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Research Article

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Evaluation of Glycoalkaloid and Phyto-Chemicals Present In Grafted and Non Grafted Eggplant Genotypes

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ABSTRACT

Solasodine a glyco-alkaloid and nitrogen containing steroidal drug (aglycone), obtained from distinctive species of the genus Solanum (Solanaceae). The Completely Randomized Design (CRD), method used for statistical analysis. The spectrometric method was used to evaluate the phytoconstituents of crude extract of dried fruits. The phytochemical content recorded highest in grafted plants as compared to non grafted eggplant genotypes. The results of grafted plants and non grafted eggplant varieties were recorded for solasodine content, total phenols and poly phenol oxidase (PPO) content had significant to each other. The solasodine content observed highest (1.66 mg/100gm) in grafted plants of Solanum xanthocarpum × Pusa Shyamala, followed by Solanum xanthocarpum × pusa hybrid-6 and the lowest 0.92 mg/100gm in non grafted eggplant genotypes. The both polyphenols and polyphenol oxidase (PPO) content were recovered maximum in grafted plants and among grafted plants Solanum xanthocarpum produced 59.69mg/100gm and 3.9OD min-1.mg-1. Based on mean performance Solanum xanthocarpum and Solanum khasianum found superior for phytochemical content and can be used as alternative for modern medicine.

Key words: *Phytochemicals, Glycoalkaloid, Grafting, Solanum sps., Crude extract.*

INTRODUCTION

Brinjal (*Solanum melongena* L.) belongs to the relatives of Solanaceae. Eggplant is native to few areas of India and Myanmar[23]. India is the world's 2nd largest producer of brinjal after China. In India, it occupies 7.22 lack hectares with the one year production of 13 lack tonnes, and common productiveness is 13.6 t/ ha[9]. It occupies nearly 26% of the worldwide production. West Bengal is the leading producer of brinjal in India and it contributes 22% of overall country production[9].

Vegetables have great potential of phytochemicals and antioxidant capacity due to fruit phenols and flavonoid constituents[19], which have been linked to various health benefits[8].

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Plant species having medicinal properties are distinctly utilized in conventional ayurvedic system on the grounds from few decades. Phytochemical survey has proven their presence in lots of monocotyledon families in particular Dioscoreaceae, Amaryllidaceae, and Liliaceae and in Dicotyledons families consist of Leguminosae and Solanaceae. Steriodal saponins are of top significance and attributable to their courting with such compounds as sex hormones and cortisones. Saponins are two types i.e. nitrogen containing and non nitrogen containing glcoalkaloids. The nitrogen containing steroidal saponins are source of glycoalkaloid content. Those are of particular in drug industry, as they are used as beginning fabric for the synthesis of 16-DPA a singular precursor for anti-fertility and anti-inflammatory steroidal drug[14].

The genus *Solanum* contains near about 1400 species across the exceptional climatic conditions from temperate to tropical areas of the world can be used in ayurvedic system to update the cutting-edge drugs[2]. The belongings of anti septic, anti-inflammatory activity, anti dysenteric and anti diuretic activity are determined generally in herb of *Solanum* species and used in the treatment of cardiac and infection of kidney[16]. The whole plant is effective as antibacterial pargative, and is powerful towards ulcers on the neck, Thermal heat of frame, burning of throat, infection of liver and chronic fever[22]. Solasodine (Fig.1) has been said as a treasured precursor of steroidal tablets and used as supplementary supply[3]. The total glycoalkaloid content above 20mg /100g fresh weight is considered unsuitable for human consumption because of toxicity. The genotype and environment are among the most important factors, influencing level of glycolalkaloids in *Solanum* species. The Solasodine has biological impact of antifungal, insect boom regulation and enzyme inhibition, and it cures skin lesions[10]. Solasodine acts on lady genital tract mechanism which include in gestation and pregnant, in male effect on testacles by reducing amount of sialic acid and epididymides and shrunken ley dig mobile nuclei[24]. It suggests cytotoxic activities in in-vitro against most of cancer cell lines, antiproliferative activities against human colon (HT29), liver (HepG2) and cancer cells[12, 13&18].

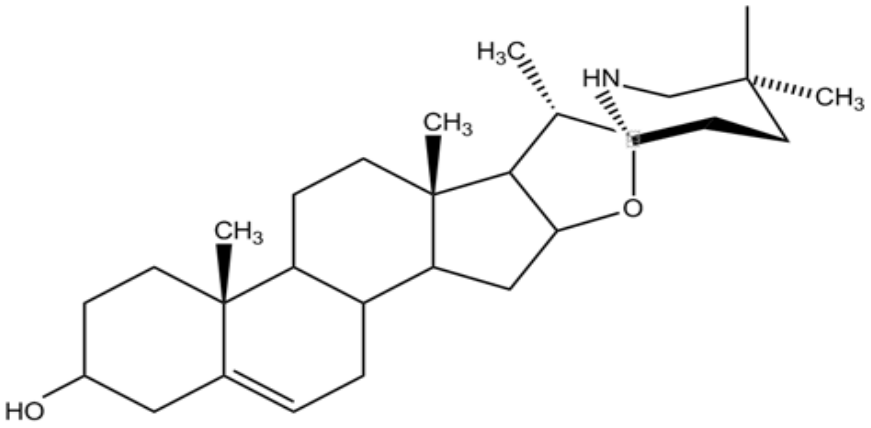

Fig. 1: Chemical structure of solasodine[24]

The total phenol content in eggplant may be responsible for health benefits as they can scavenge free radicals and inactivate other pro oxidants. Phenolic content of within most favourable reported for *S. melongena* is optimum for inducing edible quality of fruit. Polyphenol Oxidases are extensively spread and well-known enzymes and their impact on browning in damaged and fresh cut fruits and vegetables have been known for decades.

Polyphenol oxidases (PPO's) are copper containing enzymes and cataluses the hydroxylation and oxidation of diphenols and phenols to produce quinines which in turn develops black, brown or red pigments (polyphenols) that cause food browning[4].

Considering overview of facts; present investigation was conducted to elucidate the presence of solasodine from various grafted plants beside the total phenol content and one antioxidant enzyme (PPO's) to consider differences among grafted and non grafted eggplant genotypes studied.

MATERIALS AND METHODS

The study was conducted at vegetable research farm, College of Horticulture & Forestry, (CAU), Pasighat, Arunachal Pradesh (India). The experimental materials consists for the present study were comprised of four wild *Solanum* species namely *Solanum torvum*, *Solanum xanthocarpum*, *Solanum khasianum*, and *Solanum surathense* raised on plastic pots filled with homogeneous cultivable soil kept in low cost poly house and two eggplant genotypes i.e. Pusa Shyamala and Pusa Hybrid-6 were grown in nursery beds as a scion material for grafting. The non-grafted plants were used as control. Before sowing, the seeds of both cultivated varieties and wild species were treated with GA_3 solution (100ppm) for 24 hrs at room temperature for quick germination. Forty to fifty days old rootstock seedlings (4-5 leaf stage) and seedlings of eggplant varieties at 3-4 leaf stage were grafted. All plants were grafted by cleft method of grafting[11]. A Completely Randomised Design (CRD) was adopted with four replications consisting of 3 plants. The grafted plants recorded for the glycoalkaloid, Total phenol content and poly phenol oxidase activity. The principle spectrophotometric method was adopted to estimate of solasodine content of dried fruits of *Solanum* species through calorimetric method.

The aglycone, solanidine, is an important precursor for hormone synthesis, the method for extraction hydrolysis and isolation of solanidine in one step process[15]. In this system, hydrolysis was performed in 10% (w/v) hydrochloric acid in 50% methanol, after which solanidine was transferred to a phase of chloroform. Under these conditions, more than 98% of the glycoalkaloids were transformed to solanidine after 90 minutes of extraction.

Extraction and Estimation: The fresh unripe green yellow or red unripe fruits were collected and shade dried at room temperature. The dried berries were reduced to a coarse powder using a grinder and passed through sieve no. 40 for isolation purpose. Sap portion next to seeds were retained and dried at room temperature and then reduced to coarse powder. Dry powder (25 mg) was refluxed in a sealed test tube with 4 ml of 1N HCl for 2 hours in a water bath at 100^0C. The hydrolysates thus obtained were basified by adding 0.5ml of 60 percent (w/v) sodium hydroxide. The mixture was shaken well and then centrifuged at about 1000g for 5 minutes. To the supernatant 5ml of chloroform was added. The hydrolyzed derivatives were extracted into chloroform by vigorous shaking. The lower chloroform layer was taken and made upto 5 ml. To this 2.5 ml of 2×10^{-4} M bromothymol blue (BT) in borate buffer (P^H 8.0) was added and shaken for 10 seconds. The straw coloured lower layer was drawn out. To this 1ml of 0.2 M sodium hydroxide in methanol was added to develop blue colour, owing to formation of aglycone-BT blue complex.

Experimental standardization: A primary standard of 1mg/ml solasodine prepared in chloroform was used for preparing working standards of concentrations, 20, 40, 60, 80, 100 µg by pipette out suitable volumes and the final volume made up to 4 ml. The remaining procedure was the same as described above. The absorbance of each concentration was noted at 610nm and plotted against the quantity of solasodine to construct the calibration curve.

Calculation of solasodine content: The value of absorbance was substituted as 'x' in the

regression equation and the quantity of solasodine in plant sample was worked out. The data obtained were analyzed by using the analysis of variance for Completely Randomised Design (CRD) [17].

The total phenolic content (TPC) was determined using Folin–Ciocalteu reagent and expressed as gallic acid equivalents (GAE) [20]. The extracts were diluted with a 40:60 (v/v) methanol: water solution, to a suitable concentration for analysis and 0.5 ml of commercial Folin–Ciocalteu reagent was added. The mixture was mixed well and kept for 5 min at room temperature before adding 1 ml of 20% sodium carbonate in water. After incubation at room temperature for 90 min, the absorbance at 760 nm was measured against reagent blank (Beckman DU640 UV–vis Spectrometer), and the result was expressed as gallic acid equivalents (mg 100 g−1 of eggplant). Appropriate amount of fruit pulp tissue were ground in 5 ml of 100mM sodium phosphate buffer, pH 6.5 the homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used for enzyme assay. The reaction mixture contained 2.9 ml of catechol (10mM catechol in 10 mM phosphate buffer pH 6.5) and reaction was initiated by the adding enzyme extract. The changes in colour due to the oxidized catechol were read at 490 nm for one minute at an interval of 15 second. Blank was carried out without substrate.

RESULTS AND DISCUSSION

Present study focused on isolation of glycoalkaloid from eggplant with wide combinations of grafted and non grafted plants of eggplant and correlating this property with eggplant total phenol content and antioxidant enzyme.

The results of grafted plants were compared with non-grafted eggplant genotypes. The results of solasodine content were significant to and highest solasodine content of 1.66 mg/100gm in grafted plants of *Solanum xanthocarpum* × Pusa Shyamala followed by 1.65 mg/100g recorded in *Solanum xanthocarpum* grafted with Pusa Shyamala and the lowest 0.92 mg/g obtained in non grafted eggplant genotypes as shown in the table 1. The Similar results also obtained for free radical scavenging property of *Solanum xanthocarpum*[5]. The grafted plants with wild *Solanum* species had maximum recovery of glyco alkaloid while adsorption efficiency of rootstock is more than cultivated eggplant varieties and leads the maximum recovery of solasodine from grafted plants; it is in line with the previous results[6].

The total phenolic content (TPC) was lowest in non grafted plants (53.99 mg /100 g) as shown in table.1. In grafted plants *Solanum xanthocarpum* with Pusa Shyamala had the highest TPC (59.37 mg /100 g) and confirms that rootstocks had little effect on fruit phenolic content[7]. These considerable differences among the varietal types confirm important intra specific variation in fruit composition.

The PPO (polyphenol oxidases) activity significantly varies with fruits of wide grafted and non grafted plants. The result of PPO's varied from 1.39 to 1.22 OD. /min.gm as shown in table.1. The highest value recorded in grafted plants of *solanum xanthocarpum* and lowest value observed in plants of non grafted eggplant genotypes. The PPO (polyphenol oxidase) is an enzyme responsible for browning reaction and it is important concern of reducing fruit quality in brinjal[1]. The higher yield of PPO in our study may be attributed with type of rootstock plant. The optimum pH and temperature for higher PPO in tomato juice is 4.8 and 30°C respectively[21]. The results of PPO activity depend on plant source, extraction methods, purity of enzyme, buffer, and substrate. Spectrophotometric as well as statistical analysis of solasodine, PPO and total phenol content production at nine different plant types also confirms that they plays its part as phytoconstituents in grafted plants while less content in nongrafted plants.

Table 1: Comparative analysis of phytochemicals present in grafted and non grafted plants

Treatment combinations	Solasodine Content (mg/100gm)	Total Phenol content (mg/100gm)	PPO (OD. min-1. g-1)
Solanum torvum × Pusa Shyamala (T1)	1.38	55.83	1.31
Solanum torvum × Pusa Hybrid-6 (T2)	1.36	56.01	1.28
Solanum xanthocarpum × Pusa Shyamala (T3)	1.66	59.69	1.36
Solanum xanthocarpum × Pusa Hybrid-6 (T4)	1.65	58.93	1.39
Solanum khasianum × Pusa Shyamala (T5)	1.55	56.28	1.33
Solanum khasianum × Pusa Hybrid-6 (T6)	1.54	54.78	1.35
Solanum surathense × Pusa Shyamala (T7)	1.41	57.06	1.26
Solanum surathense × Pusa Hybrid-6 (T8)	1.39	55.58	1.27
Control (T9)	0.92	53.99	1.22
C.D. (at 5%)	0.065	2.084	0.019
SE(m)	0.022	0.71	0.007
SE(d)	0.031	1.004	0.009
C.V.	3.075	2.514	0.995

CONCLUSION

Amongst all *Solanum xanthocarpum* and *Solanum khasianum* are the primary sources for solasodine. The evolved method for assessment of glycoalkaloid content material is applicable to all solasodine bearing Solanaceae plants. The eggplant from different species had antioxidant and anticancer properties. A more detailed study on the bioactive compound in these plant extracts that contribute to these biological activities as well as their possible mechanism of action are therefore suggested. Consequently, *Solanum* species have been suggested as secondary metabolites in drug and ayurvedic industry. Keeping in mind the compound's anticancer activity of solasodine, we can further design nanoparticle formulation to target its effect on cancerous cells inherited with other advantages of nanoformulations.

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