GC-MS Analysis of Bioactive Compounds Present in Marketed Herbal Formulation Pykure Capsule

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ABSTRACT

It is more acceptable to believe that natural remedies are safer than synthetic subjects that with fewer side effects. The global market demand was increasing due to fusion of herbs. Standardization parameters and evaluation of herbal formulation is essential in order to assess the quality of drug of therapeutic value. This study was carried out in order to determine the bioactive compounds present in the marketed herbal formulation pykure capsule by using the GC-MS instrument. The extract was prepared by using 20 capsules by removing the capsule shell with ethanol solvent by simple maceration method. Capsule extract was analyzed by using Agilent 6890 GC with 59739N MSD and GC -MS equipped with Elite-I fused with silica capillary column (Cpsil 8cb:30mm×25mm×0.25mm). The result of GC-MS analysis confirmed the presence of 74 compounds.

Keywords: Pykure capsule, Ethanolic extract, GC-MS analysis.

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1. INTRODUCTION

Herbal medicines can be defined as any medicinal product which contains active ingredients as one or more herbal preparation or herbal substances [1]. Herbal medicine is an ancient form of ailment. The use herbal medicine is increased tremendously over past 2 decades. In this field, there is a significant lack of research data. Since 1990 three volumes of WHO monographs has been published on selected medicinal plants [2]. Herbal medicine has become an important alternative medicine. In today's world it has become popular because of its natural remedies. In many countries, traditional herbal medical system is of economic importance. 80% of the population of Africa uses herbal drugs in order to meet the health care needs. Asia and America uses herbal medicine because of cultural benefits. In china around 40% of herbal medicines of all health cares are delivered. About 70-80% of African and Asian population is using herbal medicine as a primary medicine which is estimated by WHO [3]. Pykure capsules are the pure herbal drugs manufactured by natural Ayurvedic herbs. In the last ten years more than 1,50,000 patients are treated to the

entire patients satisfaction. Each 750mg of Pykure capsule contains the following ingredients: Terminalia chubularrets - 0.10 grams, Abutilon indicum - 0.10 grams, Mesua ferrea - 0.08 grams, Azadirachta indica - 0.19 grams, Saraca indica - 0.12 grams, Cuminum cyminum - 0.05 grams. It is used to treat different types of Ano-rectal disorders [4, 5].

2. MATERIALS AND METHODS:

2.1. Preparation of sample:

Take 20 capsules, gently remove the cap and body of capsules then transfer all the capsule content in 250ml volumetric flask. To the above drug contents add 150ml ethanol, all the contents of the flask should be mixed well with stirrer and tightly closed and should be kept for 24hrs with continuous shaking. After 24hrs filter the contents of the flask and discard the solid residue and filtrate is used for analysis.

2.2. Methods of Analysis of sample by Gas chromatography–Mass spectrometry (GC-MS):

GC-MS analysis of sample was performed by using Agilent 6890 GC with 59739N MSD and GC-MS equipped with Elite – I, fused with silica capillary column (cpsi 8cb :30 mm x 25mm x 0.25 mm) composed of 100% Dimethyl polysiloxane. For GC-MS detection an electron ionization system with ionizing energy of 70 eV was used. Hydrogen (99.99%) was used as the carrier gas at a flow rate of 1.5 ml and an injection volume of 2 micro liters. It was employed with split ratio of 10:1, Initial injection temp of 40 °C to final 280 °C. Ion source temp 230 °C. The oven temp was

programmed from 110 °C (isothermal for 2 minute) with a rate of 10 °C per min to 300 °C. And a scan interval 0.5 sec and fragments from 20-200 Dalton. Total GC running time was 36 minutes. The relative average peak area and retention time, molecular formula with that of molecular weight were obtained. The interpretation on mass spectrum GC-MS was conducted by using the data base of national institute standard and technology (NIST), having 62,000 patterns. The spectrum of unknown component was compared with spectrum of known component stored in NIST library. The name, molecular weight and structure of the components of the sample material were identified [6].

3. RESULTS AND DISCUSSION:

GC-MS analysis of ethanolic extract of pykure capsules:

Figure1: GC-MS chromatogram of the ethanolic extract of pykure capsules.

3,3-Diethoxy-1-propanol (Methoxymethyl) trimethyl silane Formamide, n-methoxy

Ethyl Acetate

2-Pentanone, 4-hydroxy

Acetaldehyde, methoxy-

Diethyl carbonate

Styrene

Bicyclo[4.2.0]octa-1,3,5-triene

Benzaldehyde

Ethanedioic acid, diethyl ester

Propanedioic acid, oxo-diethyl ester

D-Arabinitol

Xylitol

3,3-diethoxy-1-propanol

propane, 1,1,3,3-tetraethoxy

Data File: G:\Sarathi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 13 at 12.340 min Area: 45758926 Area % 0.08

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 Isobutyl (1-((1-methoxypropan-2-yl)oxy)propan-2-yl) carbonate	109274	1000378-28-8	43
2 (Methoxymethyl)trimethylsilane	8949	014704-14-4	40
3 Propane, 1,1,3,3-tetraethoxy-	82929	000122-31-6	38

Propane, 1,1,3,3, tetraethoxy

Data File: G:\Sarathi documents\Sarathi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 15 at 13.843 min Area: 54096964 Area % 0.09

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 1,2,3-Butanetriol	5055	004435-50-1	50
2 1,3-Butanediol, (S)-	2333	024621-61-2	47
3 13,13-Dimethyl-3,6,9-trioxo-13-s...	124243	1000213-34-9	43

1,3 butane

1,2,3-butanetriol

Data File: G:\Sarathi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 16 at 14.411 min Area: 43655719 Area % 0.08

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 Benzaldehyde diethylacetal	47687	000774-48-1	83
2 1,5,6,7-Tetrahydro-4-indolone	15895	013754-86-4	43
3 S-(-)-1-Phenylpropanol	16796	000613-87-6	42

1,5,6,7 tetrahydroindolone

S-(-)-1-phenyl propanol

Peak Number: 17 at 14.638 min Area: 777084156 Area % 1.35

The 3 best hits from each library.

The 3 best hits from each library.	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 5-Hydroxymethylfurfural	11338	000067-47-0	95
2 5-Hydroxymethylfurfural	11337	000067-47-0	85
3 5-Hydroxymethylfurfural	11339	000067-47-0	85

5- Hydroxy methyl furfural

Trans- cinnamic acid

2-propenoic acid, 3-phenyl

Peak Number: 19 at 16.342 min Area: 61390636 Area % 0.11

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 3-Buten-2-one, 4-phenyl-, (E)-	22142	001896-62-4	97
2 3-Buten-2-one, 4-phenyl-, (E)-	22137	001896-62-4	96
3 3-Buten-2-one, 4-phenyl-, (E)-	22140	001896-62-4	96

3-buten-2-one, 4-phenyl

Data File: G:\Sarathi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 21 at 17.744 min Area: 301479569 Area % 0.52

The 3 best hits from each library.	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 2-Propenoic acid, 3-phenyl-, eth...	44349	000103-36-6	98
2 2-Propenoic acid, 3-phenyl-, eth...	44370	004192-77-2	95
3 2-Propenoic acid, 3-phenyl-, eth...	44367	004192-77-2	94

2-propenoic acid, 3-phenyl-ethyl ester

Data File: G:\Sarathi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 22 at 17.971 min Area: 54551820 Area % 0.09

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 Benzaldehyde diethylacetal	47687	000774-48-1	55
2 4-Methylphenoxyacetonitrile	23181	033901-44-9	50
3 Hexestrol, O-acetyl-	171350	1000374-87-8	30

4-methyl phenoxy acetonitrile

hexestrol, o-acetyl

Tricosane

2-methyl docosane

Tetradecane, 2 methyl-

Data File: G:\Saradhi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 24 at 18.160 min Area: 92499917 Area % 0.16

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 C ₆ H ₅ CH(OH)CH ₂ C(O)OC ₂ H ₅	59212	005764-85-2	89
2 Ethanone, 2-hydroxy-1,2-diphenyl...	90434	000441-38-3	64
3 Benzenepropanenitrile, .beta.-hy...	23219	017190-29-3	50

Ethanone , 2-hydroxy-1,2-diphenyl,oxime

Benzenepropanenitrile, beta hydroxy

Dodecane, 2,6,11 -tri methyl-

(3-methoxy-2-nitrophenyl)acetic acid, 7-nitro 2,3-dihydro-1H-indazole-3-one Methyl ester

Peak Number: 28 at 20.407 min Area: 41112923 Area % 0.07

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 Heptadecane	102598	000629-78-7	59
2 4-Pentenoic acid, 2-methyl-, dod...	142136	1000406-11-5	46
3 Pentatriacontane	265354	000630-07-9	45

Pentatriacontane

3-Phenylprop-2-enoic anhydride

trans-1-cinnamoylimidazole

Undecane, 2-methyl

docosane, 7-hexyl-

Isopropyl octadecyl ether

eicosane 10-methyl-

heptadecane 2-methyl-

Data File: G:\Sarathi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 32 at 22.781 min Area: 46346808 Area % 0.08

The 3 best hits from each library.

Ref\# CAS\# Qual

C:\Database\NIST14.L

1 Hexadecanoic acid, methyl ester	130817	000112-39-0	98
2 Hexadecanoic acid, methyl ester	130822	000112-39-0	97
3 Hexadecanoic acid, methyl ester	130820	000112-39-0	96

Hexadecenoic acid, methyl ester

Data File: G:\Sarathi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 33 at 22.869 min Area: 18906830 Area % 0.03

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 8-Methoxy-[1,2,4]triazolo[4,3-a]...	48932	1000186-80-8	53
2 2-Heptanone, 6-(3-acetyl-1-cyclo...	87464	078091-85-7	53
3 7,9-Di-tert-butyl-1-oxaspiro(4,5...	136513	082304-66-3	50

7,9-di-tert-butyl-1-oxaspiro(4,5)deca-5,9diene-2,8-dione

Data File: G:\Sarathi documents\Saradhi Documents\GC-MS DATA\756-PYK-Ethanol.D\PYK-Ethanol.D
Sample : 756

Peak Number: 34 at 23.235 min Area: 2686327858 Area % 4.65

The 3 best hits from each library.

	Ref\#	CAS\#	Qual
C:\Database\NIST14.L			
1 n-Hexadecanoic acid	117417	000057-10-3	99
2 n-Hexadecanoic acid	117419	000057-10-3	99
3 n-Hexadecanoic acid	117418	000057-10-3	99

n-Hexadecanoic acid

Hexadecenoic acid, methyl ester

Ethanone, 1-(2, 6-dihydroxy-4-methoxyphenyl)-

2,2'-Dimethylbiphenyl

Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)-4-Ethylbiphenyl

Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)-5-Tert-Butylpyrogallol

Table 1. Phytocomponents found in pykure capsule by gc-ms analysis.

S.NO.	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT
1	3,3-Diethoxy-1-propanol	C ₇ H ₁₆ O ₃	48.20gm/mol
2	2-Pentanone, 4-hydroxy	C ₅ H ₁₀ O ₂	102.132gm/mol
3	Acetaldehyde, methoxy-	C ₃ H ₆ O ₂	74.01gm/mol
4	Carbonic acid, ethyl-, methyl ester	CH ₂ O ₃ C ₈ H ₁₅ NO ₄	62.024 30189.211
5	Diethyl carbonate	C ₅ H ₁₀ O ₃	118.132gm/mol
6	Bicyclo[4.2.0]octa-1,3,5-triene	C ₈ H ₈	104.152gm/mol
7	Benzaldehyde	C ₇ H ₆ O	106.12gm/mol
8	Ethane dioic acid, diethyl ester	C ₆ H ₁₀ O ₄	146.14gm/mol
9	Propane dioic acid, oxo-, diethyl ester	C ₅ H ₈ O ₃	116.116gm/mol
10	Pentane-1,2,3,4,5-pentaol	C ₅ H ₁₂ O ₅	152.145gm/mol
11	D-Arabinitol	C ₅ H ₁₂ O ₅	152.246gm/mol
12	3,3-Diethoxy-1-propanol	(CH) ₃ CCH ₂ OH	88.15gm/mol
13	Propane, 1,1,3,3-tetraethoxy	C ₇ H ₈ O ₈	220.133gm/mol
14	1,2,3-Butanetriol	C ₄ H ₁₀ O ₃	106.121gm/mol
15	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11gm/mol
16	2-Heptenal, 2-methyl-	C ₂ H ₄ O ₂	60.252gm/mol
17	2-Propenoic acid, 3-phenyl ethyl ester	C ₁₁ H ₁₂ O ₂	176.125gm/mol
18	4-Methylphenoxyacetonitrile	C ₉ H ₉ NO	147.177gm/mol
19	Tricosane	C ₂₂ H ₄₈	324.637gm/mol
20	Tetradecane, 2-methyl-	C ₁₅ H ₃₂	212.421gm/mol
21	Ethanone, 2-hydroxy-1,2-diphenyl-, oxime	C ₁₄ H ₁₃ NO ₂	227.269gm/mol
22	2-Bromo dodecane	C ₁₂ H ₂₅ Br	249.236gm/mol
23	Dodecane, 2,6,11-trimethyl-	C ₁₅ H ₃₂	212.421gm/mol
24	Hexadecane, 2,6,10,14-tetramethyl-	C ₂₀ H ₄₂	282.556gm/mol
25	Acridine	C ₁₃ H ₉ N	79.13gm/mol
26	(3-Methoxy-2-nitrophenyl)acetic acid, methyl ester	C ₉ H ₉ N O ₅	211.173gm/mol
27	Pentatriacontane	C ₃₅ H ₇₂	492.96gm/mol
28	3-phenylprop-2-enoic anhydride	C ₁₈ H ₁₄ O ₃	278.307gm/mol
29	trans-1-Cinnamoylimidazole	C ₉ H ₈ N ₂	144.177gm/mol
30	Undecane, 2-methyl-	C ₁₈ H ₃₀	246.438gm/mol
31	Isopropyl octadecyl ether	C ₉ H ₂₂ O ₂	162.273gm/mol
32	Docosane, 7-hexyl-	C ₂₈ H ₅₈	394.772gm/mol
33	Eicosane, 10-methyl	C ₂₁ H ₄₄	296.583gm/mol
34	Hexadecenoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45gm/mol
35	8-Methoxy-[1,2,4]triazolo[4,3-a]pyridine-3-thiol	C ₆ H ₅ N ₃	119.127gm/mol
36	2,2'-Dimethylbiphenyl	C ₁₄ H ₁₄	182.144gm/mol
37	4-Ethylbiphenyl	C ₁₂ H ₁₄	182.266gm/mol
38	Hydroquinone, TMS derivative	C ₆ H ₆ O ₂	110.11gm/mol
39	Benzene, 1-methyl-4-(phenylmethyl)-	C ₈ H ₁₀ S	138.228gm/mol
40	5-tert-Butylpyrogallol	C ₁₀ H ₁₄ O ₃	182.219gm/mol
41	Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl	C ₉ H ₁₀ O ₃	185.175gm/mol
42	2-Nonyl-1-ol, diethyl acetal	C ₁₁ H ₂₄ O ₂	188.311gm/mol
43	9-Octadecenoic acid (Z)-, methyl ester	C ₁₃ H ₂₄ O ₂	212.333gm/mol
44	11-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.495gm/mol
45	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	282.48gm/mol
46	1,1'-Biphenyl, 2,2'-diphenoxy-	C ₁₂ H ₁₂ N ₂	184.242gm/mol
47	17,21-Dimethylheptatriacontane	C ₃₉ H ₈₀	549.069gm/mol
48	trans-Geranylgeraniol	C ₂₀ H ₃₄ O	290.491gm/mol
49	S-(-)-1,2,4-Butanetriol, 2-acetate	C ₄ H ₁₀ O ₃	106.121gm/mol
49	Styrene	C ₈ H ₈	104.152gm/mol
50	1,3-Butanediol, (S)-	C ₄ H ₁₀ O ₂	90.122 gm/mol
51	S-(-)-1-Phenyl propanol	C ₉ H ₁₂ O	136.194 gm/mol
52	1,5,6,7-Tetrahydro-4-indolone	C ₈ H ₉ NO	135.168 gm/mol
53	Benzaldehyde diethylacetal	C ₁₁ H ₁₆ O ₂	180.247 gm/mol
54	Trans-Cinnamic acid	C ₉ H ₈ O ₂	148.158 gm/mol
55	Tridecane, 7-hexyl-	C ₁₉ H ₄₀	268.529 gm/mol
56	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.4 gm/mol
57	Resorcinol, TMS derivative	C ₆ H ₆ O ₂	110.1 gm/mol
58	8-(2-Nitrophenoxy)octan-1-ol	C ₁₄ H ₂₁ O ₄	267.325 gm/mol
59	Eicosane, 2,6,10,14,18-pentamethyl	C ₂₅ H ₅₂	352.691 gm/mol
60	Oleic acid	C ₁₈ H ₃₄ O ₂	282.47 gm/mol
61	1-Bromo-11-iodoundecane	C ₁₁ H ₂₃ Br	235.209 gm/mol
62	tert- Hexadecanethiol	C ₄₈ H ₉₉ AuS ₃	969.467 gm/mol

63	Supraene	C ₁₉ H ₁₀ Br ₄ O ₅ S	331.35 gm/mol
64	Isopropyl octacosyl ether	C ₄ H ₁₀ O	74.123 gm/mol
65	2-Methyltriacontane	C ₃₁ H ₆₄	436.853 gm/mol
66	2-Methylpentacosane	C ₁₆ H ₃₄	226.448 gm/mol
67	Squalene	C ₃₀ H ₅₀	410.73 gm/mol
68	Hexadecane, 7,9-dimethyl-	C ₁₈ H ₃₈	254.502 gm/mol
69	Triacontane, 11,20-didecyl	C ₅₀ H ₁₀₂	703.366 gm/mol
70	Nonadecane	C ₁₉ H ₄₀	308.773 gm/mol
71	Disulfide, di-tert-dodecyl	C ₂₄ H ₃₀ S ₂	402.787 gm/mol
72	Tricosane	C ₂₃ H ₄₈	324.637 gm/mol
73	Eicosane	C ₂₀ H ₄₂ O	282.529 gm/mol
74	Hentricontane	C ₃₁ H ₆₄	436.85 gm/mol

4. CONCLUSION

Ayurvedic herbal formulations are widely used in allopathic era for their effectiveness and reduced risk of side effects. Pykure is a poly herbal capsule containing *Mesua ferrea*, *Terminalia chebularates*, *Cuminum cyminum*, *Azadiracta indica*, *Saraca indica*, *Abutilon indicum*, *Picrorhiza kurroa* and *Red ochre*. Label claims different dosages for varied pharmacological activity in Bleeding hemorrhoids, fissures and rectal swelling, prolapsed of rectum, fistula. For quest of compounds present in poly herbal formulation. Spectroscopic studies mainly Gas chromatography and Mass spectroscopy was carried out and 74 known compounds were reported and matched with NIST library of IICT.

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REFERENCES:

1. http://www.holistic-online.com/Herbal-med/hol_herb-intro.htm
2. <https://www.pharmatutor.org/articles/review-quality-control-herbal-drugs>
3. <https://www.mydr.com.au/complementary-medicine/herbal-medicine>
4. <https://www.indiamart.com/proddetail/pykure-capsules-for-piles-2172090130.html>
<http://www.herbalbodyindia.com/indications.html>
6. Seema Firdouse, jyoti gupta, Parwez Alam, GC-MS Analysis of *Amorphophallus Campanulatus* Decne Tuber. American journal of pharmtec research Vol. 6, issue 3, 2016, 791-797.