DEVELOPMENT OF HIGH VALUE PHYTOCHEMICALS FROM GREEN TOBACCO

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Abstract
In Indian agriculture, tobacco has a prominent place. Tobacco could be developed as an important food crop in combination with its traditional use for smoking and chewing. Use of tobacco for either smoking or chewing purpose is viewed seriously due to alleged health factors. The chemistry of tobacco is unique with the presence of a wide spectrum of chemical compounds of which nicotine, solanesol, malic and citric acid were identified as potential chemicals which could be recovered and converted to value-added products. This situation necessitated to examine the green tobacco crop as a source for recovery of phytochemicals alone. With the objective of maximizing bio-mass production for optimum recovery of proteins, nicotine, solanesol and organic acids from green tobacco, a field experiment was conducted by manipulating agronomic practices. Flue-cured tobacco variety was planted to have a high density population. Green leaf was harvested in six pauses for processing. An integrated approach was developed for extraction of above phytochemicals from green leaf and recovery potential was estimated. The green lamina was macerated and filtered through a cheese cloth, the green liquid was heat coagulated at 80°C to precipitate the crude protein along with chloroplast protein. Remaining after protein separation, brown liquid containing nicotine, was passed through a strongly cationic-exchange resin. The wet green mass left over after filtration is subjected to hexane extraction for the recovery of solanesol. Processing of chloroplast fraction for recovery of solanesol will be standardized. The column effluent after nicotine recovery is treated with lime to precipitate organic acids. Results indicated that tobacco cultivation for phytochemicals is a prospective proposition. The need to evolve proper marketing systems for the phytochemicals or their value-added products is also discussed.
Key words : Green tobacco, Phytochemicals, Solanesol, Coenzyme Q10.

Introduction

Tobacco is an important crop cultivated in India and tobacco plays a vital role in economy by contributing about Rs.10,000 crores annually from excise revenue and to the foreign exchange to a tune of Rs.2,000 crores. Different types of tobaccos are grown commercially in India. In the present scenario of world wide decline in the conventional uses of tobacco, exploitation of the crop for extraction of phytochemicals is a viable alternative and the presence of substantial amounts of useful phytochemicals like proteins, nicotine, solanesol & organic acids have enhanced the scope for alternative uses of tobacco. Tobacco green leaf as a source of protein, has attracted the attention world over **[1, 2, 3].** Green tobacco as potential sources for extraction of photochemicals like, nicotine, solanesol & organic acids **[4].** The proteins from tobacco are highly unusual in the plant kingdom in that they complete and well balanced in their amino acid content **[5].** Tobacco protein is potentially the most valuable component, as it can be crystallized in to a pure powder which has no odor or taste **[6].** Tobacco protein consists of two major components – Fraction –1(10 %) and Fraction – 2 (90%). Fraction – 1 protein is the most valuable for medical & pharmaceutical applications, Fraction – 2 protein is animal feed. A major proposed use for tobacco protein is for kidney dialysis patients who have severely restricted diets **[7, 8].** Nicotine is the source for the botanical pesticide, nicotine sulphate. Nicotine sulphate is eco-friendly and is easily bio-degradable and as such leaves no hazardous residues on vegetables and fruits bound to market and nicotine could be the starting material for synthesis of nicotinic acid, nicotinamide, nikethamide – used in pharmaceutical industry **[9].** An integrated approach for isolation of nicotine & solanesol from tobacco waste has been proposed **[10].** Solanesol is a naturally occurring trisesquiterpene alcohol present in tobacco (*Nicotiana tabacum* L) **[11]** and tobacco is the richest source of this chemical. The presence of a long chain of repeating isoprene units (nine) in the solanesol molecule **[12]** makes it a valuable source material for synthesizing metabolically active quinines and other drugs **[13].** Solanesol has excellent prospectus in the future as drug intermediate, particularly being the starting for the production of coenzyme Q_{10} (Co Q_{10}) **[14,15].** In this backdrop, a study was conducted to examine the phytochemicals content in green tobacco.

Materials and Methods

During the season 2008-09, field experiment was conducted in an area of 1900 Sq.m in the M/s. Bio-Pharma Laboratories Experimental farm, variety Gauthami was planted at a spacing of 70 x 35 cm to give a plant population of 40,816 plants / ha. As the population is high a higher dose of nitrogen is 150 Kg/ha was given to the crop. 100Kg N was given at the time of planting and the remaining 50Kg N was given on the 30^{th} day after planting. The crop was once irrigated on the 30^{th} day after planting up to the field capacity. As the leaves attaining maximum size, six harvests were taken and the steam leaf yield from all the harvests was recorded as 22,450 Kg/ha.

From the each harvest 5kg of green leaf lamina was processed for the recovery of phytochemicals viz. proteins, nicotine, solanesol, and organic acids. Some part of the green leaf is air cured and the balance was flue-cured. Green leaf samples from each harvest were quickly dried under IR radiation to estimate nicotine, solanesol. The range of these phytochemicals were observed to be nicotine 1.27 to 3.25%, solanesol 0.25 to 2.14% on the dry basis and protein 0.88 % to 3.08 % (Table - 1).

An integrated scheme was adopted for the recovery of phytochemicals from the tobacco green leaf (Fig. 1, 2). The green lamina (about 5kg of from each harvest) was macerated and filtered through a cheese cloth. The green liquid was heat coagulated at 80^0C to precipitate the crude protein along with chloroplast protein.

This is filtered and the crude protein is thoroughly washed with isopropyl alcohol (IPA) and then with acetone and its weight was recorded (Table - 1). The crystalline Fraction - 1 contributes more than 30% of the total protein in tobacco leaves. It is soluble in water, is tasteless and odorless, and is composed entirely of amino acids of high nutritional quality. The water soluble proteins have unique nutritional and functional properties that could make them valuable for the package food industry and for medical use. When washed free of sodium and potassium, the crystals might be used in patients with kidney failure to meet their amino acid requirement without taxing the kidney's inability to eliminate cations. Brown liquid containing nicotine remaining after protein separation was passed through a strongly cationic exchange resin (Seralite-SRC-120 Std.grade) at a flow rate of 30ml/min. After passing the extract the resin was thoroughly washed wih DM water. The nicotine captured on the resin column was eluted with a mixture of 25% Aq.ammonia solution and alcohol (1:9) at a flow rate of 15ml/min. The elute was concentrated by distillation and the recovered alcohol was reused by adding required quantity of ammonia. The concentrated nicotine solution was converted to nicotine sulphate (40%) by adding necessary quantity of sulphuric acid under cold condition **[16].** The column effluent after nicotine recovery is treated with lime to precipitate organic acids and calcium salts.

The wet green mass left over after filtration is subjected to hexane extraction for the recovery of solanesol. The wet green mass is dried up to 10% moisture content. Dried tobacco mass was placed in soxhlet thimble, solvent is hexane and refluxed for 4hrs. The contents were cooled and the hexane was removed on a rotary evaporator. The crude extract containing 20% of solanesol was purified by saponification followed by silica gel column chromatography. The crude was initially saponified by methanolic KOH to get 40% of solanesol which was further purified by silica gel column to get 90% of solanesol. As the chloroplast fraction contained significant amount of solanesol, this fraction is also processed to recover solanesol **[17].** Processing of chloroplast fraction for recovery of solanesol **[18]** will be standardized. (Table 2).

Results and Discussion:

The range of these phytochemicals were observed to be nicotine 1.27% to 3.25%, solanesol 0.26% to 2.14% on the dry basis and protein 0.91% to 3.08%. Green leaf was harvested in seven pauses for processing. An integrated approach was developed for extraction of above phytochemicals from green leaf and recovery potential was estimated. About 384.5 kg of crude protein, 41.2 kg of nicotine (97.8 kg of nicotine sulphate(40%)), 151.8 kg of crude solanesol, 18.9 kg of pure solanesol(90%)) (Table - 3) could be extraction from one hectare crop grown under the modified agronomic practices, with a green leaf yield of 22,450 kg/ha. Based on the preliminary investigations, growing tobacco for phytochemicals appears to be a feasible proportion. With the advent biotechnology, new vistas are opened for utilization of tobacco crop for production of bioengineering products like functional food proteins and highly desirable enzymes, flavors and pharmaceuticals. Thus, prospectus of growing tobacco for alternative uses seems to be encouraging. However, to exploit the immense economic potential, evolving proper marketing systems for all the phytochemicals or their value added products is imperative. There is also every need to take up research on finding new uses for these chemicals and undertake pilot-plant studies so that the bench-scale technologies can be perfected for effective transfer of technology.

Table 1: Grown tobacco: Levels of phytochemicals in green leaf

Harvest	Nicotine (%)	Solanesol (%)	Protein (%)
1	1.27	0.26	0.91
2	1.39	0.33	1.65
3	1.91	0.88	2.23
4	2.51	0.96	2.27
5	2.94	1.71	3.08
6	3.25	2.14	1.64

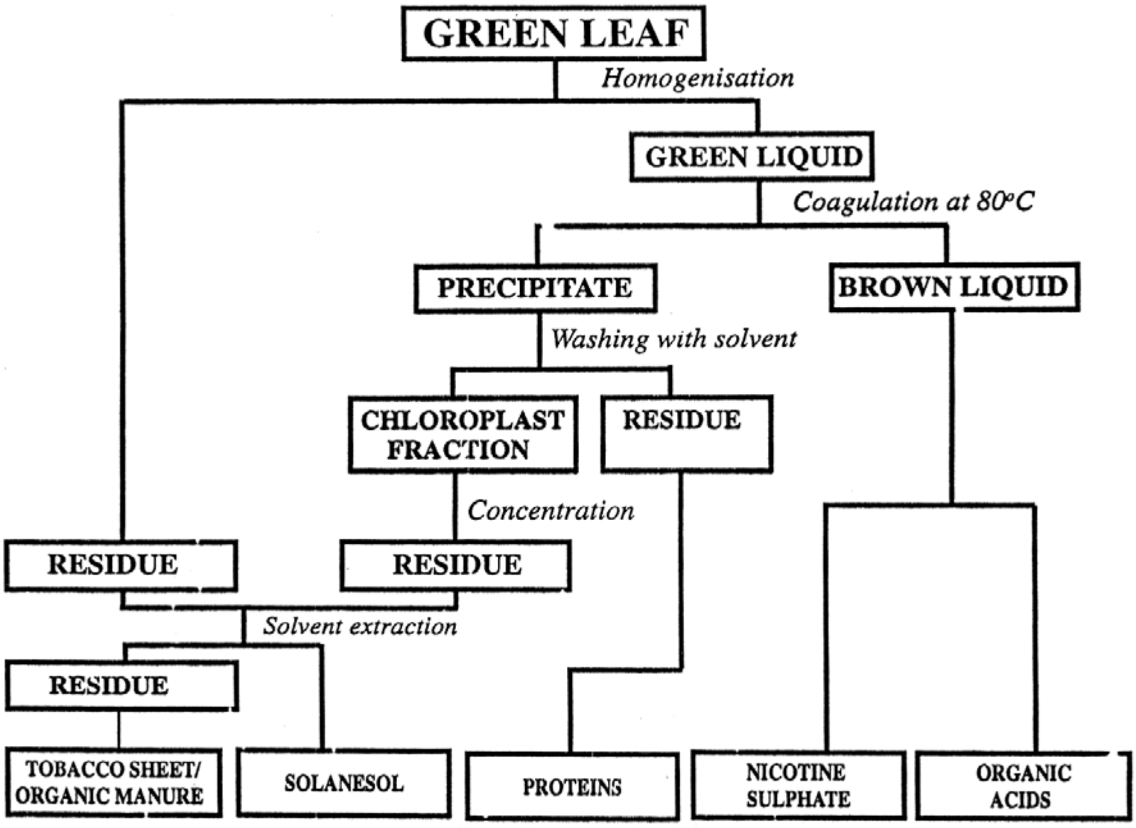
Table 2: Distribution of solanesol in green residue and chloroplast fraction (harvest wise)

Harvest	Residue			Chloroplast Fraction			Total	
	Hexane Extract (gm)	Solanesol in		Hexane Extract (gm)	Solanesol in		Hexane Extract (gm)	Solanesol (gm)
		Hexane (%)	Extract (gm)		Hexane (%)	Extract (gm)		
1	9.1	7.36	0.67	11.5	10.24	1.17	20.6	1.84
2	19.4	13.73	2.66	18.7	13.08	2.44	38.1	5.1
3	32.3	15.18	4.90	15.6	13.30	2.07	47.9	6.97
4	43.5	19.43	8.45	28.7	18.07	5.18	72.2	13.63
5	53.4	14.48	7.73	34.5	16.17	5.57	87.9	13.3
6	93.8	20.41	19.14	20.7	13.98	2.89	114.5	22.03

Table 3: Grown tobacco: Recovery of potential of phytochemicals

Phytochemicals	Yield (kg/ha)
Crude protein	384.5
Nicotine	41.2
Nicotine sulphate (40%)	97.8
Crude Solanesol	151.8
Pure Solanesol	18.9

FLOW DIAGRAM OF INTEGRATED APPROACH



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