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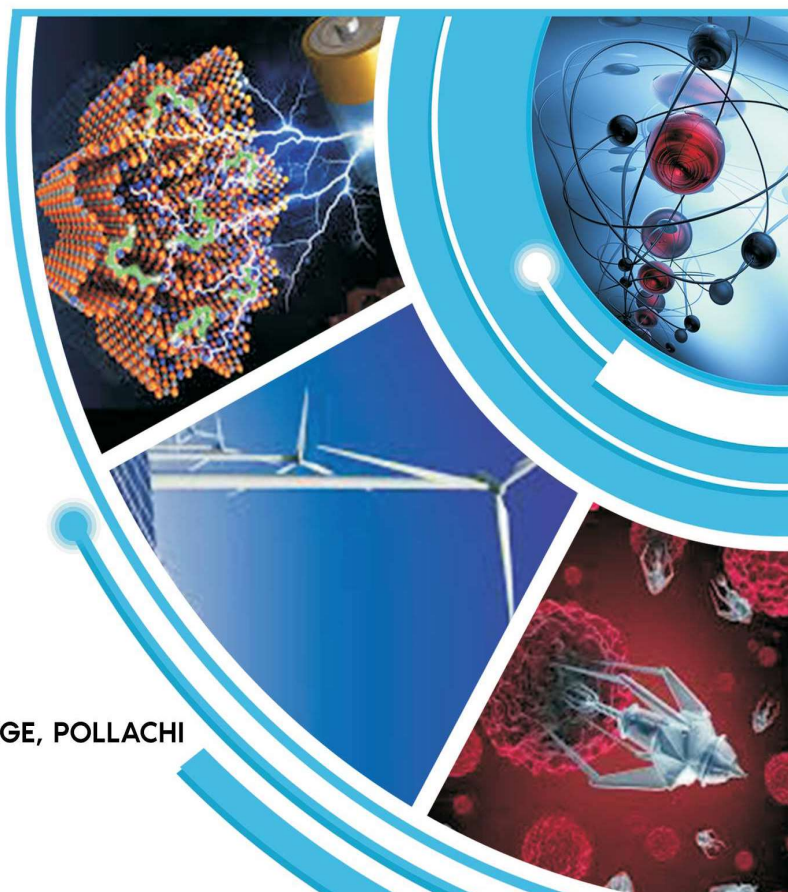
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INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS OF PAN MASALA– A SMOKELESS CHEWING TOBACCO METHANOL EXTRACT

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ABSTRACT

Every third adult in rural areas and every fifth adult in urban areas uses tobacco in some form or the other, revealed the Global Adult Tobacco Survey. So we are interested in investigating the phytoconstituents present in the smokeless chewing tobacco Pan Masala. Most of the constituents of tobacco products are soluble in methanol. We herein investigated the Phytochemical Constituents of Pan Masala–A Smokeless Chewing tobacco from its Methanol Extract which is the most selling brand in our area. About 39 phytochemicals were identified from the GC-MS analysis and most of them are already reported carcinogens.

Keywords: Phytochemical, Pan Masala, Smokeless tobacco, Carcinogens.

INTRODUCTION

The term “smokeless tobacco” refers to the consumption of un- burned tobacco, in the form of chewing, spitting, dipping, and snuff¹. Smokeless tobacco products (STPs) are used in various forms in India such as pan with tobacco, pan masala, tobacco, areca nut and slaked lime preparations, mawa, snus, mishri, and gul.² In addition to the locally prepared products, recently many commercially packed products have come into the market making it affordable and accessible to everyone, particularly for the young and poor. Moreover, STP products are also marketed and sold online³ and the use has increased from 28% to 33% among men and 12–18% among women in a decade.⁴ More than 28 carcinogens have been identified in tobacco leaves for smokeless use⁵. Consumers chew the tobacco in the mouth and spit out the juice that builds up. Nicotine and other constituents are absorbed in the lining of oral cavity and STP is responsible for cancers of the oral cavity, esophagus, pharynx, cervix, and penis. The use of chewing tobacco increases the relative risk of death by 15–30%.⁶

In chewing tobacco products the panmasala (PM) is most commonly used all over the India. The PM contains areca nut, cardamom, lime, catechu, flavouring agents with or without tobacco. The aim of the present study was analysis the phytochemical constituents present in the panmasala.

MATERIALS AND METHODS

The panmasala- A chewing smokeless product was used in all over the world. About 34.6 % existing population in Tamilnadu are continuously using smokeless tobacco products. And about 25.9% of population in India are using panmasala. Vimalpanmasala, manufactured by Vishnu pouches, liwaspur, sonipat, Haryana is the most selling brand in our region [Pollachi, Tamilnadu, India]. The tobacco material was identified and authenticated by faculty members of Department of Chemistry, NGM College, Pollachi, Coimbatore, Tamilnadu.

Methanol Extraction

In methanol most of the pan masala constituents are dissolved. The pan masala 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°C in the mangle. 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, stored in a container and kept in freezer for further investigation.

GC-MS Analysis

Gas chromatography mass spectrometry (GC/MS) is an instrumental technique, comprising a Gas Chromatograph (GC) coupled to a Mass Spectrometer (MS), by which complex mixture of chemicals may be separated, identified and quantified.

GC-MS analysis of pan masala was carried 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°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°C and 280°C respectively.

RESULT AND DISCUSSION

GC-MS Analysis of Pan Masala

The methanol extract of pan masala 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 39 compounds from methanol extract of pan masala. Most of them are carcinogenic in nature. The major components are Cyclohexanol, 5-methyl-2-(1-Methyl ethyl)-(1a',2a',5a')-(n) (28.558%), Pyridine, 3-(1methyl 2- pyrrolidinyl-(s) (13.452%), Methyl tetra decanoate(8.049%), Arecoline (5.744%), Hexadecanoic acid, Methyl ester (3.254%) and the minor components are Geraniol (1.151%), 3-Hydroxypropyl palmitate, TMS derivative (0.285%), Tonalid (0.297%), 3,9a',14,15-Diepoxy-pregn-16-en-20-one,3,11a',18- triacetoxy (0.301%), 1-Acetyl 6,8-dimethoxy-s-nitro 1,2,3,4- tetrahydroquinoline (0.312%).

Figure 1: GC-MS Chromatogram of Methanol Extract of Panmasla

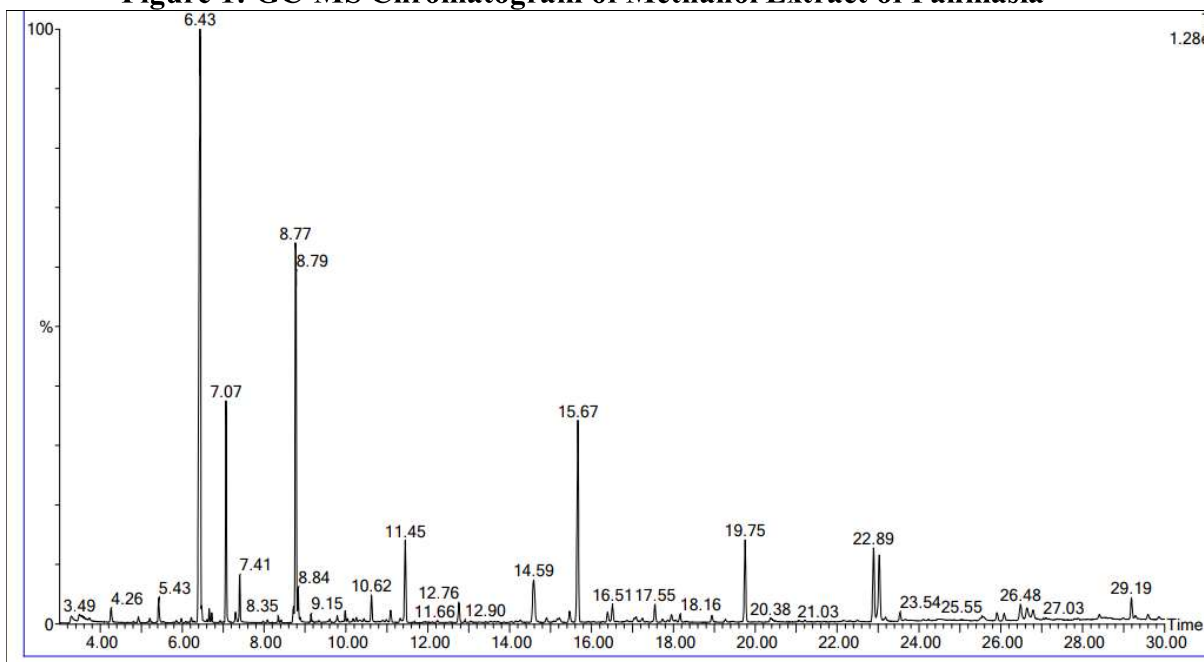


Table 1: Chemical Composition of Methanol Extract of Pan Masala

Sl.No	Name of The Compound	Molecular Formula	Retention Time (Mins)	Percentage
1	1-Butyl (dimethyl) silyloxypropane	C ₉ H ₂₂ O ₂ Si	3.289	0.674%
2	Silane, diethoxydimethyl	C ₆ H ₁₆ O ₂ Si	3.489	1.272%
3	2- Butene – 1,4 diethoxy	C ₈ H ₁₆ O ₂	3.724	0.345%
4	Eucalyptol	C ₁₀ H ₁₈ O	4.259	0.530%
5	Phenylethyl alcohol	C ₈ H ₁₀ O	5.429	0.801%
6	Cyclohexanol, 5-methyl-2-(1-Methyl ethyl)-(1a',2a',5a')-(n)	C ₁₀ H ₂₀ O	6.435	28.558%
7	Arecoline	C ₈ H ₁₃ NO ₂	7.070	5.744%
8	Guvacoline	C ₇ H ₁₁ NO ₂	7.300	0.282%
9	Geraniol	C ₁₀ H ₁₈ O	7.405	1.151%
10	Methyl anthranilate	C ₈ H ₉ NO ₂	8.721	0.402%
11	Pyridine, 3-(1methyl 2-pyrrolidinyl (s)-	C ₁₀ H ₁₄ N ₂	8.766	13.452%
12	Eugenol	C ₁₀ H ₁₂ O ₂	8.836	0.906%
13	2-Buten -1-ol, 2 ethyl-4-(2,2,3-trimethyl 3-cyclo penten-1-yl)	C ₁₄ H ₂₄ O	10.621	0.929%
14	Azulene, 1,2,3,5,6,7,8,8a'-octahydro1,4 dimethyl-7 (1-methylethyl)-[1s-(1a',7a',8a)]-	C ₁₅ H ₂₄	11.092	0.417%
15	Dodecanoicacid,methyl ester	C ₁₃ H ₂₆ O ₂	11.452	3.085%

16	Diethyl phthalate	C ₁₂ H ₁₄ O ₄	12.762	0.711%
17	Patchouli alcohol	C ₁₅ H ₂₆ O	14.588	2.616%
18	Santalol, trans-β	C ₁₅ H ₂₄ O	15.463	0.512%
19	Methyl tetra decanoate	C ₁₅ H ₃₀ O ₂	15.668	8.049%
20	Lanceol,cis	C ₁₅ H ₂₄ O	16.389	0.409%
21	Benzyl Benzoate	C ₁₄ H ₁₂ O ₂	16.514	0.665%
22	Musk ambrette	C ₁₂ H ₁₆ N ₂ O ₅	17.554	0.733%
23	5-Cyclohexadecan-1-one	C ₁₆ H ₂₈ O	17.949	0.331%
24	Tonalid	C ₁₈ H ₂₆ O	18.164	0.297%
25	1-Acetyl 6,8-dimethoxy-s-nitro 1,2,3,4- tetrahydroquinoline	C ₁₃ H ₁₆ N ₂ O ₅	18.940	0.312%
26	Hexadecanoic acid, Methyl ester	C ₁₇ H ₃₄ O ₂	19.750	3.254%
27	Musk ketone	C ₁₄ H ₁₈ N ₂ O ₅	20.375	0.336%
28	9,12-octadecadienoic acid (Z,Z)- methyl ester	C ₁₉ H ₃₄ O ₂	22.891	3.069%
29	9-Octadecenoic acid, methyl ester(E)	C ₁₉ H ₃₆ O ₂	23.026	3.032%
30	Methyl stearate	C ₁₉ H ₃₈ O ₂	23.537	0.345%
31	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	25.562	0.538%
32	Carbonic acid,(1R)-(-)-methyl tridecyl ester	C ₂₄ H ₄₆ O ₃	25.907	1.344%
33	3,7,11,15,19-Pentaoxa-2,20 disilaneicosane, 2,2,20,20 tetramethyl	C ₁₈ H ₄₂ O ₅ Si ₂	26.083	0.363
34	Myristicacid,TMS derivative	C ₁₇ H ₃₆ O ₂ Si	26.483	0.866%
35	Tetradecanoic acid, 2,3- dihydroxypropyl ester	C ₁₇ H ₃₄ O ₄	26.643	0.779%
36	1-Phenanthrene carboxylic acid, 1,2,3,4,4a,5,6,7,8,9,10,10a- dodecahydro-1,4a-dimethyl-7- (1-methylethyl)- methyl ester,(1R-(1a',4aa',7a',10aa'))	C ₂₁ H ₃₄ O ₂	26.793	0.603%
37	11-oxo-9 thiocyanato- testosterone	C ₂₀ H ₂₅ NO ₃ S	28.408	0.341%
38	3,9a',14,15-Diepoxypregn-16- en-20-one,3,11a',18- triacetoxo	C ₂₇ H ₃₄ O ₉	28.534	0.301%
39	3-Hydroxypropyl palmitate, TMS derivative	C ₂₂ H ₄₆ O ₃ Si	29.594	0.285%

CONCLUSION

The phytochemicals from methanol extract of pan masala was analysed by GC-MS method. Results revealed that around 39 constituents were present in the extract and many of them were found to be carcinogens. The chewing type Smokeless tobacco

product panmasala mostly contain carcinogenic constituents. It may cause the oral cancer in users. In this study we strongly recommend to ban and create the awareness about the panmasala smokeless tobacco product and investigate in detail about its cytotoxicity using different assays.

NO Conflict of interests for all authors**REFERENCE**

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