Research Article

Antioxidant Assay and GC-MS Analysis of One Sidha Medicine Swasa Kudori Tablets.

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ABSTRACT

Swasa kudori is a sidha preparation used for treatment of asthma and related respiratory disorders. The present study deals with the antioxidant assays and GC MS analysis of this medicine. Swasakudori is made with *Calotropis procera* flowers and *Piper nigrum* seeds. The antioxidant assays such as DDPH, FRAP and Hydrogen peroxide scavenging assays indicated that Swasakudori has strong antioxidant activity. The GC MS analysis results indicated the presence of some medicinally important biomolecules like Piperine, Pyrrolidine, 4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-, Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, n-Hexadecanoic acid, Phenol, 2,4-bis(1,1-dimethylethyl)-, Diethyl Phthalate, Octadecanoic acid, Benzoic Acid, 9, 12-Octadecadienoic acid (Z,Z)-. The properties of these molecules are known for medicinal activities similar to those of Swasakudori. Some molecules like Benzamide, Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxopentyl]-, 2-methoxy-N-(2,4-dimethoxyphenethyl)-, 5-Isopropyl-2,8-dimethyl-9- oxatricyclo[4.4.0.0(2,8)] decan-7-one, Cyclohexanamine N-cyclodecylidene- and 5. 4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]- were also found for which no medicinal activities are reported. Further work is being undertaken to investigate scientific validity of this medicine.

Keywords: Swasa Kudori, *Calotropis procera, Piper nigrum,* GC MS, Antioxidant, Piperine, Octadecanoic acid.

INTRODUCTION

The ancient science of Sidha medical practice was developed in Tamil Nadu, India by the "Sidhhars" literally meaning "accomplished people" or in other words the "Rishis." This system is similar to Ayurveda and deals with the three main humors, Vatham (Wind), Pitham (Bile) and Kapham (Phlegm). These systems are based on the premise that any disease is a manifestation of the imbalance of these three humors and medicines are given to correct the balance, or in other words homoeostasis, which leads to the cure of the disease. The system believes in customized medicine for each patient, since each individual has a different body constitution, habits and psychological disposition and may be exposed to different environmental conditions and even the spiritual aspect of the patient. Thus this system is meant for the overall well being of a patient apart from treating a particular disease.

Although quite in vogue in different parts of Tamil Nadu, like other forms of alternative medicines such as Ayurveda and Unani, not much of scientific validation has been done on the mode of action, pharmacology, pharmacokinetics, possible short terms and long term benefits or side effects etc. There is a lack of statistical data to prove the efficacy of this system. Due to these lacunae, these systems are being criticized or rejected by the scientific world. But slowly, over the recent years, scientists, Sidha practitioners, Ayurvedic practitioners, Government organizations, research organizations etc. have risen to the occasion and some reports are being published across the world.[1-23]

The present study is a modest approach in understanding the scientific basis of one such sidha medicine, namely, "Swasakudori" used for the treatment of asthma, cough and other respiratory disorders. Swasa kudori is a sidhha preparation which is made of two major constituents, *Calotropis* flowers (*Calotropis procera*) and pepper (*Piper nigrum*). One part each of pepper fruits and flowers of *Calotropis* are dried separately, ground to fine powder and mixed with sufficient quantity of leaf juice of *Calotropis* to make pills of 200 mg each. One or two pills per day along with *Calotropis* decoction or as prescribed the physician is the dosage. This medicine is effective for bronchitis, asthma, cough and consumption. There is paucity of reports on the scientific aspects of Swasakudori. Punitha have evaluated the physico-chemical and HPTLC finger printing of Swasa kudori.[24] The present study deals with the antioxidant assay and GC MS analysis of Swasa kudori to identify the active bio molecules and trying to correlate the claim of this medicine as effective treatment for asthma, cough etc.

This is one step in the direction of validating this drug scientifically.

It is a well known fact that both *Calotropis* and Pepper have many medicinal properties and are in use in traditional system of Sidhha and Ayurvedic medical practice. Among the many scientific reports available on medicinal values of *Calotropis* a few are being sighted here. Sharma have reviewed the therapeutic potential of *Calotropis*.[25] This plant is used for leprosy, elphentiasis, fever, menorrhagia, malaria and snake bite by local people. It is reported to be schizonticidal, nematocidal (Rakesh), anti microbial (Kareem 2008), antiinflamatory (Kumar and Basu, 1994), anticancer (Smith, Choedon, Mathur), antipyretic (Dewan), antioxidant (Joshi, Roy), hepato-protective (Setty) and anti-nociceptiive, (Soares).[26-36] It contains important bioactive and medicinal compounds like organic carbonate, cystine protease, procerain, alkaloids, flavonoids, sterols etc.

Pepper (***Piper nigrum***)

Black pepper is a common spice. This is a dried unripe fruit of the climbing plant belonging to family Pipereraceae. The dried or fried seeds are powered and used for various culinary and medicinal use. In Ayurveda it is known as Kapha virodhini (works against Phlegm). The decoction of Pepper is used for curing cough. This is also digestive stimulant and used for treatment of diarrhea, lack of appetite and dyspepsia.[37]

Pepper plays a great role in digestion, useful for low appetite, sluggish digestion, abdominal pain, toxins and borborygmus. Its anthelmentic qualities help to remove worms. The drug stimulates the thermal receptors and increases secretion of salvia and gastric mucous. It has an antimicrobial effect. It influences liver and metabolic function, and has been shown to have insecticide effect (Gruenwald, 1998).[38] It has also other pharmacological activities viz. antioxidant, anticonvulsant, sedative, insecticidal, pesticidal, muscle relaxant, antipyretic, anti-inflammatory, antifungal, hepatoprotective, antimicrobial, antiulcer, antibacterial, lipolytic etc. (Sharma).[39] Meghwal and Goswami, 2012 have reviewed the chemical and physiological aspects of pepper.[40]

MATERIALS AND METHODS

The medicine Swasakudori was procured from a standard Sidha pharmacy at Chennai. The medicine was subjected to antioxidant study by three methods, namely, DPPH, FRAP and Hyderogen peroxide scavenging activity assay. The medicine was also subjected to GC MS analysis by processing it suitably.

Antioxidant study

DPPH Assay (1, 1-diphenyl-2-picrylhydrazyl) (Blios, 1958)[41]

The sample was dissolved in 3 different solvents (Ethanol, Methanol and Water) in 1mg/ml concentration and used as stock. From the stock, various concentrations (100, 200, 300, 400mg) were taken for further analysis.

Respective solvents were taken as negative control.

Conc. = Concentration of the sample

OD = OD of the sample

Neg. Control = The Solvent

Activity = Neg. Control – OD / Neg. Control

% of Activity = Activity/100

IC50 = 50 – c value / m value

IC50/ml = IC50/3 (3 ml of DPPH for the assay. To find the activity in 1 ml, the value had been divided by 3).

FRAP Assay (Pulido)[42]

Sample of Swasakudori was dissolved in Methanol

Triplicates had been put for the Processes.

Conc = Concentration of the sample

OD = OD of the sample

Linearity (y) = mx + c

M = Slope

C = The point x crosses y axis

X = OD – c value / m value

mM Fe/mg = X value / concentration x 1000

Mean = Average of mM Fe/mg

STDEV = Standard Deviation for mM Fe/mg

Hydrogen Peroxide Scavenging Activity (Ruch)[43]

Sample of Swasakudori was dissolved in Methanol and water.

Triplicates had been put for the Processes.

Conc = Concentration of the sample

OD = OD of the sample

Neg. control = The Solvent

Activity = Negative control – OD / Negative control

% of activity = Activity / 100

Mean = Average of % of Activity

STDEV = Standard Deviation of % of Activity

Graph = (For Mean of % of Inhibition vs samples) Drawn using 2D clustered column

GC MS Analysis of Swasakudori.

The medicine, Swasakudori was subjected to GC MS analysis as per standard procedure. The metabolites in the samples were identified using a P2010 gas chromatography with thermal desorption system TD20 coupled with mass spectroscopy (Shimadzu). The

ionization voltage 70ev and GC was conducted in the temperature programming mode with a Restek column (0.25mm, 60m, XTI-5). The temperature in the initial column was 80°C for 1 min, and then increased linearly to 70°C to 220°C held for 3 min followed by linear increased temperature 100° C up to 290°C and held for 10min.

The injection port temperature was 290°C and the GC/MS interface was maintained at 29°C, the samples were introduced via an all glass injector working in the split mode with helium carrier gas low rate with 1.2 ml per minute.

The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS.

The relative percentage of each extract constituent was expressed with peak area normalization.

RESULTS AND DISCUSSION

DPPH Assay results are shown in Table 1.

Table 1: Indicates the results of DDPH assay with ethanol, methanol and water solutions of Swasakudori.

S. No	Solution	Conc.	OD	Neg. Control	% Activity	m value	C value	IC50	IC50/ml
1	Ethanol	100	1.063	1.295	17.915	0.063	6.25	693.889	231.2963
2		200	0.99	1.295	23.552				
3		300	0.964	1.295	25.559				
4		400	0.840	1.295	27.953				
5	Methanol	100	1.028	1.295	20.6178	0.082	6.054	535.9268	178.6423
6		200	0.951	1.295	26.5637				
7		300	0.927	1.295	28.4169				
8		400	0.809	1.295	37.5289				
9	Water	100	0.959	1.295	25.9459	0.88	9.251	463.0568	154.3523
10		200	0.859	1.295	33.6679				
11		300	0.834	1.295	35.5984				
12		400	0.786	1.295	39.3050				

From the table it is clear that the water extract of Swasakudori has indicated maximum antioxidant which was represented as the lowest value (154.3523)

FRAP Assay (Pulido)

Sample was dissolved in Ethanol for the assay.

Table 2: Indicates the antioxidant activity of Swasakudori.

	Con.	OD	m Value	c Value	X	mM Fe(II)/mg	Mean	STDEV
Ethanol	100	0.165	0.0274	0.1086	2.058394	20.58394161		
	100	0.152	0.0274	0.1086	1.583942	15.83941606		
	100	0.14	0.0274	0.1086	1.145985	11.45985401	15.96	4.56

The results indicate that Swsakudori is positive towards FRAP assay showing antioxidant activity.

Hydrogen Peroxide Scavenging Activity (Ruch)

Table 3 shows the hydrogen peroxide scavenging activity of Swasakudori.

The sample was dissolved in Ethanol. Triplicates had been put for all the Processes.

Table 3: Indicates the Hydrogen peroxide scavenging activity of Swasakudori.

	Con.	OD		Neg. Control	% Activity	Mean	STDEV
Ethanol	100	0.348	0.748	0.534759	53.47593583		
	100	0.324	0.748	0.566845	56.68449198		
	100	0.358	0.748	0.52139	52.13903743	54.10	2.34

The above assay results also indicate that Swasakudori has good antioxidant potential.

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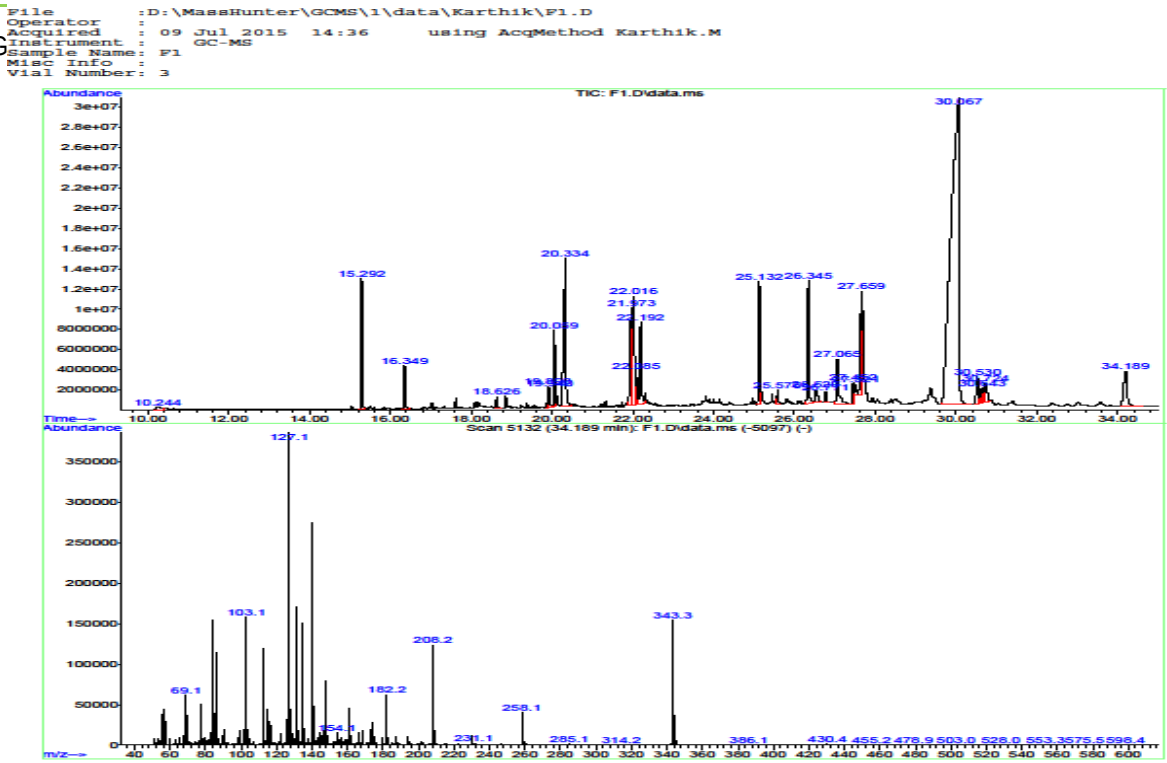

Figure 1: The GC MS graphs of Swsakudori.

Table 4: Shows the GC MS analysis results indicating retention time, name of the compound, molecular formula, molecular weight and the peak percentage value.

S. No	Retention Time (Min)	Compound	Molecular formula	Molecular weight	Peak (%)
1.	10.244	Benzoic acid	$C_7H_6O_2$	122	0.274
2.	15.292	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	2.635
3.	16.349	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	1.006
4.	18.626	3-Hexadecanol	$C_{16}H_{34}O$	242	0.266
5.	19.890	Eicosane, 2-methyl-	$C_{21}H_{44}$	296	0.434
6.	19.940	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0.382
7.	20.059	5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)]decan-7-one	$C_{14}H_{22}O_2$	222	1.734
8.	20.334	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.270
9.	21.973	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	4.613
10.	22.016	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	3.429
11.	22.085	Cyclohexanamine, N-cyclodecylidene-	$C_{16}H_{29}N$	235	0.962
12.	22.192	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.473
13.	25.132	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	3.200
14.	25.576	4-Hexadecenoic acid, DMOX derivative	$C_{20}H_{37}NO$	307	0.356
15.	26.345	Benzamide, 2-methoxy-N-(2,4-dimethoxyphenethyl)-	$C_{16}H_{17}NO_4$	287	3.825
16.	26.520	Agaricic acid	$C_{22}H_{40}O_7$	416	0.648
17.	26.771	17-Pentatriacontene	$C_{35}H_70$	490	0.327
18.	27.065	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	358	2.067
19.	27.452	4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-			0.694
20.	27.521	Piperine	$C_{17}H_{19}NO_3$	285	0.319
21.	27.659	4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-	$C_{20}H_{31}NO_3$	333	4.266
22.	30.067	Piperine	$C_{17}H_{19}NO_3$	285	53.208
23.	30.530	Pyrrolidine, 1-(1-oxo-11,14-eicosadienyl)-	$C_{24}H_{43}NO$	361	1.276
24.	30.643	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	$C_{10}H_{10}O_2$	162	0.657
25.	30.724	Pyrrolidine, 1-(1-oxo-11,14-eicosadienyl)-	$C_{24}H_{43}NO$	361	1.010
26.	34.189	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxopentyl]-	$C_{17}H_{23}NO_3$	289	2.609

1. Pyrrolidine, 1-(1-oxo-11, 14-eicosadienyl)-

Johari have found the inhibitory and cytotoxic activities of 28 Pyrrolidine type of compounds.[44]

2. Piperine and Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxopentyl]- are related compounds having diverse biological and supportive therapeutic activities like radio-protective, immuno-modulatory and anti tumor activities, antidepressant, anticonvulsant, anti-nociceptive and anti-arthritic.

It helps in the absorption of selenium, vitamin B and Beta carotene as well as other nutrients.

Among the various properties of piperine, the most important is that it facilitates the bioavailability of medicines by depressing the activity of drug metabolizing enzymes. It helps in the absorption of selenium, vitamin B and Beta carotene as well as other nutrients. Dendrite elongation inhibition activity of piperine was reported by Rao.[45-53]

3. 4, 5, 6, 7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-

This compound belongs to isoxazole-substituted compounds which are reported to have significant activities like anticancer (Rouvier, Rastogi, Chen) antimalarial (Gamage, 1994), anti-inflammatory (Chen), analgesic [Sondhi] etc.[54-59]

4. Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

This ester of unsaturated fatty acid is reported to have skin and nerve tonic properties thus keeping the cells healthy (Dhevi and Elango, 2015; Okwu and Morah 2006).[61,62]

5. n-Hexadecanoic acid

N-Hexadecanoic acid (palmitic acid)- This is a good Antibacterial, Cytotoxic (Dinesh Kumar, 2015) and antioxidant (Lalitharani).[63-64]

6. Phenol, 2, 4-bis (1,1-dimethylethyl)-

Phenol, 2, 4-bis (1, 1-dimethylethyl)- This is a good antioxidant, (Ajayi), antifungal (Rangel-Sanchez) and Anti-bacterial compound (Yogeshwari).[64-66]

7. Diethyl Phthalate

Diethyl Phtalate is used in cosmetics but higher concentration may result is teratogenic and endocrine disrupting activity (Koo and Lee, 2004).[67]

It also has antibacterial activity (Premjanu).[68]

8. Octadecanoic acid

Octadecanoic acid is Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, anti - eczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, antipsychotic and insectifuge (Dandekar).[69]

9. Benzoic Acid

Benzoic acid is reported to have anticancer, and benzoic acid derivatives possess antibacterial, antifungal properties (Vidhu and Evans, 2015; Terreaux).[70,71]

10. 9, 12-Octadecadienoic acid (Z,Z)-

This compound has a number of medicinal properties like Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Hemolytic and 5-Alpha reductase inhibitor (Rajeswari).[72]

The reports of medicinal role is not available for Benzamide, Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxopentyl]-, 2-methoxy-N-(2,4-dimethoxyphenethyl)-, 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)] decan-7-one, Cyclohexanamine N-cyclodecylidene- and 5. 4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-.

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