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The GC MS Study of on Siddha Formulation, Nilavembu Kudineer (Kashayam)

Nandini MS[1*], Mudiganti Ram Krishna Rao[2], K Prabhu[3], Vijayalakshmi N[4] and Lakshmi Sundaram R[5]

[1]Department of Microbiology, Sree Balaji Medical College and Hospital, Chennai, India

[2]Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Chennai, India

[3]Department of Anatomy, Sree Balaji Medical College and Hospital, Chennai, India

[4]Department of Chemical and Biotechnology, SASTRA Deemed University, Tamil Nadu, India

[5]Department of Biotechnology, Sri Ramachandra Institute of Higher Education and Research, Chennai, India

[*]Corresponding e-mail: drnandini@bharathuniv.ac.in

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ABSTRACT

Introduction: *This present study is about the GC MS analysis of one important Siddha formulation, Nilavembu Kudineer.*

Methods: *Nilavembu Kudineer powder was obtained from standard Siddha medical vendor at Chennai, India and was suitably processed for GC MS analysis.*

Results: *Some important biomolecules such as Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, piperine, ursodeoxycholic acid, ethyl iso-allocholate, 9,19-cyclolanostan-3-ol, 24,24-epoxymethano, acetate and hexadecanoic acid, 2-(hexadecyloxy)ethyl ester were observed in the GC MS profile which are found to have medicinal roles that are far reaching, which supports this medicine as an effective formulation towards the cure of many inflammatory diseases.*

Conclusion: *This medicine, due to the presence of medicinally important molecules indicates its effectiveness as a potent medicine for which it is usually prescribed.*

Keywords: GC MS, Nilavembu Kudineer, Siddha, Piperine, Ursodeoxycholic acid, Ethyl iso-allocholate

INTRODUCTION

Siddha and Ayurvedic forms of medicine are in practice since time immemorial and their importance has become extremely important after the advent of some viral diseases such as swine flu, chikungunya, SARS and latest, the

COVID-19 [1]. When the whole world is grappling to solve the problem of these deadly diseases, India has shown that we do have our native medicines to cure, ward off or develop immunity towards these diseases. Nilavembu Kudineer and another siddha formulation, Kabasura Kudineer have been accepted by department of Ayush, government of India as standard medicines and are being supplied in hospitals in many parts of the country. It will be of interest to understand the medicinal roles of these formulations so that the approach towards the diseases could be more focused and results could be still effective. The powder of Nilavembu Kudineer is prepared by drying equal parts of the following plants: *Andrographis paniculata, Vetiveria zizanoides, Cyperus rotundus, Satalalum album, Zingiber officinalis, Poper nigrum, Trichosanthus curcumerina, Mollugo ceruana* [2]. After cleaning and washing with water these plants are shade dried and made into fine powder. One or two table spoons of this powder is mixed with 240 ml water and boiled to get $1/4^{th}$ volume. 30 ml to 60 ml of this decoction is consumed twice a day or as suggested by the consulting physician. There are some reports on the efficacy of Nilavembu Kudineer. Anbarasu et al., have reported the anti-inflammatory, antipyretic and analgesic properties of Nilavembu kashyam. Parthiban and Kumar, have worked on the clinical aspects of Siddha medicines. Similarly, Satya et al., have reviewed the Siddha herbs and herbo mineral formualtions. Lavekar and Padhi have discussed the management of chikungunya through Ayurvedic approach. Rubeena et al., have reported the antiviral activities of Nilavembu Kashayam on chicugunya and dengue fevers. Ramanathan et al., have evaluated the capsules of Nilavembu Kudineer. Similarly, Christian et al., 5 have reported the antiviral effect of Nilavembu Kashayam. Rajashekhar et al., have also reviewed the therapeutic potential of *Andrographis panniculata* on viral infections. Rubeena et al. and Jain et al., have evaluated the antiviral potential of Nilavembu Kudineer on chikungunya and dengue [3]. This work is in continuation of our endeavour to find out the types of molecules present in Siddha and Ayurvedic medicine to have a better understanding of the action of these medicines. 10-30 we have subjected the medicine, Nilavembu Kashayam or Kudineer to GC MS analysis and tried to understand the roles of chemical molecules present in them [4].

MATERIALS AND METHODS

Nilavembu Kudineer was resourced from standard Ayurvedic shop vendor at Chennai. The later this was subjected to GC MS analysis by standard procedure.

Instrument Gas Chromatography (Agilent: GC: (G3440A) 7890A. MS MS: 7000 Triple Quad GCMS,) was equipped with Mass spectrometry detector [5].

Sample preparation

100 µl of sample is taken and dissolved in any 1 ml of suitable solvent and the solution is vigorously stirred for about 10 seconds. The clear extract is then subjected to gas-chromatography for analysis [6].

Protocol for GC MS

The GC MS column consisted of DB5 MS (which is 30 mm × 0.25 mm ID × 0.25 µm and is composed of 5% phenyl 95% methyl poly siloxane), electron impact mode at 70 eV; carrier gas helium (99.999%) was used at a Constant flow of 1 ml/min with Injector temperature of 280°C; and auxilary temperature: 290°C along with ion-source temperature of 280°C [7].

The oven temperature was programmed from 50°C (isothermal for 1.0 min), with an increase of 40°C/min to 170°C (isothermal for 4.0 min) and followed by 10°C/min to 310°C (isothermal for 10 min) fragments from 45 Da to 450 Da and 32.02 min was total GC running time. GC MS Library (NIST and WILEY) was used for identifying the compounds [8].

RESULTS AND DISCUSSION

The GC MS profile of Nilavembu Kashayam is represented in Figure 1. Table 1 shows the types of possible compound, retentions time, molecular mass, their molecular formulae and percentage peak area as shown in the GC MS profile of Nilavembu Kashayam [9]. The metabolites were identified by comparing the retention time and fragmentation pattern with mass spectra in NIST spectral library in the computer software (version 1.10 beta, Shimadzu) of GC-MS along with the possible pharmaceutical roles of each molecules according to Dr. Duke's Phytochemical and ethnobotanical data base (national agriculture library, USA) and others. The bio-molecules as

indicated in GC MS profile show some promising chemical compounds such as Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, ursodeoxycholic acid, piperine, ethyl iso-allocholate, 24,24-epoxymethano-, 9,19-cyclolanostan-3-ol, acetate and hexadecanoic acid, 2-(hexadecyloxy)ethyl ester which have indirect or direct role which assist the function of the medicine. Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate has medicinal role like increasing zinc bioavailability, oligosaccharide provider, decreases endothelial platelet adhesion, decreases endothelial leucocyte adhesion, endocrine tonic and as energizer [10]. Piperine has medicinal properties such as radio-protective, immuno-modulatory, anti-tumor, antidepressant, anticonvulsant, anti-nociceptive, anti-arthritic, help in vitamin B, selenium, Beta carotene and other nutrient's absorption. Ethyl iso-allocholate has many medicinal roles such as mucolyte, anti-coagulant, anti-dyspeptic, anti-inflammatory and proteolytic [11]. Thus it clear that these molecules are directly or indirectly help cure the diseases for which this medicine is prescribed [12]. The use of such Ayurvedic and Siddha medicines have the advantage that a group of molecules curing the disease synergistically, in contrary to single molecule modern medical treatment [12]. Thus the disease is acted upon on a number of targets, which have direct or indirect bearing on the pathology [13]. This approach of treatment also reduces the burden on the patient's physiology due to less side effects, being natural. The usually the traditional and alternative medicines approach the disease in an altogether different perspective of maintaining the homeostasis and thus treating the disease holistically [14].

It is also interesting there are many molecules, such as the medicinal roles are not known 9,12-octadecadienoyl, 1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-tri hydroxy -1,4-bis(hydroxymethyl)-1,7,9 trimethyl, [1S (1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha., 9a.alpha.,10a.alpha.)]-,triarachine, lycoxanthin, 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-,5H-cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9atris(acetyloxy)-3 [(acetyloxy)methyl] 2 chloro 1,1a,1b,2,3,4,4a,7a,7b, 8,9,9a dodecahydro4a,7bdihydroxy1,1,6,8 tetramethyl, [1aR(1a.alpha.,1b.beta.,2.alpha.,3.beta.,4a.beta.,7a.alpha., 7b.alpha.,8.alpha.,9.beta., 9a.alpha.)], Eicosanoic acid, 2 [(1 oxohexadecyl)oxy] 1 [[(1 oxohexadecyl)oxy]methyl]ethyl ester etc., the medicinal roles of which are not known, warrant further work that could indicate useful results. Further work is in progress to understand the roles of each molecule as displayed in the profile of GC MS (Figure 1 and Table 1) [15].

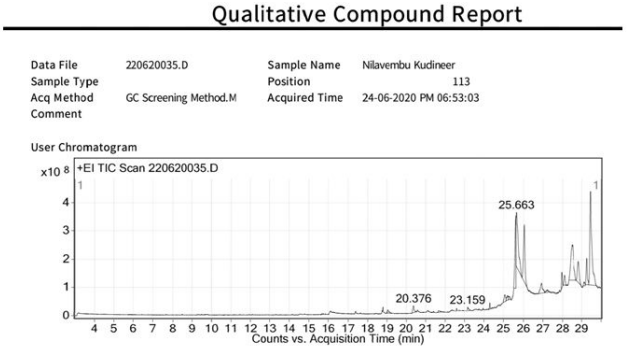

Figure 1 Indicates the GC MS profile of Nilavembu Kudineer.

Table 1 Shows retention values, possible bio-molecules types, their molecular formulae, peak area, molecular mass and their medicinal roles each bio-molecules plays as displayed in the GC MS profile of Nilavembu Kashayam.

S.No	Retention time	Compound name	Mol. formula	Mol. weight	% Peak area	Possible medical role
1	19.05	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268.2	1.52	Increase zinc bioavailability, oligosaccharide provider, decreases endothelial leucocyte adhesion, decreases

						endothelial platelet adhesion, endocrine tonic, energizer
2	20.38	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298.2	2.86	Not known
3	21.67	Piperine	$C_{17}H_{19}NO_3$	285.1	1.04	Radioprotective, immuno-modulatory, anti-tumor, antidepressant, anticonvulsant, anti-nociceptive, anti-arthritic help in selenium, Beta carotene and vitamin B absorption and also many other nutrients
4	23.16	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a, 2,5,5a, 6,9,10,10a-octahydro-5,5a, 6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha., 1a.alpha., 2.alpha.,5.beta., 5a.beta.,6.beta., 8a.alpha.,9.alpha., 10a.alpha.)]-	$C_{20}H_{28}O_6$	364.2	1.19	Not known
5	24.29	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	392.3	1.22	Inhibitor of arachidonic acid, inhibits the production of uric acid, Acidifier and increases the activity of aromatic amino acid decarboxylase
6	25.06	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.3	3.05	Anti-coagluant, antidyspeptic,

						anti-inflammatory, mucolyte, proteolytic
7	25.21	Hexadecanoic acid, 2-(hexadecyloxy)ethyl ester	$C_{34}H_{68}O_3$	524.5	1.82	Acidifier, inhibit uric acid production, inhibits arachidonic acid production and increase aromatic amino acid decarboxylic activity
8	25.28	9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate	$C_{33}H_{54}O_3$	498.4	1.3	Oligosaccharide provider
9	25.63	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568.5	20.47	Acidifier, arachidonic acid production is inhibited, increases the activity of aromatic amino acid decarboxylic and inhibits production of uric acid
10	26.06	Triarachine	$C_{63}H_{122}O_6$	974.9	23.99	Not known
11	27.25	Lycoxanthin	$C_{40}H_{56}O$	552.4	1.61	Not known
12	28.51	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	$C_{57}H_{104}O_6$	884.8	30.12	Not known
13	29.14	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9atris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1,6,8-tetramethyl-, [1aR-8.alpha., 9.beta., 9a.alpha.)]-(1a.alpha., 1b.beta., 2.alpha.,3.beta.,	$C_{28}H_{37}ClO_{11}$	584.2	1.23	Not known

		4a.beta., 7a.alpha., 7b.alpha.,				
14	29.26	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[(1-oxohexadecyl)oxy]methyl]ethyl ester	$C_{55}H_{106}O_6$	862.8	8.58	Not known

CONCLUSION

As of the above discussion it's now clear that Nilavembu Kudineer, which is common term in Tamil Nadu does contain some very important molecules, as shown in profile of GC MS towards curing a number of inflammatory diseases. It is concluded from the data that extracts of NKC exhibited significant role in medicinal chemistry for the formulation of life-saving drugs. The analysis of GCMS on the ethyl acetate extract of NKC exposes the existence of vital medicinal bioactive components. The medicinal value of these components in ethyl acetate extract of NKC is similar to the components presents in the other plant extracts which have been already proved. So it is also proved that the extract taken is also equally effective. The work is in the process of evolution to determine its genetic activity and thereby to brighten its pharmacological profile in the field of traditional medicines. A plant having phytochemicals gains pharmaceutical reputation. The scope of the ongoing research is to isolate the phytochemicals and test their medicinal activities.

CONFLICT OF INTEREST

Conflict of interest declared none.

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ETHICAL STATEMENT

No human subjects in this article hence no informed consent is applicable.

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