



Phytochemical and GC MS Analysis of an Ayurvedic Formulation, Patolakaturohinyadi Kwatham

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ABSTRACT

Medicinal efficacy of most of the ayurvedic and siddha preparations is time tested. Patolakaturohinyadi kwatham is a known medicine for anorexia, vomiting, skin diseases, jaundice, viral infections and liver diseases. Phytochemical and GC MS analytical study was conducted on this medicine. It was observed that saponin was present in all the three extracts whereas phytochemicals like steroids, amino acids, flavonoids, coumarins and carbonyl compounds were absent in all the three extracts. The GC MS analysis revealed the presence of some very important bio molecules like Propanoic acid 3, 3'-thiobis- didodecyl ester, Piperine, Ergosterol, 1 H- Imidazole, 1-(1-oxooxtadecyl)-derivative, Trideca-1,7,11-triene-1,1-dicarbonitrile, 4,8,12-trimethyl derivative and Cyclopropanecarboxylic acid undec-2-enyl ester, which have known medicinal properties. These results substantiate the medicinal effects of Patolakaturohinyadi kwatham as claimed by Ayurveda.

Keywords: Patolakaturohinyadi kwatham, Phytochemical, Ayurvedic, Sidha, Piperine, Ergosterol.

INTRODUCTION

Patolakaturohinyadi Kwatham is a very famous Ayurvedic medicine in paste form. It is used in the treatment of anorexia, vomiting, skin diseases, jaundice, viral infections and liver diseases. It is widely used in the treatment of skin diseases involving itching, pigmentation and burning sensation. It is used in the treatment of fever of Kapha and Pitta origin. It is a potent antitoxic medicine used for liver detoxification. It improves digestion and relieves anorexia. It is a potent antimicrobial medicine. The main ingredients of Patolakaturohinyadi kwatham are, Patola (*Trichosanthes dioica*), Katurohini (*Picrohiza kurroa*), Chandana - Sandal wood (*Santalum album*), Madhusrava (*Leptadenia reticulata*), Guduchi (*Tinospora cordiafolia*) and Patha (*Cissampelo spariera*). All these ingredient plants have many medicinal properties and the combination of these plants in formulating Patolakaturohinyadi kwatham is of great significance. It is observed that the medicinal potential of this medicine not only reflects the individual role of each plant but also enhances the role, significantly.

Patola(Patal): *Trichosanthes dioica*

Trichosanthes dioica is a vegetable used extensively in north India, known as Patal or Parwal. Gupta and Pagoch 2014, have reviewed its various medicinal properties.¹ This plant contains Vitamin A, Vitamin C, Tannins, Saponins, Alkaloids, Peptides and Triterpenes. The seed extract contained 7-oxidihydrokaro undiol-3-benzoate

and one main phytosterols, namely, ethyl cholest-7-enol. The seeds contain lectin.

This plant has apoptogenic, antiinflammatory, antileishmanial, chemopreventive and antiproliferative, Anti-hyperglycemic & antihyperlipidemic activity and laxative properties.³⁻⁷

Katurohini: *Pycorrhiza kurroa*

Pycorrhiza kurroa also known as Kutki has two active bitter compounds, Picroside I and Picroside II. These molecules are known for their hepatoprotective activity and also against toxins.^{8,9} The antioxidant role of this plant was studied by Tiwari and Kant.^{10,11}

Chandana (Sandal): *Santalum album*

Sandal is an age old medicinal plant and it is used for many diseases. Rao have reviewed the various medicinal properties of Sandal.¹² The antihyperglycemic and antihyperlipidemic effect of sandal on diabetes was reported by Kulkarni.¹³ Cardioprotective role of sandal was studied by Khan.¹⁴ It functions as a brain tonic.¹⁵ Its antiulcerogenic property was demonstrated by Ahmed in Rats.¹⁶

Madhusrava: *Leptadenia reticulata*

The roots and whole plant is mainly used as general tonic (Rasayana) which revitalizes the body for weak, convalescing patients.¹⁷ Its medicinal value has been studied by Bhatt for general debility, involuntary seminal discharge and as anti venom against snake bite.¹⁸ This



plant exhibits properties like antiepileptic, hepatoprotective, anti-anaphylactic and antibacterial.¹⁹⁻²²

Guduchi: *Tinospora cordifolia*

This plant is known for its variety of uses in Ayurveda. Some of the active components present in the plant are alkaloids, steroids, diterpenoid lactones, aliphatics and glycosides.²³

This plant is of great interest to researchers across the globe because of its wide ranging medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritis, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities.²⁴⁻²⁶

Patha: *Cissampelos pareira*

This plant also has a variety of medicinal values due to the presence of important medicinal components like Pelosin, O-methylcurine, I-curine Cissamine, Cissampareine, Hyatin, Bebeerine, Cycleanine, Tetrandine and Beriberine, Cissampeline, Cissampoline, Dicentrine, Insularine, Pareirine, Hyatinine, Pareirubrine A, Pareirubrine B, Pareitropone, Norimeluteine, Cissampeloflavone, D-Quercitol, and Grandirubrine.²⁷

This plant is used for cramps, gynecological problems, digestive problems like colic pains, poor digestion, constipation, diuretic etc.

There is an accumulation of scientific evidence to prove this plant's multifarious medicinal properties.

Reports on this plant as antinociceptive, antiarthritic, cardiotoxic, antihemorrhagic, antifertility, antioxidant, neuroprotective, antidiuretic, hepatoprotective and antioxidant.²⁸⁻³⁵

In the present study Patolakaturohinyadi kwatham is subjected to phytochemical analysis, antioxidant study and GC MS analysis.

This knowledge gained thereby could lead to a better evaluation of this medicine at scientific level.

MATERIALS AND METHODS

Collection of samples: Patolakaturohinyadi kwatham was obtained from standard Ayurvedic shop from Chennai.

Patolakaturohinyadi kwatham Ingredients:

This medicine is prepared from the herbs in Patoladi gana of Ashtanga Hrudayam, an Ayurvedic treatise.

- Patola - *Trichosanthes dioica*
- Katurohini - *Picroohiza kurroa*
- Chandana - Sandal wood- *Santalum album*
- Madhusrava - *Leptadenia reticulata*
- Guduchi - *Tinospora cordiafolia*
- Patha - *Cissampelos pareira*

Preparation of sample

About 5g of sample was taken and dissolved in 50ml of distilled water and it was kept undisturbed for 10 hours. Another 5gm of sample was taken and dissolved in mixture of ethanol and water in the ratio of 1:1. Raw sample was taken directly for tests.

Phytochemical analysis

The preliminary phytochemical analysis of the herbal medicine was conducted on raw sample, for the presence of alkaloids, flavonoids, anthroquinones, triterpenoids, cardiac glycosides, amino acids phytosterols, carbonyls, quinines, coumarins, phlobatanins, phenolic compounds etc. based on the protocols of Eazhisaivallabi, Adetuyi and Trease and Evans).³⁶⁻³⁸

Test for alkaloids

The extract of the powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, one portion was treated with equal amount of Dragendorff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively.

The appearance of creamy precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids.

Test for saponins

About 0.5 g of the extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.

Test for tannins

About 0.5 g of extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for steroids

2 ml of acetic anhydride was added to 2 ml of extract of each sample along with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids

1 ml of 10% NaOH was added 3 ml of the extract. Appearance of yellow colour would indicate the presence of flavonoids.

Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red



colour in the ammonical layer indicates the presence of anthraquinones.

Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy-sugar characteristic of cardioids.

Test for amino acids

To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO₄ solution was added. A violet colour indicated the presence of peptide linkage of the molecule.

Test for Tri-Terpenoids

5ml of each extract was added to 2ml of chloroform and 3ml of con. H₂SO₄ to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids.

Test for Phlobatannins

When crude extract sample was boiled with 2 % aqueous HCl the formation of red precipitate was taken as evidence for the presence of phlobatannins.

Test for Quinones

Dilute NaOH was added to the 1 ml of crude extract. Blue green or red coloration would indicate the presence of quinones.

Test for Coumarin

10 % NaOH was added to the extract and chloroform was added for observation of yellow colour, which shows the presence of Coumarin.

Phenolic compounds

The extract (500 mg) was dissolved in 5 ml of distilled water.

To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Detection of Phytosterols

Liebermann- Burchard's test: The extract (50mg) was dissolved in 2ml acetic anhydride. To this 1-2 drops of conc. Sulfuric acid was added, along the sides of the test tube. An array of color changes showed the presence of phytosterols.

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of Patolakaturohinyadi kwatham

S. No.	Phytochemicals	(Raw Sample)	(Sample in Distilled Water)	(Ethanol:Water)
1	Saponins	+	+	+
2	Tannins	+	-	-
3	Triterpenoids	-	+	+
4	Quinones	+	-	-
5	Steroids	-	-	-
6	Amino acids	-	-	-
7	Cardiac glycosides	-	+	+
8	Anthroquinones	-	-	+
9	Flavonoids	-	-	-
10	Alkaloids	-	-	-
11	Phytosterol	+	-	-
12	Coumarines	-	-	-
13	Phenolic compounds	+	-	-
14	Phlobatannin	-	-	+
15	Carbonyl	-	-	-

Present = +; Absent = -

The result of phytochemical analysis is shown in Table 1. It was found that saponin was present in all the three extracts whereas phytochemicals like steroids, amino acids, flavonoids, coumarins and carbonyl compounds were absent in all the three extracts.



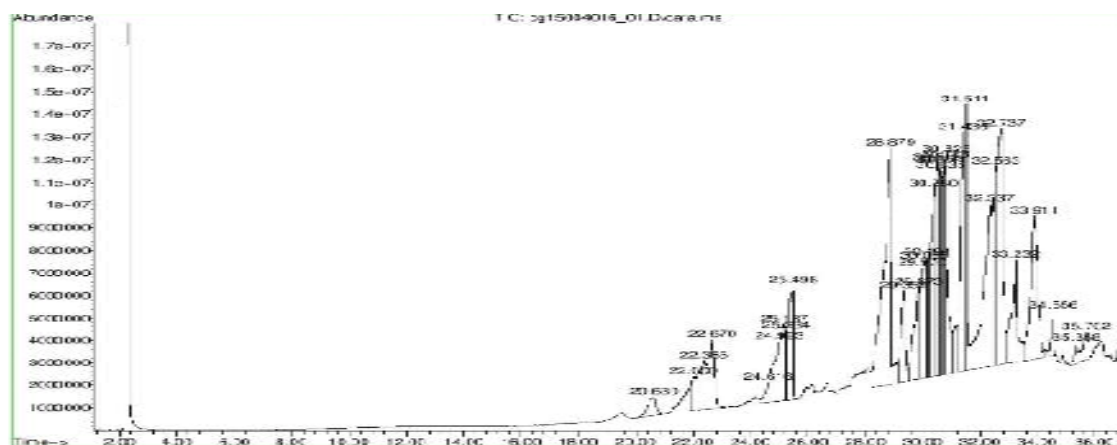


Figure 1: The GC MS Graph of Patola katurohinyadi Kwatham.

Table 2: GC MS Profile of Patola katurohinyadi kwatham (Reference Library-NIST05a.L)

S.No.	Phyto-component	% Peak Area	Retention Time (RT) In Min.
1.	Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy) propyl ester	0.94	20.634
2.	1, 2- Benzenedicarboxylic acid, diisooctyl ester	0.60	22.002
3.	1, 2- Benzenedicarboxylic acid, diisooctyl ester	1.46	22.360
4.	Phthalic acid, dodecyl 2-ethylhexyl ester	2.96	22.666
5.	1 H- Imidazole, 1-(1-oxooctadecyl) – derivative	0.48	24.617
6.	Hexadecaneperoxoic acid, 1, 1-demethyl-3-[(1-oxohexadecyl) oxy] propyl ester	2.38	24.995
7.	Hexadecaneperoxoic acid, 1, 1-demethyl-3-[(1-oxohexadecyl) oxy] propyl ester	2.16	25.189
8.	Hexadecaneperoxoic acid, 1, 1-demethyl-3-[(1-oxohexadecyl) oxy] propyl ester	0.33	25.229
9.	1-pentene-3-one, 2-methyl- derivative	4.19	25.495
10.	Phthalic acid, 2-ethylhexyl tridecyl ester	11.01	28.875
11.	1, 3, 5- Traiazin-2-amine, N-cyclohexyl-4, 6-bis[2,2,2-trifluoro-1-(trifluoromethyl) ethoxy] derivative	2.59	29.355
12.	2, 6-octadine, 4- methyl derivative	3.15	29.876
13.	Cyclopropanecarboxylic acid undec-2-enyl ester	1.79	29.968
14.	Cyclopropanecarboxylic acid undec-2-enyl ester	2.25	30.080
15.	5-Thia-10-azaspiro decane-7-carbonitrile, 7,8-bis(trifluoromethyl)-1,1,3,3,- tetramethyl-2,9-dioxo-derivative	1.29	30.193
16.	Cyclopropanecarboxylic acid undec-2-enyl ester	4.64	30.377
17.	Cyclopropanecarboxylic acid undec-2-enyl ester	3.87	30.520
18.	Cyclopropanecarboxylic acid undec-2-enyl ester	1.83	30.550
19.	Trideca-1,7,11-triene-1, 1-dicarbonitrile, 4,8,12-trimethyl derivative	2.07	30.632
20.	Trideca-1,7,11-triene-1, 1-dicarbonitrile, 4,8,12-trimethyl derivative	2.96	30.714
21.	Cyclopropanecarboxylic acid undec-2-enyl ester	1.55	30.785
22.	Cyclopropanecarboxylic acid undec-2-enyl ester	3.85	30.826
23.	Bis (2-ethylhexyl) Phthalate	5.18	31.429
24.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	3.96	31.510
25.	Cyclopropanecarboxylic acid undec-2-enyl ester	8.16	32.337
26.	4-Tosyl-8,9,17,18-dibenzo-1, 7-diehia-4,10,16-triazacyclooctadeca-8,17-diene	4.81	32.562
27.	Trideca-1,7,11-triene-1, 1-dicarbonitrile, 4,8,12-trimethyl derivative	8.07	32.736
28.	1 H- Imidazole, 1-(1-oxooctadecyl) – derivative	2.97	33.236
29.	1, 4-Napthalenedione, 2- (3,7,11,15,19,23,27-heptamethyl-2,6,10,14,18,22,26-octacosaeptaenyl)-3-methyl- derivative	6.58	33.910
30.	Ergosterol	0.76	34.554
31.	Piperine	0.53	35.360
32.	Propanoic acid, 3, 3'-thiobis-didodecyl ester	0.94	35.697

From the GC MS analysis as represented in Figure 1 and Table 2, it was observed that some major components like propanoic acid 3 3'-thiobis- didodecyl ester, Piperine, ergosterol, 1 H- Imidazole, 1-(1-oxooxtadecyl) – derivative, Trideca-1,7,11-triene-1, 1-dicarbonitrile, 4,8,12-trimethyl derivative, Cyclopropanecarboxylic acid undec-2-enyl ester were obtained.

Propanoic acid 3 3'-thiobis- didodecyl ester used as antioxidant.

Piperine has diverse biological and supportive therapeutic activities like radioprotective, immunomodulatory and anti tumor activities, antidepressant, anticonvulsant, antinociceptive, and anti-arthritis.⁴⁰⁻⁴⁸ 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.⁴⁹ In Patola the role of Piperine could be its anti-inflammatory, antioxidant and its role as facilitator of bio-availability of other medicines.

Ergosterol is a sterol found in cell membranes of fungi and protozoa, serving many of the same functions that cholesterol serves in animal cells. Because many fungi and protozoa cannot survive without ergosterol, the enzymes that create it have become important targets for antifungal drugs. Ergosterol is a provitamin form of vitamin D₂ and exposure to ultraviolet light causes a chemical reaction that produces vitamin D₂. The role of ergosterol in Patolakaturohinyadi kwatham could be its facilitating antifungal drug by mimicking with the ergosterol present in fungal cell wall.

Imidazole is incorporated into many important biological molecules. The most pervasive is the amino acid histidine, has an imidazole side-chain. Histidine is present in many proteins and enzymes and plays a vital part in cell metabolism, particularly intracellular buffering and functions of hemoglobin, in the structure and binding functions of hemoglobin.⁵⁰ Histidine can be decarboxylated to histamine which is a cause of allergies. Imidazoles are used in antifungal preparations.⁵¹

Other biological activities of the imidazole pharmacophore relate to the downregulation of intracellular Ca⁺⁺ and K⁺ fluxes, and interference with translation initiation.⁵² 1, 2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester is a cytotoxic compound which was tested on human cancer cell lines.⁵³ Cyclopropanecarboxylic acid derivatives are antidepressant agents.⁵⁴

Trideca-1, 7, 11-triene-1, 1-dicarbonitrile, 4, 8, 12-trimethyl derivative molecules are considered to be good radiation absorbing agents.

1, 4-Naphthalenedione, 2- (3,7,11,15,19,23,27-heptamethyl-2,6,10,14,18,22,26-octacosaeptaenyl)-3-methyl- derivative is Vitamin K2 type.

The presence of such compounds like piperine, ergosterol, imidazole etc in Patola clearly indicates that these compounds were formed during the preparation of this medicine from the constituent plants. It is of interest to find that the medicinal values of each constituent plant and that of Patolakaturohinyadi kwatham match thus indicating scientific pharmacological efficacy of this medicine. Further work is in progress to ascertain other parameters like pharmacology, pharmacokinetics and toxicology of this medicine.

CONCLUSION

The results revealed that the presence of compounds like piperine, ergosterol and imidazole clearly substantiate the medicinal value of Patolakaturihinyadi kwatha as claimed by Ayurveda. Further investigation on the isolation and characterization of the molecules for their medicinal role is under way.

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