Screening of Influenza A (H1N1) neuraminidase inhibitor for Kabasura Kudineer, Nilavembu Kudineer and the novel formulation JACOM

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Abstract

Due to erratic climate change, vector-borne diseases started flaring up from the second half of the last decade. Siddha medicine has been used as a public health tool to effectively manage chikungunya and dengue in the epidemics that happened in 2008 and 2016. Tamil Nadu government has made enormous efforts to control vector-borne diseases. Due to which morbidity and mortality due to vector borne diseases came down compared with other states. Two official Siddha formulations, namely Kabasura Kudineer Chooranam and Nilavembu Kudineer Chooranam and novel herbal formulation – JACOM, are used to combat vector-borne diseases. These decoctions lack an evidence base as a formulation. Screening has been done to check the efficacy of the formulation in inhibiting neuraminidase. Neuraminidase inhibition assay was performed to determine the activity of Siddha formulations. The Kabasura Kudineer Chooranam, Nilavembu Kudineer Chooranam and JACOM showed excellent inhibitory activity. The Kabasura Kudineer and Nilavembu Kudineer and JACOM aqueous extract showed maximum neuraminidase inhibition of 80.35%, 91.78% and 87.97%, respectively.

Keywords: Neuraminidase inhibition assay, Kabasura Kudineer Chooranam, Nilavembu Kudineer Chooranam, JACOM, H1N1, Influenza A

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Introduction

The effect of climate change on the distribution and intensity of vector-borne diseases has been a controversial topic for the last 20 years. Climate change might increase risk areas for infectious diseases like dengue fever and increase the burden of common diseases, putting many individuals in danger [1]. Climate change has impacted sudden disaster in Chennai during 2015, causing heavy floods raising the incidence of vector-borne diseases. However, proper action of the government and dispensing of Nilavembu Kudineer as prevention has inhibited the virulence of vector-borne diseases [2]. Nilavembu Kudineer has an antiviral property which is used by people long back. Kabasura Kudineer is also used to treat vector-borne diseases, but it has been used to treat Swine flu. However, there is no existing evidence to support the claim of using these decoctions in swine flu. Andrographolide from *Andrographis paniculata* has been tested for its efficacy of inhibiting neuraminidase earlier [3]. However, Nilavembu Kudineer, Kabasura Kudineer and JACOM as a formulation have not been tested. The present research will warrant justice to the anti-influenza therapy from Siddha products. The study's main aim is to evaluate the neuraminidase inhibition activity of Kabasura Kudineer Chooranam, Nilavembu Kudineer Chooranam and JACOM.

MATERIALS AND METHODS

Kabasura Kudineer Chooranam is a polyherbal Siddha formulation consisting of fifteen ingredients (Tale.1), Nilavembu Kudineer Chooranam consists of nine ingredients (Table.2), and JACOM formulation consists of five ingredients (Table.3). Kabasura Kudineer Chooranam, Nilavembu Kudineer Chooranam and JACOM procured from GMP Siddha Central Research Institute Pharmacy, Arumbakkam, Chennai - 600106. Tamil Nadu, India.

TABLE: 1 Kabasura Kudineer Chooranam.

S.NO	TAMIL NAME	SCIENTIFIC NAME	QUANTITY
1.	Chukku	*Zingiber officinale*	1part
2.	Thippili	*Piper longum*	1part
3.	Ilavangam	*Syzygium aromaticum*	1part
4.	Cirukancori Ver	*Tragia involucrate*	1part
5.	Akkirakaram Ver	*Anacyclus pyrethrum*	1part
6.	Mulli Ver	*Hygrophilla auriculata*	1part
7.	Kadukkaithol	*Terminalia chebula*	1part
8.	Adathodai Elai	*Adathoda vasica*	1part
9.	Karpooravalli Elai	*Coleus amboinicus*	1part
10.	Kostam	*Saussurea lappa*	1part
11.	Seenthil Thandu	*Tinospora cordifolia*	1part
12.	Siruthekku	*Clerodendron serratum*	1part
13.	Nilavembu Samoolam	*Andrographis paniculata*	1part
14.	Vattathiruppi Ver	*Sida acuta*	1part
15.	Korai Kizhangu	*Cyperus rotundus*	1part

TABLE: 2 Nilavembu Kudineer Chooranam.

S.NO	TAMIL NAME	SCIENTIFIC NAME	QUANTITY
1.	Nilavembu	*Andrographis paniculata*	1part
2.	Vettiver	*Chrysopogon zizanioides*	1part
3.	Vilamicha ver	*Plectranthus vettiveroides*	1part
4.	chandanam	*Santalum album*	1part
5.	Korai kizhangu	*Cyprus rotundus*	1part
6.	Chukku	*Zingiber officinale*	1part
7.	Pei putal	*Trichosanthes cucumerina*	1part
8.	Milagu	*Pepper nigrum*	1part
9.	Parppatakam	*Mollugo cerviana*	1part

TABLE: 3 JACOM Chooranam.

S.NO	TAMIL NAME	SCIENTIFIC NAME	QUANTITY
1.	Nilavembu	*Andrographis paniculata*	1part
2.	Adathodai Elai	*Adathoda vasica*	1part
3.	Thulasi	*Ocimum tenuiflorum*	1part
4.	Malaivembu	*Melia azedarach*	1part
5.	Papali ilai	*Carica papaya*	1part

EXTRACTION

The powdered sample of the three polyherbal Siddha formulations was extracted with 500 mL of aqueous using the Soxhlet apparatus. After completing the extraction process, the extract will be filtered, and the solvent will be removed by distillation under reduced pressure. It will be subjected to neuraminidase inhibition assay.

NEURAMINIDASE INHIBITION ASSAY:

The NA-FluorTM Influenza Neuraminidase Assay Kit (Life Technologies, No: 4457091) was employed to test the efficacy of aqueous extracts on the viral neuraminidase inactivated H1N1 as per the manufacturer's instructions. The inactivated virus stock was titrated by performing an NA activity assay, and the optimum virus dilution for the neuraminidase inhibition assay was selected. Serial dilutions of aqueous extract of Kabasura Kudineer, Nilavembu Kudineer Chooranam and JACOM (1280µg/ml to 2.5µg/ml) were tested for NA inhibitory activity. Oseltamivir was included as a positive control in the assay. Fluorescence was measured using a fluorescence polarization microplate reader (excitation 355 nm, emission 460 nm.[4][5] Neuraminidase inhibition percentage was determined using dose-response data using sigmoidal curve fitting with the formula:

%Activity= Sample intensity /positive control intensity; % Inhibition = 100-% Activity

GC-MS analysis

GC-MS technique was used to analyze the presence of active compounds in the Siddha formulations. Elucidation on the mass spectrum of GC-MS will be prepared using the database NIST08 and WILEY8.[6]

RESULTS & DISCUSSION

The minimal inhibitory concentration of Kabasura Kudineer extract was found to be 2.5µg/ml, exhibited 80.35% of NA inhibition. The maximum inhibition was observed at 1280 µg/ml and revealed 87.35% of NA inhibition. (Fig.1). The minimal inhibitory concentration of Nilavembu Kudineer extract was found to be 2.5µg/mL and presented 91.78% of NA inhibition. The maximum inhibition was observed at 1280 µg/ml and revealed 90.32% of NA inhibition. (Fig.2). The minimal inhibition percentage of JACOM at least concentration 2.5µg/ml exhibited 87.97% of inhibition. The maximum was observed at 1280 µg/ml and revealed 88.49% inhibition (Fig.3). Thus, we conclude that all the two formulations can inhibit

neuraminidase, among which Nilavembu exhibited maximum activity with the highest inhibition of 91.78%.

The newer class of neuraminidase inhibitors, oseltamivir and zanamivir, has an inhibition range of about 70-80 %. Earlier influenza had been treated with adamantanes such as amantadine and rimantadine, which was slightly toxic to the patients. The minimal cytotoxicity was observed in the plant extracts due to cytoprotective components [5]. In our study, this is an indication that these extracts might serve as potential in developing safe and less toxic neuraminidase inhibitor.

The phytochemical compounds resolved in the GC MS analysis revealed active principles in the Kabasura Kudineer, Nilavembu Kudineer and JACOM extracts. (Fig.4, Fig.6 & Fig.8). GC-MS analysis of Kabasura Kudineer extract showed 6 compounds with 1 major constitutes namely 1,1,1,3,5,7,9,11,11,11 - decamethyl-5-(trimethyl siloxy) hexasiloxane (MW-490). (Fig.5) whereas the Nilavembu Kudineer extract showed 11 compounds with I major constitute as Cyclotetrasiloxane, octamethyl. (Fig.7) and the JACOM extract showed ten compounds with I major constitute as 13208(5-methyl-2-phenyl-1,3-dioxan-4-yl) methanol (Fig.9)

The retention time, molecular mass and chemical structures are represented. (Table.4 ,5 & 6)

Overall, our study reports provide substantive care for the consumption of three polyherbal Siddha formulations as hopeful sources of novel anti-influenza drug candidates. Future studies are recommended to clinically prove the efficiency of the Nilavembu Kudineer, Kabasura Kudineer Chooranam and JACOM as neuraminidase inhibitors.

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Competing interest declaration:

The authors declare that they have no competing interests.

Ethical approval statement:

NA

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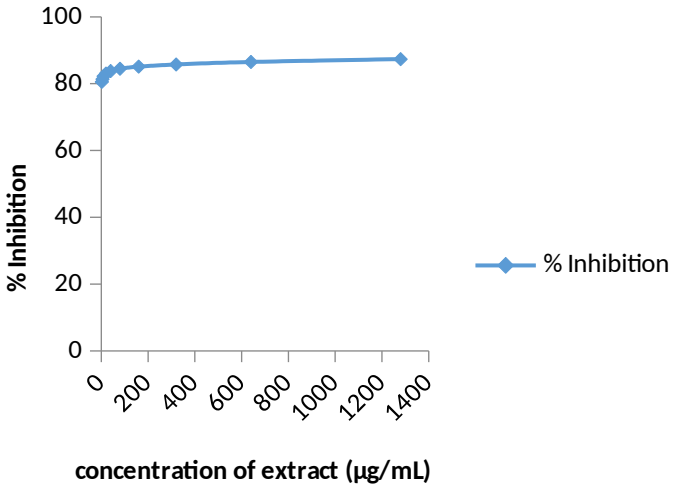

Fig 1: Neuraminidase inhibition by Kabasura Kudineer Chooranam

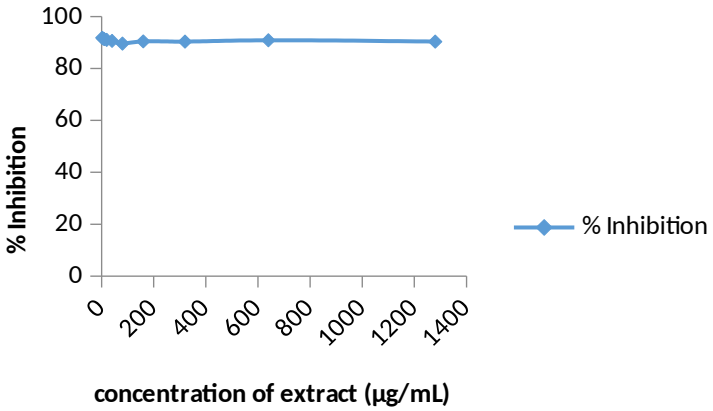

Fig 2: Neuraminidase inhibition by Nilavembu Kudineer Chooranam

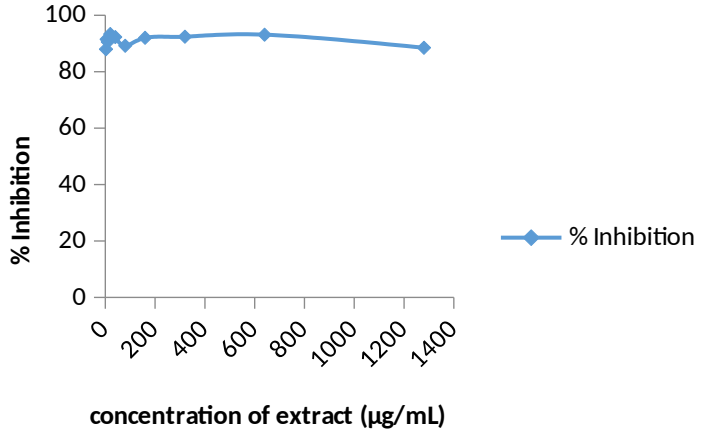

Fig 3: Neuraminidase inhibition by JACOM Chooranam

Table 4: GC-MS analysis of Kabasura Kudineer Chooranam extract

S.No.	Compound name	Retention time	Molecular weight	Molecular formula
1	1,1,1,3,5,7,9,11,11,11-decamethyl-5-(trimethylsiloxy)hexasiloxane	401.1	490	$C_{13}H_{42}O_6Si_7$
2	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	407	504	$C_{14}H_{44}O_6Si_7$
3	Octasiloxane ,1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15,15-hexadecamethyl-	399	578	$C_{16}H_{50}O_7Si_8$
4	Cyclohexasiloxane, dodecamethyl-	377	444	$C_{12}H_{36}O_6Si_6$
5	1,1,1,5,7,7,7, heptamethyl-3,3-tris(trimethylsiloxy)tetrasiloxane	349	444	$C_{13}H_{40}O_5Si_6$
6	Trimethylsilyl-di(timethylsiloxy)-silane	321	280	$C_9H_{28}O2Si_4$

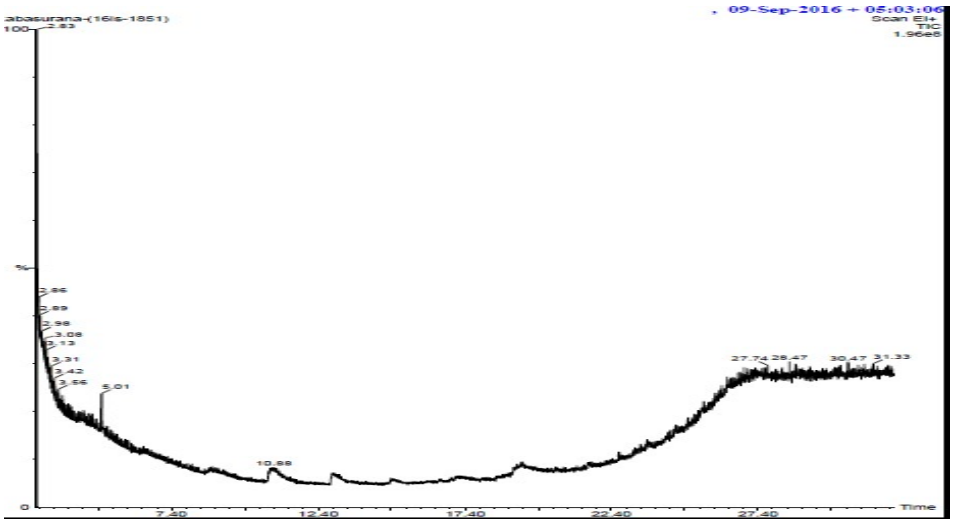

Fig 4: Chromatogram of Kabasura Kudineer Chooranam extract

114901,1,1,3,5,7,9,11,11,11-DECAMETHYL-5-(TRIMETHYLSILOXY)HEXASILOXANE

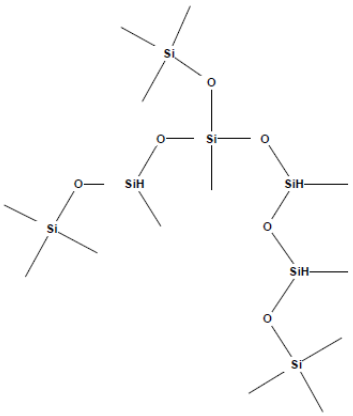

Fig 5: Chemical structure

Table 5: GC-MS analysis of Nilavembu Kudineer Chooranam extract

S.No.	Compound name	Retention time	Molecular weight	Molecular formula
1	Cyclotetrasiloxane,octamethyl	735	296	$C_8H_{24}O_4Si_4$
2	Cyclotetrasiloxane,octamethyl	704	296	$C_8H_{24}O_4Si_4$
3	2'6'-dihydroxyacetophenone,bis(trimethylsilyl)ether	499	296	$C_8H_{24}O_4Si_4$
4	2,5-dihydroxyacetophenone,bis(trimethylsilyl)ether	500	296	$C_{14}H_{24}O_3Si_2$
5	3-ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)teisiloxane	532	340	$C_{11}H_{32}O_4Si_4$

6	Benzoic acid ,4-methyl-2-trimethylsilyoxy-,trimethylsilyl ester	418	296	$C_{14}H_{24}O_3Si_2$
7	5-(p-aminophenyl)-4-(p-tolyl)-2-thiazolamine	352	281	$C_{16}H_{15}N_{36}$
8	Benzoic acid ,3-methyl-2-trimethylsilyoxy-,trimethylsilyl ester	380	296	$C_{14}H_{24}O_3Si_2$
9	Pentasiloxane ,dodecamethyl-	412	384	$C_{12}H_{36}O_4Si_5$
10	3-isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	410	354	$C_{12}H_{36}O_4Si_4$
11	1-phenazinecarboxilic acid ,6-(1-methoxyethyl)-methyl ester	319	296	$C_{17}H_{16}O_3N_2$

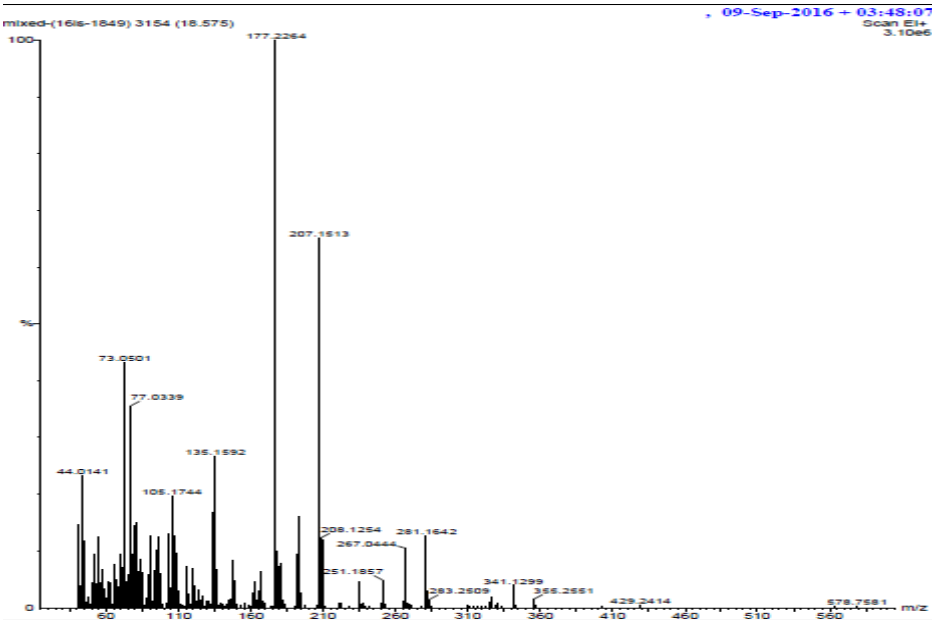

Fig 6: Chromatogram of Nilavembu Kudineer Chooranam extract

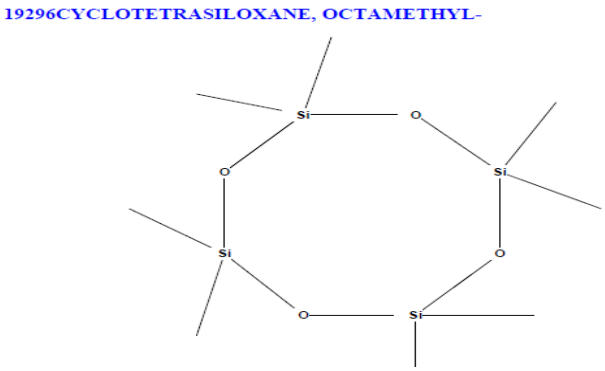

Fig 7: Chemical structure

Table 6: GC-MS analysis of JACOM formulation

S.No.	Compound name	Retention	Molecular	Molecular

		time	weight	formula
1	5-methyl-2-phenyl-1,3-dioxan-4-yl)methanol	374	208	$C_{12}H_{16}O_3$
2	1,2-dimethoxy-4-(1-methoxy-1-propenyl)benzene	401	613	$C_{12}H_{16}O_3$
3	1,4-cyclohexandiene-1,2-dicarboxylic acid ,4,5-dimethyl-dimethyl est	330	611	$C_{12}H_{16}O_3$
4	Benzeneacetic acid ,alpha-oxo-trimethylsilyl ester	377	222	$C_{11}H_{14}O_3Si$
5	2-thiazolamine,n-[2-(3,4-dimethoxyphenyl)ethyl]-4-(2-pyrinyl)-	305	341	$C_{18}H_{19}O_2N_3S$
6	1(3H)-isobenzofuranone,6,7-dimethoxy-3-[2-(2-methoxyphenyl)-2-oxoe	328	342	$C_{19}H_{18}O_6$
7	Phenol,2,4-bis(1-methylpropyl)-	300	206	$C_{14}H_{22}O$
8	2,4-benzyldiene-d-glucose	317	268	$C_{13}H_{16}O_6$
9	N-methyl-1-adamantaneacetamide	348	207	$C_{13}H_{21}ON$
10	Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-17-[o-(phenylmethyl)	323	481	$C_{29}H_{43}O_3NSi$

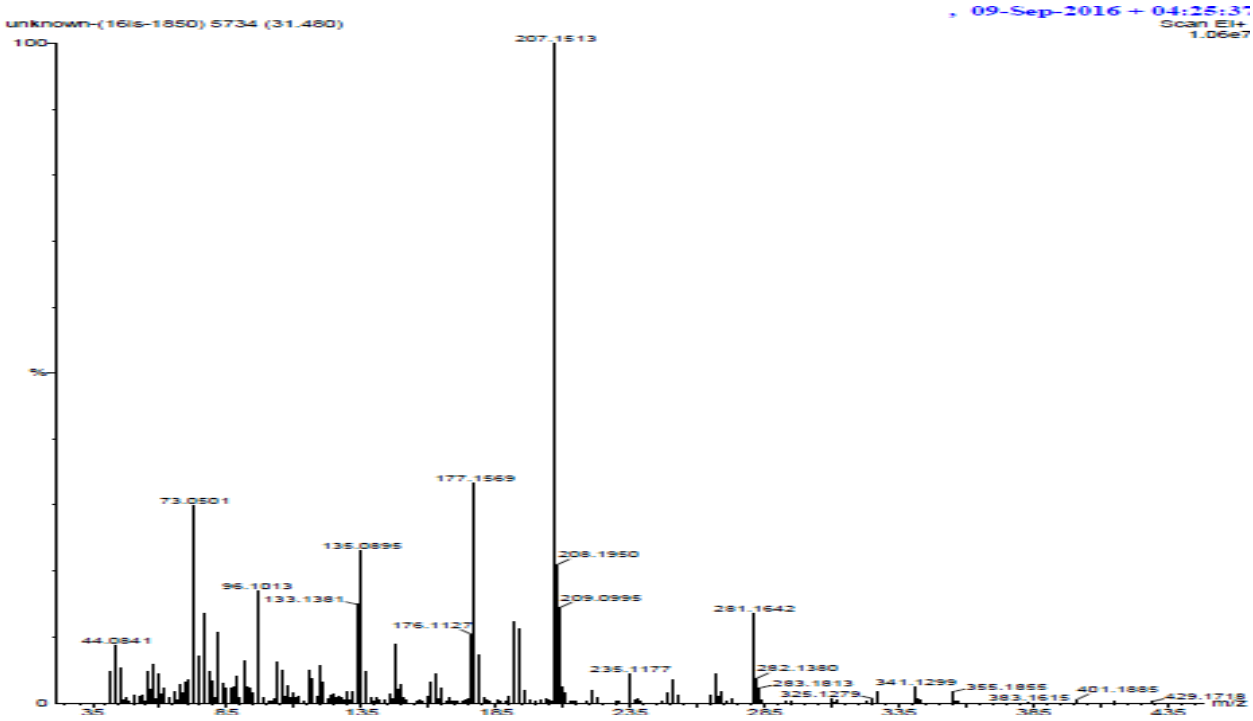

Fig 8: Chromatogram of JACOM

13208(5-METHYL-2-PHENYL-1,3-DIOXAN-4-YL)METHANOL

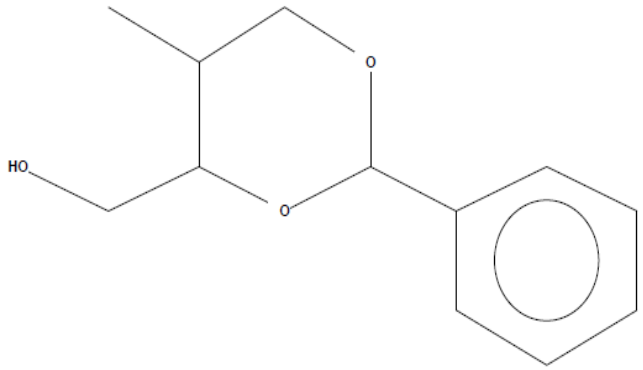

Fig 9: Chemical structure