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Review on *Rawlofia serpentina*-An Endangered Plant Species

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Abstract

Rauwolfia serpentina has long being used in India for the treatment of snakebites, mental illness, hypertension, blood pressure, etc. The root extract of this plant is also used to hasten the expulsion of the fetus, to treat painful affection of bowels, diarrhoea, dysentery, cholera and colic. *In vitro* propagation studies of different plant species have shown that the technique may be a solution for rapid propagation of such selected useful plant species and subsequent exploitation. It also has been found that explant of an alkaloid producing plant, cultured ***in vitro*** retain the capacity to synthesis alkaloids to that in the intact plant.

Keywords *Rauwolfia serpentine, In vitro* propagation,Antimicrobial activity

INTRODUCTION

Rauwolfia serpentina L. Benth is an important medicinal plant (woody perennial shurb) belonging to Apocynaceae family. The plant is indigenous to the Indian subcontinent and south East Asian countries, commonly known by different names; Sarpagandha, Snake root plant, Chotachand, Chandrika, etc. *R. serpentina* contains some 50 indole alkaloids and most of the total alkaloid content present mainly in root bark. Among all the alkaloids reserpine, yohimbine, serpentine, deserpidine, ajmalicine, ajmaline, etc. are used to treat hypertension and breast cancer. In this review different aspects of this plant in the areas of tissue culture, phytochemistry, pharmacocology and genetic studyhas been reviewed.

Medicinal Properties of *Rauwolfia serpentina*:

Rauwolfia serpentina contains a variety of compounds with antioxidant capacity and other health benefits like treatment of diabetes, cardiovascular disease, cancer and hypertension. The antibacterial and antifungal activities were high in petroleum ether and acetone extract of *Rauwolfia serpentina* [1]. *Rauwolfia serpentina* has long being used in India for the treatment of snakebites, hypertension, high blood pressure and mental illness. Different ethnic groups use this plant to treat snake, insect and animal bite, mental illness, schizophrenia, hypertension, blood pressure, gastrointestinal diseases, circulatory disorders, pneumonia, fever, malaria, asthma, skin disease, scabies, eye diseases, spleen diseases, AIDS, rheumatism, body pain, veterinary diseases etc. this plant is also being used to prepare fermented food products[2]. It has been stated that this plant is used as antidote against snakebite [3]. Plant derived indole inhibitors were identified from the extracts of Raulofia serpentine which has the capacity to acts as aldose reductase, a potent drug for diabetes [4]. The methanol root extract of this plant provides the high antioxidant compound phenol of 233mg/gm. So this plant has significant antidiabetic and hypolipidemic activity [5].

Antimicrobial Activity of *Rauwolfia serprntina*:

The aqueous and methanol extracts of *Rauwolfia vomitoria* show antimicrobial activity against *Klebsiella, Pneumonia, Staphylococcus aureus, Enterobacter, Pseudomonas aeruginosa* and Escherichia coli. Propionibacterium acnes and Staphylococcus epidermidis have been recognized as pus-forming bacteria triggering an inflammation in acne. In a study antimicrobial activities of some Indian medicinal plants were against these bacteria were evaluated. Among others the ethanolic extracts of *Rauwolfia serpentina* roots had greatest antimicrobial effect and produced strong inhibition zone against Propionibacterium acnes. Phytochemical screening of Rauwolfia serpentine revealed the presence of alkaloid could be responsible for activity [6]. Antibacterial activity of *Rauwolfia serpentina* plant extract against Escherichia coli, Staphylococcus aureus, Salmonella typhi and Klebsiella pneumonia by disc diffusion method showed the alcoholic extract was found effective against Staphylococcus aureus only. The antibacterial activity is due to presence of alkaloids which confirmed by gas liquid chromatography and positive alkaloid test [7].

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Chemical contents of *Rauwolfia serpentina*:

Major alkaloids *Rauwolfia serpentina* were Reserpine, Rauwolfine, Serpentine, Sarpagine, Ajmaline, Yohimbine and Ajmalicine. Insulin binds to insulin receptors that is present on different cells of the body and mediates the absorption of glucose in to the cells. Docking studies of insulin receptor with alkaloids of *Rauwolfia serpentina* revealed that few of the alkaloid present in *Rauwolfia serpentina* may be potential activators of Insulin receptor [8]. Reserpine content were analysed by HPLC using methanol extracts and was reported that the Reserpine content varies with different geographical location [9]. Reserpine is an indole alkaloid and is detected, monitored and quantified by HPTLC. The average recovery was found to be 98.78% [10]. Reserpine content was measured in the different parts of *Rauwolfia serpentina* plant including leaf, stem, flower and root. 90% of total reserpine content was produced from root, whereas stem and root contains 10% [11]. From cell suspension culture the yield of the main alkaloid vomilenine was 51 times more than that of differentiated plants. Crude enzymes isolated from cell suspension culture completely metabolize the biogenetic precursor strictosidine under formation of several alkaloidal compounds [12]. Comparision of alkaloid extraction in normal Rauwolfia root bark and cell suspension reveals both systems produced different alkaloids and that of amount of total alkaloid is lower in hairy root than in the cell suspension [13]. Seven new indole alkaloids were isolated from dried roots of *Rauwolfia serpentina*, namely methylajmaline, methylisoajmaline, 3-hydroxysarpagine, yohimbinic acid,

yohimbinic acid, isorauhimbinic acid and 7-epiloganin and glomeratose A. The structures of these compounds were determined by spectroscopic and chemical means [14]. It has been reported that β-Glucosidase enzyme was extracted from different parts of *Rauwolfia serpentina* and purified using ammonium sulphate fractional precipitation and Sephadex G-25 column chromatography. It has been found that mature leaf has the highest activity of crude enzyme in *Rauwolfia serpentine* [15]. Raucaffricine-O-beta-D-glucosidase was extracted from cell suspension cultures of *Rauwolfia* by five step procedure using anion exchange chromatography,chromatography on hydroxylapatite, gel filtration and FPLC- chromatography on mono Q and mono P. proteolysis of the pure enzyme with endoproteinase revealed six peptide fragments with 6-24 aminoacids. Two largest fragments showed sequences, of which the motif Val-Thr-Glu-Asn-Gly is typically for beta-glucosidases. Sequence alignment of these fragments demonstrated high homologies to linamarase from Manihot esculenta (81 % identity) and to beta-glucosidase from Prunus avium (79 % identity) [16]. Solvents play vital role in extraction process. In a study aqueous solutions of sodium cumene sulfonate gave quantitative and faster extraction of *Rauwolfia vomitoria* as compared to the extraction using methanol. The extraction rate is influenced by intraparticle diffusion and increases with increasing temperature and hydrotrope concentration [17]. Alkaloid profiling of *Rauwolfia serpentina* root, leaves and callus were performed which were collected from different localities. The crude alkaloid fraction (CAF) suggests that roots are rich in alkaloid content as compared to leaves and callus. The TLC analysis showed that roots are rich in reserpine [18].

Tissue Culture Studies:

Various parts of the *Rauwolfia serpentina* plant were used as explant and inoculated in Murashige and skoog medium containing 2, 4-D and 6-BAP. Maximum callus inductions of 93.65% were obtained in leaf and stem explants. The callus was inoculated in shooting medium containing BAP and NAA and maximum shoots were obtained. The rooting was induced in invitro regenerated shoots in MS medium containing IBA and NAA and 100% rooting was obtained [19]. Callus was induced from root explants of *Rauwolfia serpentina* using 2mg/l BAP and 0.8mg/l NAA and later induced to bud formation which further developed into shoots. The plantlet formed were transferred to soil which initially watered with half strength Knop's solution till they became autotrophic and were noticed to grow well in open field condition [20]. High frequency of 96.43% callus was induced when nodal segments from invitro raised shoots were cultured on MS medium supplemented with 0.5mg/l BA and 2.0mg/l NAA [21]. Varying concentration of BAP and combination of BAP with IBA produced multiple shoots.

Maximum multiple shoots (85.6%) were obtained when MS medium supplemented with 5.0mg/l BAP and 0.5mg/l IBA along with 2.5% sucrose and 0.85% agar. The developed shoots were excised and implanted on MS medium with varying concentration of IBA. Maximum rooting of 76.6% was obtained and 74% of regenerated plantlets survived in open field condition [22]. Low concentration of IBA, NAA and 2,4-D stimulated root formation and propagule development from stem cuttings.2,4-D at 5 ppm had the highest impact on root formation and propagule development (100%), followed by IBA (83% at 50 ppm) and NAA (66% at 10 ppm). Double or triple doses of NPK, especially increased N nutrition releaved better growth activities. Crude alkaloid contents of the roots increased significantly only under increased N level [23]. Switching from surface to submerged cultivation in liquid medium polymorphism of the RAPD spectra is discovered following 4-6 growth passages of tissues. This polymorphism may reflect both changes in the DNA sequence and in genetic structure of the cell population [24]. It has been reported that callus was induced from leaf, stem explants, whereas direct regeneration observed when apical and nodal explants were used. Combination of IBA (0.125 mg/l) + BAP (1.0 mg/l) produced better results for both callus induction and direct regeneration [24].

A study reveals that callus of *Rauwolfia serpentina* consists of cell colonies with different fluorescence (yellow-green and blue-white) under 365nm UV- light. HPLC analysis showed that the yellow-green fluorescent strains produced more reserpine, whereas the blue-white strains produced more 3, 4, 5-trimethoxy benzoic acid. Combination of 10μM NAA and 10μM BA enhanced the production of reserpine in the yellow-green fluorescent cell strains [25]. During tissue culture stress induced by alteration of medium composition (hormones) for several generations' results in changes the in level of production of alkaloids. Such changes were noted in a study that *Rauwolfia serpentina* cells that have been cultured and maintained on modified Linsmaier-skoog medium for over 13 years produced more ajmaline(0.005-0.012g dw) than reserpine (0-0.003g dw) [26]. Somatic Hybrid cell suspension culture by combining *Rauwolfia serpentina* and Rhazya stricta produced a number of monoterpenoid indole alkaloids compared with the parental cultures. The alkaloids were identified as tubotaiwine, an isomeric mixture of vallesiachotamine, stemmadenine and vomilenine and were all produced in small amount <2mg/l cell suspension [27]. Growth hormones play vital role in somatic embryogenesis. In a study leaf explants were transferred to MS medium containing different combinations of PGRs. Among the various combinations of BAP (1.0 – 3.0 mg/L) and IAA (0.1 – 0.5 mg/L) the intensity of callus induction was highest in 2.5 mg/L BAP + 2.0 mg/L IAA and 1.0 mg/L BAP + 0.5 mg/L IAA. The frequency of callus induction was highest 77.77% in 1.0 mg/L BAP + 0.5 mg/L IAA. Among the various combinations of BAP and IAA shoot regeneration was highest 75% in 2.5 mg/L BAP + 0.4 mg/L IAA.The shoot was transferred to MS medium for root regeneration containing BAP (2.5 mg/L) + IAA (0.3 – 0.5 mg/L) + NAA (0.3 – 0.5 mg/L). The frequency of root regeneration was 100% in MS medium containing BAP (2.5 mg/L) + IAA (0.5 mg/L) + NAA (0.5 mg/L). After rooting on shoots the plantlets were shifted to sterile soil field pots and the survival percentage of plants after hardening was 67% [28].

In another study young leaves of *Rauwolfia serpentina* inoculated on MS medium supplemented with NAA induced callus under 24 hour light while 2, 4-D induced callus under 16 hour light [29]. Direct root induction from leaf explants of *Rauwolfia serpentina* was performed by combinations of two auxins namely PABA + NAA and IBA +NAA promoted better root growth compared to single auxin treatment. Liquid MS media gave slower growth, reduced number of roots, shorter root length as well as absence of reserpine, using the same combination of growth regulators, compared to solid MS media. The culture incubated under dark conditions

conditions produced thin roots [30]. In a study high frequency callusing was induced in leaf and stem explants of *Rauwolfia serpentina* when inoculated on modified Murashige and Skoog (MS) medium supplemented with 2.5mg/l 2, 4-D. maximum regeneration of shoots from callus (90%) was observed in MS medium supplemented with 0.2mg/l NAA and 1.5mg/l BA. Direct regeneration (96%) was recorded best in MS medium supplemented with BAP 2.5mg/l. higher induction of root (100%) was observed in MS medium supplemented with NAA 0.5mg/l [31].

Genetic Studies:

The most important characteristic feature of *Rauwolfia serpentina* was tetrasporangiate anther, dicotyledonous type of anther wall formation, occurrence of both successive and simultaneous cytokinesis during the meiosis in microspore mother cells, uninucleate and highly vacuolated glandular tapetum,tetrahedral, tetragonal and decussate pollen tetrad and three- celled mature pollen grains during shedding time [32]. Analysis of 18S, 25S and 5S rRNA genes in intact plants and cultured tissues of *Rauwolfia* species shows the presence of RFLP in intact plants and long term *Rauwolfia serpentina* tissue cultures. In addition changes in amount of 18S-25S rRNA gene were observed in long term *Rauwolfia serpentina* tissue cultures [33]. Major alkaloids *Rauwolfia serpentina* were Reserpine, Rauwolfine, Serpentine, Sarpagine, Ajmaline, Yohimbine and Ajmalicine. Insulin binds to insulin receptors that is present on different cells of the body and mediates the absorption of glucose in to the cells. Docking studies of insulin receptor with alkaloids of *Rauwolfia serpentina* revealed that few of the alkaloid present in *Rauwolfia serpentina* may be potential activators of Insulin receptor [34]. Reserpine and rescinnamine in *Rauwolfia serpentina* powders and tablets are detected by Liquid chromatographic (LC) method which uses fluorescence detection. Methanol is used as mobile phase to which a small volume of aqueous solution of pentasulphonic acid sodium salt is added to achieve desired elution characteristics. Reserpine is determined at an excitation wavelength of 280nm and an emission wavelength of 360nm, because rescinnamine is completely non-fluorescent at these wavelengths. Recinnamine is determined at an excitation wavelength of 330nm and an emission wavelength of 435nm, because reserpine is completely non-fluorescent at this wavelength [35].

Enzymes play important role in biosynthetic pathway. The enzyme strictosidine synthase (STR1) from the Indian medicinal plant *Rauwolfia serpentina* is of primary importance for the biosynthetic pathway of the indole alkaloid ajmaline [36]. The effect of *Rauwolfia vomitoria* root bark extract and its interaction with vitamin E on the liver function enzymes , such as alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (γ-GT) and the histological architechtures of the liver tissues of normal animals. The result showed significant increase in the activity of AST by the extract [37].

Conclusion

Conserving of the endangerd plant species is challenge to the researcher to protect them. They conserved by using invitro method of micropropagation. *Rauwolfia serpentina* is one such plant we can conserve them by invitro propagation.

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