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LIPOPHILIC CONSTITUENTS IN HDBRG TOBACCO

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Harvel De Bouxo Rio Grande **(HDBRG) tobacco, popularly known as HD Burley tobacco, which is a high yielding, sun-cured, burley type of tobacco grown under irrigated conditions in the heavy black soils of Andhra Pradesh and used in blend in cigarette manufacture. It has specific characteristics of responding to nitrogen fertilization, producing the highest biomass and seed. Lipophilic constituents play an important role in quality/aroma of tobacco. Representative cured leaf samples of HDBRG tobacco were analyzed for lipophilic constituents by soxhlet hexane extraction, alkaline hydrolysis and fractionation by column chromatography.**

The total hexane extractables in HDBRG tobacco was high (9.94%) and solanesol accounted for 24.24% of the extract. The general lipid profile of fractions indicated the presence of alkanes, fatty acids, sterols, terpenes and fatty alcohols. In the non-polar fraction, the odd-numbered paraffinic homologues were predominant, accounting for ~66% of the fraction. In respect of the combined total of normal and iso alkanes, the relative content of hentriacontane was the highest (~40%) followed by dotriacontane (~19%). In the polar fraction of the hydrolysate from powder, the proportion of saturated fatty acids ($C_{14:0}$ + $C_{15:0}$ + $C_{16:0}$ + $C_{17:0}$ + $C_{18:0}$) was ~58%, while that of unsaturated fatty acids ($C_{18:2}$ + $C_{18:3}$) was ~42%. Palmitic acid ($C_{16:0}$: 585 µg/g) was the major fatty acid. In terms of relative contents, stigmasterol (41.6%) was the major phytosterol, followed by campesterol (20.8%), cholesterol (20.4%), β-sitosterol (12.5%) and fucosterol (5.1%). HDBRG tobacco had higher levels of α-tocopherol (vitamin E) (50.7%) and fatty alcohols. The LC-MS analysis of solanesol in the APCI (+) mode revealed abundant stable (M-H_2O+H) ion (m/z at 613.7) with low abundance of other fragmentation ions thus confirming the presence of solanesol in the fractions. The results showed that, higher levels of total hexane solubles and solanesol in the fraction are the characteristic features of this tobacco. Among the lipids, isohentriacontane, palmitic acid, stigmasterol, α-tocopherol and solanesol were the principal constituents in the respective groups.

Key words: HDBRG tobacco, Lipophilic constituents, Non-polar and Polar compounds

INTRODUCTION

Harvel De Bouxo Rio Grande (HDBRG) tobacco, popularly known as HD Burley tobacco, which is a high yielding, sun-cured, burley type of tobacco supposed to be introduced from Brazil. The heavy bodied HDBRG tobacco, which is closely related to burley in chemistry, grown under irrigated conditions in the heavy black soils in Guntur district of Andhra Pradesh and it has specific characteristics of responding to nitrogen fertilization, producing the highest biomass and seed. This leaf is mainly used in the domestic cigarette blends and only a small quantity is exported. The different types of tobacco have distinct physical, chemical and organoleptic characteristics which are primarily governed by the soil, climate, variety and crop husbandry. The important chemical constituents are alkaloids, carbohydrates, nitrogenous compounds, acids, bases and lipids influencing the leaf quality. Some of the compounds have a positive impact on the leaf aroma and smoke flavour, while the others have negative impact.

A wide variety of chemical constituents were reported in different types of tobacco leaf including lipids and sterols. Quantitatively, the non-polar lipids as extracted by petroleum ether exhibit a wide variability and they ranged from 6.51 to 15.30% in flue-cured tobacco germplasm (Chaplin and Miner, 1980). In addition to genetic factors, cultural practices, weather conditions and curing methods determine the quantitative and

qualitative composition of different classes of components comprising the non-polar lipids. The quantitative composition of the hexane extractives were investigated in detail by various workers in FCV tobacco (Ellington *et al.*, 1978), *Lanka* tobacco (Kameswara Rao, 1983), *Natu* tobacco (Nagaraj and Chakraborty, 1977) and burley tobacco (Davis, 1976) by employing sophisticated analytical techniques. Liu *et al.* (2007) observed that Soxhlet extraction failed to quantify the sterol glycosides because of their polarity and also alkaline saponification was insufficient to cleave the acetal bond between the phytosterol and the carbohydrate moiety.

Total ether soluble extractives include almost all the lipid components and estimation of total ESE represents the quality of a particular type of tobacco. In general, it was regarded that higher levels of total ESE will be a positive attribute for tobacco quality. In this paper, an attempt has been made to study the total lipophilic constituents by soxhlet hexane extraction and alkaline hydrolysis methods and their fractionation in HDBRG tobacco grown under irrigated conditions in heavy black soils of Andhra Pradesh.

MATERIALS AND METHODS

HDBRG tobacco was grown in a bulk crop with three replications, at CTRI Research Station, Guntur, Andhra Pradesh with recommended package of cultural practices. Leaf samples were collected during 2009-10 and 2010-11 seasons. Cured leaf samples were collected from all primings. The leaf midribs were removed and resultant lamina portion was dried in the hot air oven at 60°C for 6 hours, powdered, passed through 40 micron mesh and used for chemical analysis. Representative samples were prepared by mixing in relative proportions. For fractionation of total lipids, the representative samples of 2011 season were used.

Hexane extractives

Hexane extractives were extracted from 500 g of tobacco powder with 3000 ml of n-hexane by Soxhlet extraction for 8 h. The solvent was removed using a Buchi flash evaporator at 40°C, and weight of total extractives was recorded. The extraction was carried out in triplicate. Values are expressed in per cent (Grunwald *et al.*, 1977) and results were statistically analysed (Panse and Sukhatme, 1957).

Separation of polar and non-polar lipids

The total hexane extractives thus obtained was dissolved in 600 ml of n-hexane, and successively extracted four times (3 x 250 ml and 1 x 100 ml) with 90% methyl alcohol (MeOH) and pooled. The hexane layer was washed three times successively with a total of 450 ml of distilled water and the water solubles were discarded. Methanol and hexane extracts were concentrated, dried and weights were recorded as per cent of non-polar and polar fractions.

Modified base-hydrolysis procedure for extraction of total lipids

Modified base-hydrolysis procedure was adopted (Ellington *et al.*, 1977) for the quantitative recovery of lipids. Tobacco powder (10 g) was refluxed with 400 ml of 2 N KOH in 85% ethanol for 2 h. The mixture was cooled and adjusted the pH to 2 with conc. HCl after treating with 400 ml of water. The mixture was filtered through a Whatman No. 1 fluted filter paper and the hydrolysis flask and the residue were washed with 30 ml mixture of benzene - 85% ethanol (1:1) five times. Saturated aqueous KCl solution (250 ml) was added and the mixture was extracted with 250 ml of hexane four times. Hexane extract of the hydrolysate was concentrated to dryness on a Buchi flash evaporator at 40°C. Two consecutive 8 ml portions of benzene were added and the mixture was taken to dryness under vacuum after each addition for removal of residual water or ethanol. Simultaneously, modified base-hydrolysis procedure was adopted for the quantitative recovery of lipids in Soxhlet hexane extractives.

Column chromatography

Hexane extract of the hydrolyzate (1g) was layered on the Silica gel (60 – 120 mesh) column (45 2 cm I.D.) in hexane and eluted with 1000 ml of hexane to yield the non-polar lipid fraction (F1). The semi-polar fraction (F2) was then separated with 2500 ml of hexane:benzene (3:1) and the polar lipid fraction (F3) with 1000 ml of

benzene:diethyl ether (3:1). The eluates were evaporated to dryness and weight of each fraction was recorded. The GC-MS analysis was carried out by taking 5 mg of F1 and 10 mg each of fractions F2 and F3 and dissolving in 1 ml of hexane. Hexane extract of the hydrolysate was subjected to silica gel column chromatography with the following solvent eluents successively petroleum ether (SF1), petroleum ether: acetone [95:5 (SF2), 90:10 (SF3), 80:20 (SF4), 60:40 (SF5) and 40:60 (SF6)]. Ten mg each of the two fractions were dissolved in hexane and transferred into a 1 ml volumetric flask and made up to the volume with hexane for GC-MS analysis. Due to the polarity of components in semi-polar and polar fractions, fractions F2 and F3, 40 µl each were derivatised by reacting with BSTFA [N,O-bis (trimethylsilyl) trifluoro acetamide] at 50°C for 30 min.

GC-MS analysis

The GC-MS analysis was carried out on Agilent 6890 GC system equipped with a 5973 N inert mass selective detector and 7863 auto sampler (Agilent Technologies, USA). A ZB-5 MS (5% Phenyl, 95% Dimethyl polysiloxane) (ZebronTM – Phenomenex, USA) column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness was used. The oven was programmed from an initial temperature of 50°C (held for 2 min) to the final temperature of 300°C @ of 10°C/min. The final temperature was held for 5 min. Hydrogen was used as the carrier gas with a flow rate of 1.2 ml/min. The inlet and interface temperatures were kept at 270°C. The EI source was operated at 230°C and the quadrupole temperature was 150°C. The MS was scanned from 30 to 600 units for recording full scan spectra. One micro liter of the sample was injected in split-less mode by the auto sampler. The peaks obtained were identified using U S National Institute of Standards and Technology (NIST) standard mass spectral library database. As authentic standards of the compounds are not available for quantification, the area normalization method was adopted and the proportion of a particular compound in the total lipid fraction was calculated.

Fatty acids

Hexane extractives (100 mg) were used for extraction and esterification (10% H_2SO_4 in absolute methanol) by the method of Kates (1975). The hexane extractives were made up to 25 ml for GC-MS analysis. The GC-MS analysis was performed using a QP 2010 Plus GC-MS system equipped with AOC - 20i auto sampler (Single quadrupole, Shimadzu Corporation, Kyoto, Japan). A ZB-5 MS (5% Phenyl, 95% Dimethyl polysiloxane) (Zebron™ – Phenomenex, USA) capillary column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness was used. The oven was programmed from an initial temperature of 100°C (held for 3 min), ramped @ 8°C/min up to 220°C (held for 5 min) to the final temperature of 240°C @ 2°C/min. The final temperature was held for 2 min and the total run time was 35 min. Helium was used as the carrier gas with a flow rate of 0.78 ml/min. The inlet and interface temperatures were kept at 250°C. The EI source was operated at 225°C and the MS was scanned from 50 to 500 units for recording full scan spectra. For calculating fatty acids, the MS was operated in selected ion monitoring (SIM) mode. In the SIM mode, for valid characterization, the following ions were selected as quantifiers and qualifiers for the respective fatty acid (m/z 74, 87 and 55 for $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$; 55, 69 and 74 for $C_{18:1}$; 67, 81 and 95 for $C_{18:2}$; 79, 67 and 95 for $C_{18:3}$). One micro liter of the sample was injected in split mode with the ratio of 1:20 by the auto sampler. Standard fatty acids were purchased from Sigma (St. Louis, MO 63178, USA). Five calibration standard mixtures of fatty acids were prepared by serial dilution with hexane ranging from 0.04 to 96 ppm.

Solanesol

HPLC analysis

Solanesol content in the tobacco powder was estimated (Narasimha Rao *et al.*, 2000) employing Shimadzu LC 8A HPLC with UV-VIS detector, at 210 nm. The mobile phase was HPLC grade isopropyl alcohol: methyl alcohol (60: 40) at a flow rate of 1 ml/min. The retention times and area per cent of different constituents were recorded.

LC–MS analysis

Forty mg each of the polar fractions of the tobacco powder and soxhlet hexane extract hydrolysates were dissolved in IPA and transferred

to 1 ml volumetric flasks and made up to the volume with IPA for identification of solanesol by LC-MS.

LC-MS (Agilent 1100 MSD ion-trap-SL mass spectrometer) coupled with atmospheric pressure chemical ionization (APCI) source in positive ion mode, equipped with a degasser (G1379A), binary pump (G1312A), auto-sampler (G1329A), auto-sampler thermostat (G1329B) and diode array detector (G1315B) of wave length 210 nm was employed for the qualitative and quantitative determination of solanesol in the fractions. Solanesol and other compounds were separated on an Agilent - Eclipse XDB -C18, 4.6 150 mm, 5 µm column using the isocratic mode of elution. For isocratic elution, 50% acetonitrile in isopropanol as mobile phase was pumped at a flow rate of 1.0 ml/min; the sample injection volume was 2 µL with column temperature maintained at ambient conditions. Nitrogen was employed as the nebulizer gas. The ion source conditions were set as follows: temperature, 335 C; nebulizer gas, 35 psi; dry gas, 10.0 l/min; skimmer 40.0 V; capillary exit 128.0 V; trap drive 44.5; max accu time 200 ms; Icc target 20000. The data were acquired and processed using Chemstation 5.3 (Agilent Technologies, Waldbronn, Germany).

RESULTS AND DISCUSSION

Hexane extractables

A large number of components identified in tobacco leaf belong to the broad group of lipids which may be polar or non-polar. The liphophilic constituents *viz.*, paraffins, polyenes, esters, solanesol, sterols, tocopherols and fatty acids are important because they are related to the leaf quality/aroma and smoke flavour. The total hexane extractables or PEE content in HDBRG tobacco was high (9.94%) and solanesol accounted for 24.24% of the extract. Further serial extraction of the total hexane solubles with 90% methanol and water resulted in three fractions *i.e.*, hexane solubles (69.3% of total hexane extractables and 6.9% on the basis tobacco), 90% methanol solubles (17.3% of total hexane extractables and 1.7% on the basis of tobacco (Table 1) and water solubles (13.3% of total hexane extractables and 1.4% on the basis of tobacco). Higher levels of PEE are positively correlated with aroma in FCV tobacco (Grunwald *et al.*, 1977). Even in chewing tobacco, higher levels of PEE are positively correlated with aroma as these extracts contain all lipids and fatty acids (Murthy and Gopalachari, 1984). In sun-cured chewing tobacco varieties grown in Tamil Nadu, PEE levels varied from 5.90 to 7.80% with a mean value of 6.97% (Siva Raju *et al.*, 2012). Gangadhar *et al.* (2011) reported significant positive correlations of nicotine with solanesol and PEE and PEE with solanesol.

The above total lipid extracts were characterized into non-polar and polar fractions employing techniques like GC-MS and LC-MS. Column chromatography of 1 g of the fraction obtained after base hydrolysis of tobacco powder (10 g) yielded non-polar (70 mg), semi-polar (300 mg) and polar (500 mg) fractions accounting for 88% of the total eluates collected. In the case of column chromatography of 30 g of fraction obtained after base hydrolysis of soxhlet hexane extract resulted in non-polar (3.17 g) and polar (24.15 g) fractions, accounting for 91% of the total eluates. The general lipid profile of fractions after column chromatography indicated the presence of alkanes, fatty acids, sterols, terpenes and fatty alcohols.

In the non-polar fraction containing aliphatic alkanes obtained from the hydrolysate fraction of powder, the odd-numbered homologues *viz.*, C_{27}, C_{31} and C_{33} were predominant accounting for ~66% of the fraction (Table 2; Fig.1). In this fraction, the per cent composition of normal (C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32} and C_{33}) and iso (C_{31} and C_{32}) series was 62.8 and 37.2%, respectively. The branched chain hydrocarbon, isohentriacontane was the major compound in the paraffin fraction with a relative content of 26.3%, followed by the linear paraffin n-tritriacontane (20.3%). Straight chain (normal) alkanes, hentriacontane (13.5%), triacontane (8.5%) and dotriacontane (8.2%) accounted for ~30% of the fraction. In respect of the combined total of normal and iso alkanes, the relative content of hentriacontane was the highest (~40%) followed by dotriacontane (~19%).

Similar trends of relative contents were observed in the case of the non-polar fraction resulted from the base hydrolysis of soxhlet hexane

Table 1: Lipid fractions of HDBRG tobacco

Sample	Total hexane extractables		Hexane solubles (after extraction)		90% Methanol solubles		Water solubles	
	(g)	(%)*	(g)	(%)**	(g)	(%)**	(g)	(%)**
2009-10								
S1	37.85	7.57	28.76	75.98	5.50	14.53	3.59	9.49
S2	47.19	9.44	32.78	69.46	5.50	11.66	8.91	18.88
S3	59.72	11.94	41.54	69.56	8.00	13.40	10.18	17.04
S4	49.96	9.99	34.34	68.73	9.00	18.01	6.62	13.25
Mean	**48.68**	**9.74**	**34.36**	**70.93**	**7.00**	**14.40**	**7.33**	**14.67**
SD	**9.00**	**1.80**	**5.34**	**3.39**	**1.78**	**2.68**	**2.89**	**4.17**
CV (%)	**18.49**	**18.46**	**15.53**	**4.77**	**25.42**	**18.62**	**39.47**	**28.43**
2010-11								
S1	54.62	10.92	37.22	68.14	10.00	18.31	7.41	13.56
S2	53.00	10.60	32.65	61.60	10.40	19.62	9.95	18.77
S3	46.50	9.30	33.00	70.97	10.00	21.51	3.50	7.53
S4	48.50	9.70	34.00	70.10	10.50	21.65	4.00	8.25
Mean	**50.66**	**10.13**	**34.22**	**67.70**	**10.23**	**20.27**	**6.22**	**12.03**
SD	**3.79**	**0.76**	**2.08**	**4.24**	**0.26**	**1.60**	**3.04**	**5.24**
CV (%)	**7.49**	**7.47**	**6.08**	**6.26**	**2.57**	**7.91**	**48.85**	**43.55**
Seasons								
Mean	**49.67**	**9.93**	**34.29**	**69.32**	**8.61**	**17.34**	**6.77**	**13.35**
SD	**6.48**	**1.29**	**3.75**	**3.95**	**2.09**	**3.75**	**2.81**	**4.60**
CV (%)	**13.05**	**13.03**	**10.94**	**5.70**	**24.24**	**21.61**	**41.49**	**34.50**

*Per cent of tobacco

** Per cent of total hexane extractables

extract of HDBRG tobacco (Table 2). The odd-numbered paraffins were more (63.7%) in this fraction. The ratio of normal: iso alkanes was 58.8:41.2, isohentriacontane (32%) being the major hydrocarbon, followed by n-tritriacontane (22.7%). The paraffins, hentriacontane (39%) and dotriacontane (19%) were more in respect of the sum of normal and branched chain alkanes.

Nagaraj and Chakraborty (1977) reported that in *Natu* tobacco, the odd-numbered n-paraffins constituted 91% of the total and n-hentriacontane was the major paraffin accounting for 55.6%. Dotriacontane was the major even-numbered compound with a relative content of 7.6%. Devrex and Esnault (1974) reported that in the essential oil from tobacco steam distillate, n-heptacosane ($C_{27}H_{56}$) was the major linear paraffin while isohentriacontane ($C_{31}H_{64}$) was the major branched chain paraffin. According to Chortyk *et al.* (1975), the combined totals for the normal and iso compounds were 37 and 19%, respectively in the case of cigarette and flue-cured tobacco. In *Lanka* tobacco, the odd-numbered alkanes viz., C_{27}, C_{29}, C_{31} and C_{33} were predominant accounting for ~77% of the fraction. The per cent composition of normal, iso and anteiso series was 60, 27 and 13%, respectively. The straight chain hydrocarbon, n-hentriacontane was the major compound in the paraffin fraction with a relative content of 26.5%, followed by n-tritriacontane (9.7%) and n-nonacosane (8.7%) (Kameswara Rao, 1983).The findings emanated from the present study are in consonance with the reported findings.

HDBRG tobacco had higher levels of á-tocopherol (vitamin E) (50.7%) and fatty alcohols *viz.*, decanol (15.8%), hexadecane-4-ol (13.9%),

Table 2: HDBRG tobacco — Composition of lipophilic constituents

Rt (min)	Compound	Powder hydrolysate	Soxhlet hydrolysate
		Relative content (%)	
Non-polar			
22.33	Nonadecane ($C_{19}H_{40}$)	0.42	0.11
23.85	Hexacosane ($C_{26}H_{54}$)	4.34	3.66
25.25	Heptacosane ($C_{27}H_{56}$)	3.57	3.59
25.75	Octacosane ($C_{28}H_{58}$)	2.06	1.02
25.92	Nonacosane ($C_{29}H_{60}$)	1.87	1.58
26.34	Triacontane ($C_{30}H_{62}$)	8.54	9.36
26.58	Hentriacontane (Iso $C_{31}H_{64}$)	26.31	31.99
27.05	Hentriacontane ($C_{31}H_{64}$)	13.45	7.26
27.21	Dotriacontane (Iso $C_{32}H_{66}$)	10.87	9.11
27.64	Dotriacontane ($C_{32}H_{66}$)	8.24	9.66
27.92	Tritriacontane ($C_{33}H_{68}$)	20.34	22.66
Semi-polar			
10.61	Decanol	15.77	-
19.21	Phytol	13.08	78.72
22.35	4-Hexadecanol	13.89	21.28
24.28	Squalene	3.37	-
26.42	á-Tocopherol	50.72	-
28.14	Cycloartenol	3.17	-
Polar			
16.11	Tetradecanoic acid (Myristic acid) - $C_{14:0}$	4.65	3.80
17.10	Pentadecanoic acid $C_{15:0}$	1.77	2.21
18.06	Hexadecanoic acid (Palmitic acid) $C_{16:0}$	39.58	33.55
18.71	Heptadecanoic acid $C_{17:0}$	3.11	3.87
19.55	9,12-Octadecadienoic acid (Z,Z) (Linoleic acid) $C_{18:2}$	7.63	13.05
19.61	9,12,15-Octadecatrienoic acid (Z,Z,Z)(Linolenic acid) $C_{18:3}$	34.51	31.22
19.84	Octadecanoic acid (Stearic acid) $C_{18:0}$	7.26	10.01
21.49	Eicosanoic acid (Arachidic acid) $C_{20:0}$	1.51	2.30
26.40	Cholesterol	20.39	32.25
27.06	Campesterol	20.81	15.97
27.22	Stigmasterol	41.22	28.50
27.63	â-Sitosterol	12.45	13.35
27.73	Fucosterol	5.14	9.94

phytol (13.1%), cycloartenol (3.2%) and a triterpene *i.e.*, squalene (3.4%) (Fig. 2). Squalene was reported as a constituent of burley tobacco (Rodgman *et al.*, 1961). Kameswara Rao *et al.* (1988) reported the presence of the minor terpenes like phytol, squalene, cycloartenol, 24-methylene cycloartanol and beta-amyrin in *Lanka* tobacco and its smoke.

Fatty acids

In the polar fraction of the hydrolysate from powder, the proportion of saturated fatty acids ($C_{14:0}$ + $C_{15:0}$ + $C_{16:0}$ + $C_{17:0}$ + $C_{18:0}$) was ~58%, while that of unsaturated fatty acids ($C_{18:2}$ + $C_{18:3}$) was ~42%. In terms of relative content, palmitic acid ($C_{16:0}$: 39.6%) and linolenic acid ($C_{18:3}$: 34.5%) were the

major acids identified (Table 3). The ratio of unsaturated to saturated fatty acids was 0.72.

In the case of polar fraction of the hydrolysate of Soxhlet hexane extract, a similar trend was observed. The relative contents of saturated and unsaturated fatty acids were 56 and 44%, respectively, with palmitic (33.6%) and linolenic (31.2%) being the principal saturated and unsaturated fatty acids, respectively (Table 2). The ratio of unsaturated to saturated fatty acids was 0.79. In both the cases, oleic acid ($C_{18:1}$) was not detected, may be due to merger with linolenic acid.

Results of the GC-MS analysis (SIM mode) (Fig. 3) using standard fatty acid methyl esters to quantify the fatty acids are presented in Table 3. It is inferred from the mean values of two seasons that palmitic acid ($C_{16:0}$: 585 µg/g) is the major fatty acid in HDBRG tobacco, followed by linolenic acid ($C_{18:3}$: 322 µg/g), Oleic ($C_{18:1}$), linoleic ($C_{18:2}$), myristic ($C_{14:0}$) are the other important fatty acids, with saturated acids accounting for 55.4% while the unsaturated acids accounted for 44.6% of fatty acids. The ratio of unsaturated to saturated fatty acids was 0.80.

Nagaraj and Chakraborty (1979) analyzed fatty acids in *Natu* tobacco by GC and reported the presence of decanoic (6.1%), lauric (8.5%), myristic (14.1%), myristoleic (17.0%), palmitic (10.9%), palmitoleic (6.4%), stearic (9.1%), oleic (8.0%), linoleic (1.6%) and linolenic (18.2%) acids. The saturated acids constituted about 48% of the total whereas the remaining 52% was accounted for by unsaturated fatty acids. Kameswara Rao (1983) reported the following major fatty acids in *Lanka* tobacco leaf, $C_{18:3}$, $C_{16:0}$, $C_{18:2}$ and $C_{18:1}$, the unsaturated acids accounting for 47%. The higher fatty acids (myristic, palmitic, stearic, oleic, linoleic and linolenic) comprised about 0.75 - 1.1% in Virginia tobacco and about 0.5% in Burley, with palmitic being about 25% of these total acids (Leffingwell, 2001).

Sterols

In terms of relative contents, stigmasterol (41.6%) was the major phytosterol, followed by campesterol, cholesterol, â-sitosterol and fucosterol in HDBRG tobacco in the polar fraction separated from the hydrolysate of powder (Table 2). However, differences were observed in the relative content of sterols in the hydrolysate of hexane extract which was obtained by Soxhlet extraction, where cholesterol was the major phytosterol, followed by stigmasterol, campasterol, â-sitosterol and fucosterol (Table 2).

Liu *et al.* (2007) observed that Soxhlet extraction failed to quantify the sterol glycosides because of their polarity and also alakaline

Table 3: Individual fatty acids – Season-wise

Fatty Acid	2009-10				2010-11			
	S1 (µg/g)	S2 (µg/g)	S3 (µg/g)	Mean (µg/g)	S1 (µg/g)	S2 (µg/g)	S3 (µg/g)	Mean (µg/g)
Lauric acid ($C_{12:0}$)	10.33	8.60	11.17	10.03	13.02	8.20	11.00	10.74
Myristic acid ($C_{14:0}$)	118.43	118.15	107.54	114.71	147.44	113.66	142.65	134.58
Pentadecanoic acid ($C_{15:0}$)	67.71	71.77	37.63	59.04	64.19	53.96	50.65	56.27
Palmitic acid ($C_{16:0}$)	585.05	587.84	504.27	559.05	660.07	525.68	648.98	611.58
Heptadecanoic acid ($C_{17:0}$)	38.37	48.06	38.98	41.80	41.15	35.17	42.60	39.64
Linoleic acid ($C_{18:2}$)	171.82	149.18	202.40	174.47	251.18	192.82	261.31	235.10
Oleic acid ($C_{18:1}$)	193.53	151.41	225.18	190.04	279.51	215.00	317.56	270.69
Linolenic acid ($C_{18:3}$)	281.08	194.12	270.01	248.40	406.12	317.78	461.59	395.16
Stearic acid ($C_{18:0}$)	119.04	122.47	118.80	120.10	128.68	109.53	134.12	124.11
Total	1585	1452	1516	1517.67	1991	1572	2070	1877.67

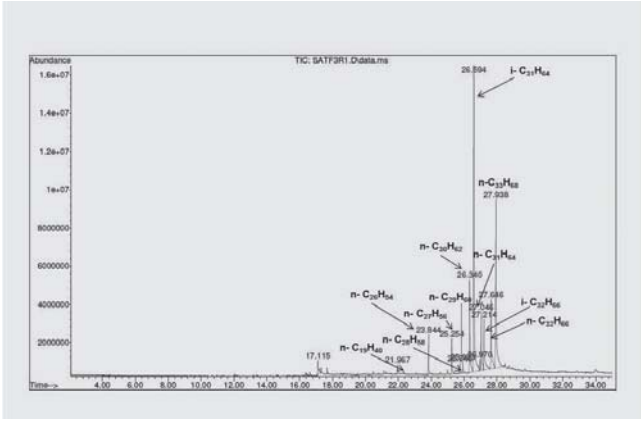

Figure 1. GC-MS chromatogram of non-polar fraction of soxhlet hexane hydrolysate in HDBRG robacco

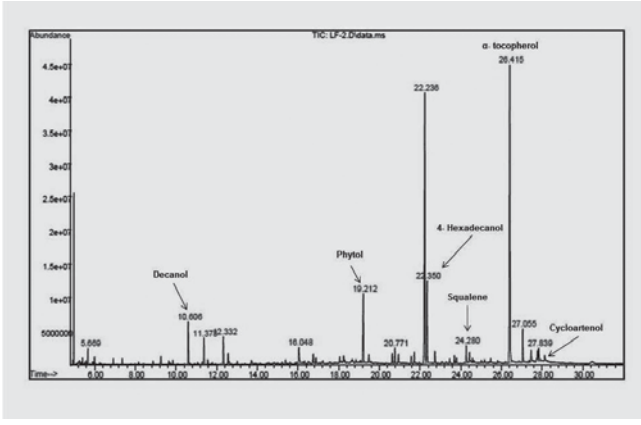

Figure 2. GC-MS chromatogram of TMS derivatives of compounds in the semi-polar fraction of powder hydrolysate of HDBRG tobacco

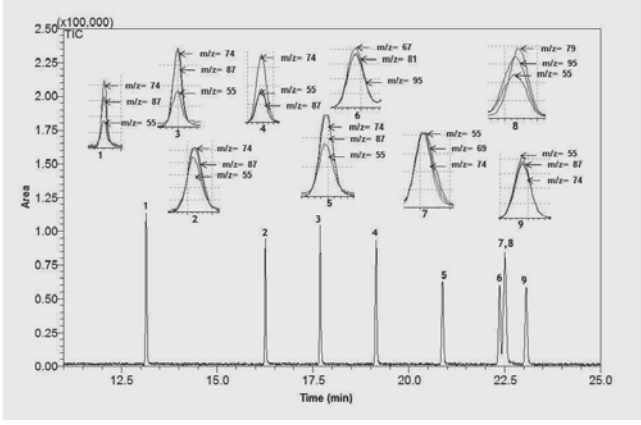

Figure 3. GC-MS chromatogram of methyl esters of FA. The labeled compounds (1-9) are listed in the Table 3.

saponification was insufficient to cleave the acetal bond between the phytosterol and the carbohydrate moiety.

Ellington *et al.* (1977) reported the levels of the four major sterols in FCV tobacco as, cholesterol (0.30 mg/g), campesterol (0.53 mg/g), stigmasterol (0.75 mg/g) and â-sitosterol (0.88 mg/g) totaling 2.46 mg/g. In the two lines of Burley tobacco, the levels of cholesterol, campesterol, stigmasterol and â-sitosterol were, L1: 0.18, 0.23, 0.72 and 0.38 mg/g and L2: 0.21, 0.33, 0.82 and 0.94 mg/g, respectively (Davis, 1976). The contents and forms of distribution of phytosterols in tobacco varied with tobacco cultivar and cultural practices (Grunwald *et al.*, 1977).

Solanesol

Solanesol content in the samples varied from 1.60 to 2.80% with mean values of 1.97 and 2.46% for the seasons 2009-10 and 2010-11, respectively. The LC-MS analysis in the APCI (+) mode revealed abundant stable (M - H_2O+H) ion (m/z at 613.7) with low abundance of other fragmentation ions, confirming the presence of solanesol in the fractions.

The data are consistent with the report of Phani Kiran *et al.* (2008) that HDBRG tobacco grown in the Guntur tract could be a rich source of solanesol (Range: 0.50 – 3.75%; Mean: 1.75%), both the maximum and mean values being the highest among different types of tobacco grown in the country. Solanesol content in various types of tobacco grown in different agro-ecological situations in India ranged from 0.09 to 3.18 % (Narasimha Rao and Prabhu, 2005). Burton *et al.* (1989) investigated the factors influencing solanesol content in burley tobacco and reported that genotype, growing conditions and agronomic practices which have profound influence on the solanesol content in the leaf at various growth stages of the plant.

It is concluded that higher levels of total hexane solubles/petroleum ether extractives and solanesol in the fraction are the characteristic features of this tobacco. Among the lipids, isohentriacontane, palmitic acid, stigmasterol, á-tocopherol and solanesol were the principal constituents in the respective groups.

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