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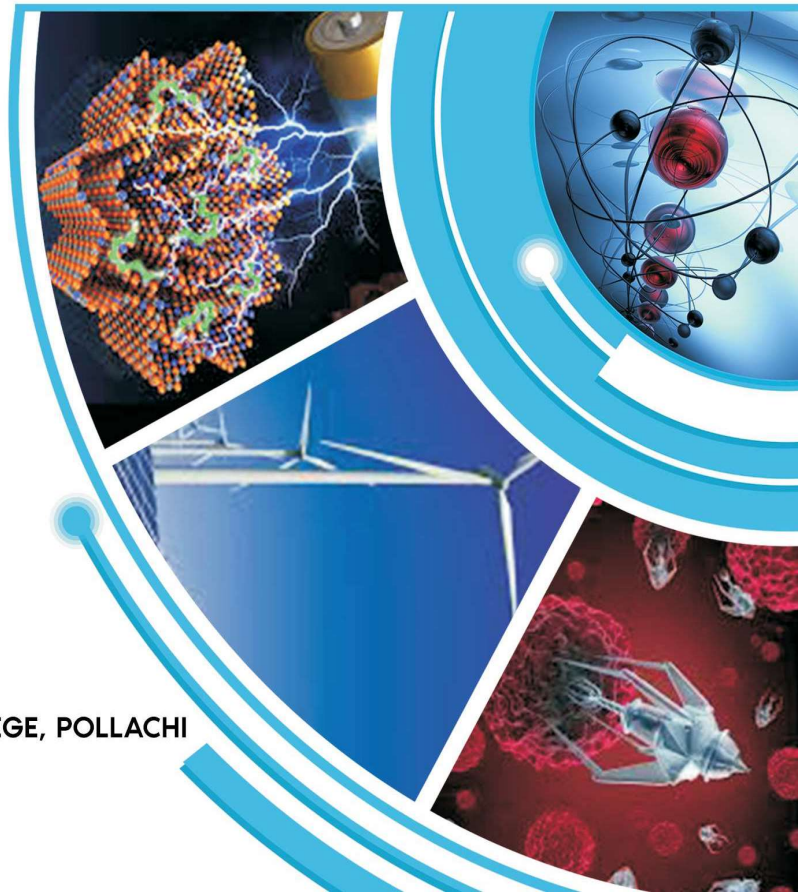

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INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS OF HANS – A SMOKELESS CHEWING TOBACCO METHANOL EXTRACT

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ABSTRACT

India is the 8th largest exporter of tobacco products in the world1. The botanical name for tobacco is *Nicotiana tabacum* and another one is *Nicotiana rustica*[2]. The leaves and juice were much used for skin disorders in earlier generation. Since tobacco is the most important avoidable plant because of large number of premature death and diseases in the world due to cancer. The tobacco plant is more seen as destructive plant than as herb. Herein we interested in studying the phytochemical constituents present in the methanol extract of Hans – a smokeless chewing tobacco commonly used in India. Most of the constituents of Hans are soluble in methanol. About 36 compounds were obtained and most of them are found to be already reported carcinogens.

INTRODUCTION

India is the second largest producer of tobacco in the world. In the year 1605 Portuguese introduced tobacco in India. Initially it was grown in Gujarat and later on it started growing in all parts of the India. All types of tobacco is cultivated in India including flue cured Virginia. Different tobacco products are devised for the use of smoked tobacco. Smokeless tobacco is consumed without burning. There is direct contact between the user's body and this tobacco. It is either chewed or directly inhaled via nose in powdered form. Smokeless tobacco includes two categories: chewing tobacco and snuffs.[3]

Hans is a commercial preparation containing areca nut, slaked lime, catechu, condiments and powdered tobacco[4]. Hans contains almost all the ingredients that go into the making of a pan, but are dehydrated so that the final product is not perishable[5]. It comes in attractive sachets and tins, which can be stored and carried conveniently. Hans is very popular in urban areas [6]. So we interested in investigating the phytoconstituents using GC-MS analysis.

MATERIALS AND METHODS

The Hans - A chewing smokeless product was used in all over the world and about 25.9% of population in India is using Hans. There are different Hans brands available in the market. Our Hans brand under investigation is HANS-CHHAP TOBACCO, manufactured by Murarilal Harish Chandra Jaiswal Private Limited, Kundli, Haryana - the most selling brand in our region [Pollachi, Tamilnadu, India]. The tobacco material wasidentified and authenticated by the faculties of Post Graduate Department of Chemistry, NGM College, Pollachi, Coimbatore, TamilNadu.

METHANOL EXTRACTION

The Hans was taken into the dry round bottom flask. Methanol was also added into the round bottom flask. The sample was heated for 3 hours at 40^{o} C in a mandle. The mother -liquid of the sample was collected in a beaker through whatman filter paper. The sample was filtered and concentrated. Then it is allowed to cool, storied in a container and kept in freezer for further investigation.

GC-MS ANALYSIS OF HANS

Gas Chromatography Mass Spectrometry (GC/MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identfied and quantified. GC-MS analysis of Hans wascarried out on Gas Chromatograph interfaced to a Mass Spectrometer equipped with a DB-5 MS capillary standard non-polar column of 30m length, 0.25 µm thickness. Helium was used as a carrier gas at a constant flow of 0.1ml/min and the temperature was 350^{o}C for 20 minutes. The sample of 100 ml was dissolved in 1ml of methanol and injected with split less mode. Mass spectra were recovered over 50-500 amu range with electron impact ionization energy 70eV, where injector and MS transfer line temperature were set at 230^{o}C and 280^{o}C respectively.

RESULT AND DISCUSSION

GC-MS Analysis of Hans

The methanol extract of Hans is subjected to GC-MS analysis. The components were identified by comparison of the retention time of the GC peaks with those obtained using NIFT library, analysis revealed that the presence of 35 compounds from methanol extract of hans. The major compounds are 1-Butyl(dimethyl)silyl oxy propane (1.187), Silane, diethoxy dimethyl (1.992), Ethanol, 2-(2- ethoxyethoxy) (1.083), 3(methylthio)-1-propanol (1.123), 2-propanol,1,1'-oxybis (0.760).The Minor compounds are 9,12,15-octadecatrienoic acid (z,z,z)(1.002), 11,13-dimethyl – 12-tetradecen-1-ol-acetate (0.759), 10,13-octadecadiynoic acid, methyl ester(0.519), Acetic acid(1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethyl naphtha-2-yl)methyl ester (0.900), Hexanedioic acid, bis(2-ethylhexyl) ester (0.646).

CHROMATOGRAM

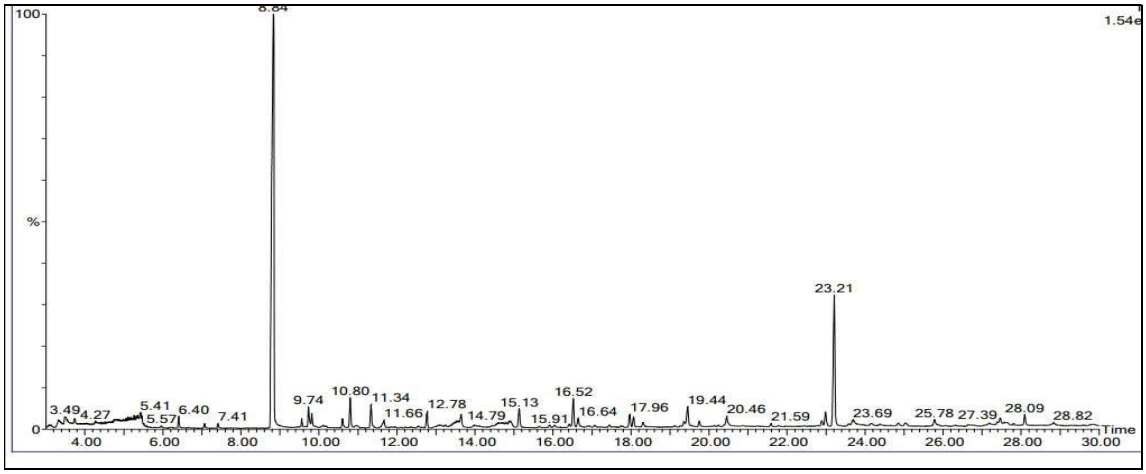

Figure 1. GC-MS Chromatogram of methanol extract of Hans

Table 1: Chemical composition of methanol extract of Hans

S.NO	NAME OF THE COMPOUND	MOLECULAR FORMULA	TOTAL PERCENTAGE	RETENTION TIME
1	1-Butyl (dimethyl)silyl oxy propane	$C_9H_{22}OSi$	1.187	3.334
2	Silane, diethoxy dimethyl	$C_6H_{16}O_2Si$	1.992	3.489
3	Ethanol, 2-(2- ethoxyethoxy)	$C_6H_{14}O_3$	1.083	3.744
4	3(methylthio)-1-propanol	$C_4H_{10}OS$	1.123	3.889
5	2-propanol,1,1'-oxybis	$C_6H_{14}O_3$	0.760	4.059
6	2,6-pyridine dicarboxaldehyde, 3-(phenyl methoxy)-bis[Methyl(2-pyridyl)hydrazone]	$C_{26}H_{25}N_7O$	0.803	4.274
7	D- Galactonic acid, ɤ-lactone	$C_6H_{10}O_6$	1.313	4.809
8	Methanamine, N-methoxy	C_2H_7NO	1.145	4.889
9	Methane, nitro	CH_3NO_2	1.104	5.104
10	1-propanol	C_3H_8O	0.599	5.139
11	Urea	CH_4N_2O	1.213	5.359
12	Phenylethyl alcohol	$C_8H_{10}O$	1.710	5.439
13	Pyridine,3-(1-methyl-2-pyrrolidinyl)-(s)	$C_{10}H_{14}N_2$	37.574	8.836
14	Anabasine	$C_{10}H_{14}N_2$	1.509	10.802
15	1,2,3,6-tetra hydro-2,3'-bipyridine	$C_{10}H_{12}N_2$	1.214	11.337
16	2,3'-dipyridyl	$C_{10}H_8N_2$	0.592	11.662
17	Diethyl phthalate	$C_{12}H_{14}O_4$	0.801	12.777
18	Phthalic acid,octyl 2- propyl pentyl ester	$C_{24}H_{38}O4$	1.078	13.107
19	Phthalic acid, di (2-propyl pentyl) ester	$C_{24}H_{38}O_4$	1.440	13.568
20	Benzophenone	$C_{13}H_{10}O$	1.038	13.648
21	1,2-benzene dicarboxylic acid, dipropyl ester	$C_{14}H_{18}O_4$	0.710	14.713
22	Diisooctyl phthalate	$C_{24}H_{38}O_4$	0.856	14.893
23	Cotinine	$C_{10}H_{12}N_2O$	1.120	15.133
24	Benzyl benzoate	$C_{14}H_{12}O_2$	1.573	16.524
25	3-ethyl-3,4- dihydro 2(1H) quinoxalinone	$C_{10}H_{12}N_2O$	0.565	16.644
26	neophytadiene	$C_{20}H_{38}$	0.677	17.964
27	2' pentadecanone, 6,10,14	$C_{18}H_{36}O$	0.545	18.669

	trimethyl			
28	Bicyclo[4.4.0]dec 2 –ene-4-ol, 2-methyl-9(prop-1-en-3-ol-2-yl)	$C_{15}H_{24}O_2$	1.388	19.455
29	n-hexadecanoic acid	$C_{16}H_{32}O_2$	1.088	20.455
30	9,12,15- octadecatrienioc acid, methyl ester(z,z,z)	$C_{19}H3_2O_2$	0.793	22.986
31	Phytol	$C_{20}H_{40}O$	8.488	23.211
32	9,12,15- octadecatrienoic acid (z,z,z)	$C_{18}H_{30}O_2$	1.002	23.687
33	11,13-dimethyl – 12-tetradecen-1-ol-acetate	$C_{18}H_{34}O_2$	0.759	25.778
34	10,13-octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	0.519	27.183
35	Acetic acid(1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethyl naphtha-2-yl)methyl ester	$C_{16}H_{26}O_2$	0.900	27.463
36	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	0.646	28.093

CONCLUSION

The Phytoconstituents from methanol extract of chhap tobacco brand Hans was analysed by GC-MS method. Results revealed that around 36 constituents were present in the extract, many of them are found to be carcinogens. Most of the chemical constituents obtained were found to be known carcinogens. The product is toxic to the cells. So it is highly recommended for further cytotoxicity analysis and to ban Hans.

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