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Assessment of Phytochemicals of Oroxylum indicum Plant Supplemented with Selected Plant Growth Regulators

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ABSTRACT
Oroxylum indicum belongs to family: Bignoniaceae commonly known as Broken bones tree is widely distributed throughout South East Asia and India. The endangered forest tree known as Oroxylum indicum is having many unexplored medicinal properties. Oroxylum indicum is small perennial extending to a height 8-15 m branched at top chief grow in evergreen forest. Its leaves are 0.5-1.5 m long, 2-3 pinnate with opposite pinnae, leaflets 2-4 pairs, 6-12 cm long and 4-10 cm broad ovate or are numerous having reddish-purple and pinkish-yellow within, about 0.3-0.6 meter long. The stalk of the flowers is one feet long. To report different phytochemicals like sterols, saponins, glycosides, flavonoids, alkaloids, tannins, phenols and quinones various tests were performed. The results of these phytochemical testing of crude methanol and ethanol extracts from leaves, root bark of Oroxylum indicum. (Chopra, R.N., et al 2002).The preliminary phytochemical screening study revealed that the leaf of Oroxylum indicum has presence of Flavonoids, Saponins Alkaloids, Sterols, Tannins, Phenolic Compounds, Fats and Oils, Glycosides, lignins.

Keywords: Oroxylum indicum. L., flavonoids, alkaloids, tannins, Phytochemicals

I. INTRODUCTION

Oroxylum indicum belonging to the family Bignoniaceae, has been used for the present study. The leaves of this plant are reported to contain flavonoids namely chrysin, oroxylin-A, scutellarin, baicalein (Sankara S. Nair 1972).This plant having much medicinal values (Anonymous 1998).Leaves are also found to contain quercetin-3-o-α-L-arabinopyranoside, 1-(2-hydroxyethyl) cyclohexane-1, 4-diol, apigenin (Haribabu 2010). Seeds of this plant are reported to contain ellagic acid (Vasanth et al 1991). Root bark is reported to contain chrysin, baicalein, biochanin-A and ellagic acid (Myitreyi 2008). The root bark has also been reported to contain two flavonoids 2, 5-dihydroxy-6, 7-dimethoxy flavone and 3, 7, 3', 5'-tetramethoxy-4'-hydroxyflavone (Kawaskar.U 2003). Stem bark is found to contain ellagic acid, chrysin, oroxylin-A, scutellarin, baicalein. (Chen LJ 2003, Maitrai. Z ,2008).

Role of SA, BRs in Growth and Development of Plants

The potential of BRs to enhance development in Brassica napus pollen led to their original discovery. It

has been shown that BRs may stimulate the elongation and division of plant cells. The term "brassin" was later used to BRs. In 1979, the most active BR, brassinolide (BL), was isolated. Brassinosteroid insensitive 1 (BRI1), a receptor kinase that initiates an intracellular signaling cascade in response to extracellular BR sensing, was isolated, which was the most important discovery. Numerous BRs with distinct chemical structures have been identified in various plant species since BL was first identified, indicating that BRs likely originated early in the history of plants. Finding A. thaliana mutants lacking BRs allowed scientists to confirm their role as plant hormones. (Praveena 2020), Teasterone (TE), 6-deoxocastasterone (6-deoxoCS), Typhasterol (TY), Brassinolide (BL), and 28-norcastasterone (28-norCS) are among the BRs that have been researched so far. These compounds are found in a wide variety of plant species and habitats. (Lee 2020).

An essential signal molecule controlling immunity and plant development, salicylic acid (SA) is a phytohormone. The isochorismate (IC) and phenylalanine ammonia-lyase (PAL) pathways are two separate methods in which plants make SA from chorismate. The IC route accounts for the vast bulk of SA production in Arabidopsis, exceeding 90%. (Fujikura 2020). SA signaling during plant immune responses has been well explored, and NPR1 was identified as a key component of this process. NPR1 is also important for SA-mediated growth regulation through the control of cell division and expansion. (Gallego 2011).

BRs, which are steroid hormones, have several functions in plant development and growth. Expansion, cell division, stem cell preservation, vascular development, elongation of diverse cell types, and floral transition are only a few of the many developmental and physiological activities regulated by BRs in plants. Additionally, they are involved in a wide variety of processes, including photomorphogenesis, pollination, development, senescence, hypocotyl elongation, root growth, shoot growth, stomata patterning, pollen tube growth, seed germination, and development. (Aldukhi 2019) BRs may stimulate excessive growth in plants cultivated in hydroponic environments by activating the cell cycle during seed germination, controlling the proliferation of leaf cells, and regulating the advancement of the cell cycle. Additionally, BRs control the formation of stomata and the responses to biotic and abiotic stress. In addition, BR is critical for controlling the ratio of male to female fertility in plants. A range of processes, including etiolation, stigma elongation, proton transport, plant architecture, thermo-tolerance, tiller number, leaf angle, and leaf size, are influenced by BR. Furthermore, crop yields might be enhanced with the external administration of BR or by modifying their production and signaling. (Sun Y.,2013).

Preliminary phytochemical assessment revealed the presence of phytocostituents like flavonoids, alkaloids, tannins and in the leaf extraction, root bark of Oroxylum indicum. (Jordan et al. 2006). The preliminary screening using TLC technique reflected the presence of four phytoconstituents such as baicalein, chrysin, Oroxylin-A in modern phytochemical analysis in terms of quantification of different bioactive phytoconstituents in the leaf extract, root bark of Oroxylum indicum was performed using reverse phase high performance liquid chromatography (RP-HPLC) fingerprint. To evaluate the quality of root bark, leaf extract of Oroxylum indicum, a simple rapid and accurate RP-HPLC method was developed for the assessment of three bioactive phytoconstituents viz. chrysin, baicalein, oroxylin-A. (Grampurohit N.D.,et al 1994). The components were quantified in different extracts viz. alcohol, petroleum ether, ethyl acetate and n-buatnol successively. Quantification of phytoconstituents was done by using RP-HPLC in petroleum ether and hydrolysed n-butanol fraction. Standard baicalein, chrysin , oroxylin –A, were employed for the development of the method. The RP-HPLC system used a base

deactivated C18 column with water, methanol, acetonitrile and orthophosphoric acid as the mobile phase and detection was performed at 262 nm. The method was precise with relative standard deviation for these constituents that ranged between 0.5-1.0%. The content of four phytoconstituents in the root bark, leaf extract of Oroxylum indicum was determined to establish the effectiveness of the method. (Mishra et al 2010).

II. MATERIAL AND METHODS:

Plant growth regulators:

A controlled environment system was used to examine the effects of three plant growth regulators on the germination of Oroxylum indicum seeds. The concentrations of GA3, BAP, and IAA were 50, 100, 150, and 200 um. A control treatment of distilled water was employed for 24 hours after twenty-four uniformly sized seedlings were soaked in three different doses of plant growth regulators. After that, the seeds were washed with distilled water as necessary to make sure they were wet enough to germinate. A temperature of 25 oC was used for incubation. Over the course of 30 days, we monitored the germination of every single seed.

Seed Germination Conditions:

Each germination treatment, including control, was performed with three each chamber at 25+_ 2 º C, with alternating light (14/10 h photoperiod). The seeds were kept moist and checked every day. Visible protrusion of the radical was the criterion to score seed germination. Final observation on the percentage germination, percentage relative germination, percentage dormancy, percentage relative dormancy and seedling morphology were taken after 30 days.

Collection of plant material:

Healthy leaves and root bark Oroxylum indicum were collected from a mother plant growing in Rangareddy dist of Telangana, washed thoroughly under running top water to remove the surface contaminants. After proper drying at room temperature transfer to the laboratory for further analysis.

Determination of phytoconstituents by RP-HPLC

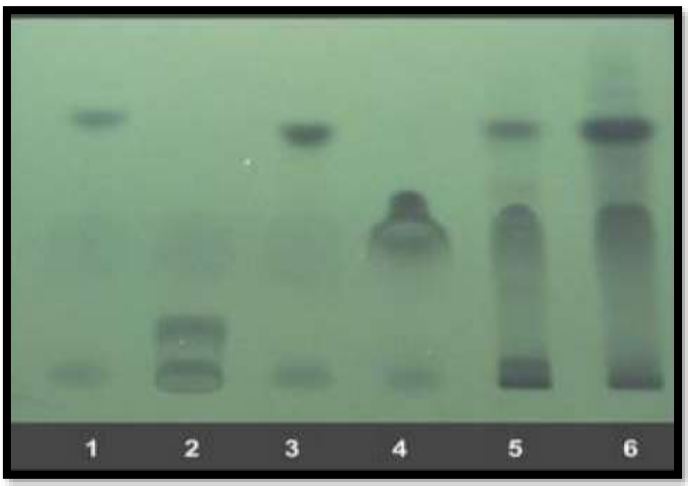
Shimadzu 2010C integrated High performance liquid chromatographic system was used for this experiment. Shimadzu 2010C system equipped with quaternary gradient pump, UV-VIS detector, Column Oven, programmable auto sampler controlled by CLASS-VP software. The quantification of phytoconstituents in petroleum ether and hydrolyzed n-butanol extracts was performed by HPLC method on a base deactivated RP-phase. Complete separation of the phytoconstituents was achieved on 250 X 4.6 mm i.d. Hypersil BDSRP-C18 5 µm column. The mobile phase consisted of Water: Methanol: Acetonitrile: Ortho phosphoric acid. Injection volume was10 µL used. The isocratic method was run for 35 min. The flow rate was 1ml/min at room temperature. The phytoconstituents were detected at 262 nm (UV-VIS detector) (Maitreyi 2008). Oven temperature was ambient. The quantification of baicalein, chrysin, oroxylin-A was estimated by using calibrated Shimadzu LC-2010 quaternary RP-HPLC system. (Lien C.,et al 2003). HPLC analysis of petroleum ether and hydrolyzed nbutanol fraction was carried out for developing finger printing and also to verify presence of chrysin, baicalein, oroxylin-A, in the root bark and leaf of Oroxylum indicum.

III. RESULTS AND DISCUSSION

On preliminary phytochemical screening, the root bark, leaf extract of Oroxylum indicum showed presence of alkaloids, flavonoids, tannins, and anthraquinones. (Subramaniyan S.S 1972). The TLC

study was aimed at checking the presence of similar kind of compound, if any, in active fractions but probably in different form. Baicalein, chrysin, oroxylin-A were reported to be present in stem bark and leaves of Oroxylum indicum. (Gokhale .M 2005).Our observations on TLC support the presence of baicalein, chrysin, biochanin-A in the root bark, leaf extract of Oroxylum indicum. Based on results of TLC study, we have suggested the presence of three bioactive phytoconstituents viz. chrysin, baicalein, Oroxylin-A, the root bark and leaf extract of Oroxylum indicum. (Polya GM 2003). To evaluate the quality of root bark of Oroxylum indicum, a simple rapid and accurate RP-HPLC.

a) Oroxylum indicum. L



b) HPLC-Chromatography with fractions

Fig :1- Showing a) Oroxylum indicum L. plant, b) RP-HPLC having Phytochemicals of Baicalein, Flavonopids (Oroxylin, Chrysin),fractions and standards (1: Biochanin A, 2:Ellagic acid, 3: Chrysin, 4: Baicalein, 5: Hydrolysed n butanol, 6: petroleum ether).

IV. CONCLUSION

Preliminary phytochemical screening indicated that the root bark of Oroxylum indicum was rich in flavonoids. The TLC observations identified the presence of four phytoconstituents such as chrysin, baicalein, ellagic acid and biochanin-A in both petroleum ether and hydrolysed n-butanol fractions. The newly established RP-HPLC method described the quantification of chrysin, baicalein, ellagic acid

biochanin-A in petroleum ether and n-butanol fraction after hydrolysis of the respective glycosides from the root bark of Oroxylum indicum. The advantages
lie in the simplicity of sample preparation. The proposed RP-HPLC conditions ensure sufficient resolution and the use of reference standard guarantees the precise quantification of the phytoconstituents. On the Chromatography of RP-HPLC having Phytochemicals of Baicalein, Flavonopids Oroxylin, Chrysin ,fractions and standards 1.Biochanin A, 2.Ellagic acid, 3.Chrysin, 4.Baicalein, 5. Hydrolysed n butanol, 6. petroleum ether. Results analysis of experiments indicative of satisfactory precision and reproducibility. Therefore, this method is accurate, precise, rapid, simple, and selective for quantitative monitoring of chrysin, baicalein, ellagic acid and biochanin-A in the root bark, leaf extracts of Oroxylum indicum.

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