenoids were present in all treatments except control treatment. Aqueous root extracts of $T_M$, $T_F$, $T_{MB}$, $T_{MF}$ $T_{BF}$, $T_{MBF}$ treatments only showed the presence of terpenoids. Glycosides were found present in the ethanolic root extract of $T_B$, $T_{MB}$, $T_{MF}$, $T_{MBF}$, while aqueous root extract of $T_{MF}$, $T_{MBF}$ only showed the presence of glycosides. The saponins were also present along all treatments in ethanolic extract, while aqueous extract of $T_F$, $T_{MB}$, $T_{MF}$ $T_{MBF}$ showed the presence of saponins. Both aqueous as well as ethanolic extracts showed the presence of tannins in all treatments except aqueous extract of control treatment. Proteins were also present in all treatments in both aqueous as well as ethanolic root extract. Similar results were also reported earlier [48]. The phytochemical screening of leaf, stems bark, root bark and callus contain most of the secondary metabolites analyzed. Phytochemical study on *Oroxylum indicum* has been reported by various workers earlier [49-53] but without any inoculation. During the establishment of the AM symbiosis, a range of chemical and biological parameters is affected in plants, including the pattern of secondary plant compounds. The accumulation of flavonoids [54], phenolic compounds [55], triterpenoids [56] in plants colonized by AM fungi has been already reported which corroborates with the results of this study. The quantitative estimation of the phytochemicals present in *O. indicum* with inoculation after 270 days has been shown in Table 2. The tannin concentration was determined with the help of tannic acid as standard (Fig.3) and it was found maximum (0.0500±0.037) in $T_M$ treatment while it was found minimum (0.0341±0.321) in control treatment. The TPC in mg/g GAE in the alcoholic root extract of *O. indicum* was derived by standard (Fig.1). It was maximum (32.24±0.384) in $T_M$ while TPC was minimum (21.12±6.15) in Control treatment. The total flavonoid content (TFC) was also determined with the help of standard quercitin (Fig. 2).The TFC mg/g in alc. root extract was found to be maximum (0.429±0.002) in $T_M$, while control sample had minimum (0.334±0.003). Maximum (0.993±0.006) chlorophyll content mg/g was estimated in $T_{MB}$ and minimum (0.639±0.01) was found in control treatment. The total alkaloid content was maximum (0.769±0.031) in $T_{MB}$ while it was minimum (0.115±0.055) in control treatment. The total protein content in (mg/g) was maximum (960.65±0.065) in $T_{MBF}$ while it was minimum (299.04±0.0943) in control treatment. The total protein content was determined by the help of standard as bovine serum albumin (Fig.4). Maximum (9.885±0.044) polysaccharide content (mg/g) was found in $T_{BF,}$ while, minimum (3.688±0.309) polysaccharide content was found in control treatment. The total polysaccharide content was determined by the help of glucose as standard (Fig.5). The ANOVA of the Table 2 shows that *F* value was 66.86 for $P \leq 0.05$ with F critical value of 2.290 which is significant in nature. Numerous studies have noticed that AMF can directly or indirectly influence the secondary metabolism of plants, causing changes in secondary metabolite levels [57, 58]. The symbiotic AM fungi can induce changes in the accumulation of secondary metabolites, including phenolics in roots and aerial parts and also essential oil of host plants [59]. Many studies have shown that some bacterial species respond to the presence of certain AMF [60], suggesting a high degree of specificity between bacteria associated with AMF. The presence of bioactive compounds indicates the medicinal importance of the plants. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against various diseases.

The plant growth-promoting microorganisms and their inoculation in the rhizosphere of medicinal plants are very useful in increasing the growth of plants through nutrients uptake *vis-a-vis* phytochemical yield by active metabolism. The indiscriminate collection, over exploitation, uprooting of whole plants, this plant has become endangered and vulnerable in different parts of the Indian subcontinent. The existence of *O. indicum* in natural population is in jeopardy [7]. Hence, alternative ways of preservation and cultivation of naturally occurring medicinal plants are required. It can be carried out by utilizing the putative bioagents present in the rhizosphere of medicinal plants. These plant growth promoting microorganisms of the medicinal plants also influence the quality and quantity of bioactive constituents. They also influence the metabolic activity and bioactivity of these medicinal plants. Hence, studies are required for the evaluation of the differences of microbial diversity and the bioactive components of medicinal plants among different habitats which will pilot ways to unearth the relationship between plants, microorganism diversity and the bioactive compounds accumulation.

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