Research Article

Antioxidant Study and GC MS Analysis of an Ayurvedic Medicine 'Talisapatradi Choornam'

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ABSTRACT

The present study deals with the antioxidant assay and GC MS analysis of one Ayurvedic medicine Talisapatradi Churnam. This medicine is used to treat respiratory and digestive disorders. The medicine was subjected to DPPH, FRAP and Hydrogen Peroxide scavenging assays and it was found that it has good antioxidant potential. The GC MS analysis results indicated the presence of twenty six bio molecules of which some of them like n-Hexadecanoic acid, Octadecanoic acid, piperine, Phenol, 2, 4-bis (1, 1-dimethylethyl), Glycerol 1-palmitate, 7, 9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Eicosane, 2-methyl- derivative, Octadecanoic acid esters, 2-(3,4-Methylenedioxyphenyl) cyclohexanone and 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)]decan-7-one were present in higher quantities whereas some minor compounds like Heptadecane, 1,3-Benzodioxole, 5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1α,3aα,4β,6aα)]-, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Heneicosane, Heptadecane, 2,6,10,15-tetramethyl-, Isopropyl myristate, Hexadecane, 2,6,11,15-tetramethyl-, Diethyl Phthalate, β-Asarone and Geranyl-α-terpinene were also found. The study tries to correlate the medicinal activity of the medicine with the antioxidant activity and with some important bio molecules known for their similar medicinal roles.

Keywords: Talisapatradi Churnam, Antioxidant, GC MS, Ayurvedic, DPPH, FRAP, Hydrogen Peroxide.

INTRODUCTION

Ayurveda is the foundation of Indian medicinal science which deals with specific properties of drugs and various aspects of science of life and the art of healing. This practice of traditional medicine is time tested and dates back to thousands of years. The attention towards the use of Ayurvedic, Sidha and other modes of traditional and alternative medicine is gaining momentum in recent times. As compared to the allopathic drugs these medicines have proved to be cost effective, easily available and produce very low or no side effects.[2] According to the WHO, 2002 report, about 70–80% of the world's population depends on local and alternative medicines which are available as local herbs and other salts for their primary health care.[3]

The world health organization (WHO) defines traditional medicine as: "the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being (WHO, 2013).[4]

Ayurvedic formulations are made from plant sources, animal sources as well from natural salts and chemicals. Although effective, these medicines have not been scientifically validated and their efficacy tested. Due to this reason, Ayurveda, Sidha and other traditional forms of medical systems are not being universally popularized and accepted. But slowly more and more reports in this regard are coming and this is a welcome trend towards better, affordable and safe health practice.[5-23] The present work envisages in understanding some of the scientific aspects of one such medicine, Talisapatradi churnam.

Talisapatradi Churnam is an Ayurvedic medicine for treatment of respiratory and digestive disorders. This powder is a classical preparation from the text Astanga Hridaya - Rajayakshma Chikitsa of Ayurveda. It is a good remedy in acute, chronic and allergic bronchitis. It is very useful in acute exacerbation of asthma. In chronic asthma it reduces the frequency and severity of asthmatic attacks.[24]

Talisapatradi churnam consists

1. Talisapatra - *Abies webbiana* – Leaves – 12 grams
2. Maricha - *Piper nigrum* – Friuts – 24 grams
3. Pippali - *Piper longum* – Fruits – 48 grams
4. Sunthi – Dry ginger - *Zingiber officinale* – 36 grams
5. Dalchini - *Cinnamomum zeylanicum* – 6 grams

6. Ela – Cardamom - *Elettaria cardamomum* – 6 grams

7. Vamsalochna – Bamboo - (*Bambusa arundinacea*) – Stem – 60 grams

8. Cane Sugar – 384 grams

The constituents are separately powdered and mixed. Sugar binders are used to make balls or tablets.

Dosage- 1 to 3 grams along with honey or hot water, thrice a day.

This is manufactured by pharmaceutical companies such as Zandu, Baidyanath, IMIS Pharma etc.

The literature about the scientific efficacy and validation of Talisapatradi choornam is scanty. Sharma have compared the different brands of Talisapatradi medicine available for their physico-chemical parameters.[25] Sharma also have elaborated the control parameters of this drug.[26]

The medicinal values of each of the constituent plants are mentioned, so that their role in the main drug could be identified.

Talisa patra (*Abies webbiana*)

This plant is reported to have medicinal properties such as antibacterial, mast cell stabilizing, anxiolytic, anti-tumor, anti-inflammatory, antitussive and as CNS depressant.[27-29] Kumar have reviewed the pharmacognostic, phytochemical and pharmacological effects of *Abeis webbiana.*[30] Some active principles mainly monoterpenes (from essential oil), flavonoids, biflavonoid glycosides, phytosterols and diterpene glycosides (taxol like compounds) were isolated from the leaves. Anti-inflammatory effect was exhibited by (+)-pinitol, isolated from leaves of the plant.[31]

Pepper (***Piper nigrum***)

Pepper plays a great role in digestions, useful for low appetite, sluggish digestion, abdominal pain, toxins and borborygmus.[32] Its anthelmentic qualities help remove worms. The drug stimulates the thermal receptors and increases secretion of salvia and gastric mucous. It has antimicrobial effect. It influences liver and metabolic function, and has insecticidal effect.[33] It has other pharmacological activities like antioxidant, anticonvulsant, sedative, muscle relaxant, antipyretic, anti-inflammatory, antifungal, hepatoprotective, antimicrobial, antiulcer and lipolytic.[34] Meghwal and Goswami have reviewed the chemical and physiological aspects of pepper.[35] The dried or fried seeds are 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 treating cough.

Pippali *Piper longum*

Kumar have reviewed the various health benefits of *Piper longum*.[36] *Piper longum* has many important medicinal values such as anticancer, antioxidant, hepatoprotective, anti-inflammatory, immunomodulatory, antimicrobial, antihyperlipidemic, analgesic, antidepressant, antiamoebic, vasodialtory, bioavailability enhancer due the presence of piperine in it, antiobesity activity, radioprotective, cardioprotective and antifungal.[37-55]

Sunthi* (Ginger) *Zingiber officinale

Ginger is also one of the household medicines used against common cold, cough and indigestion. Its medicinal values are well documented.[56] Adel and Prakash, 2010 have reported its antioxidant properties.[57] Ginger controls vomiting and nausea during pregnancy.[58] It controls blood pressure by blocking calcium channels.[59]

Bambusa arundinacea

According to Ayurveda text, the plant Bamboo is claimed to be *medhoghna* (removing or destroying excessive fat.[60] Charaka prescribed decoction of leaves or seeds in treatment of excessive fat.[61] Singhal have reviewed the medicinal role of Bamboo.[62] Fruit and seeds act on medha dhatu and are useful in fat metabolism and obesity.[63] The other traditional uses of the plant are as emmenagogue, anti-inflammatory, astringent, anti-spasmodic, tonic and to check cattle diarrhea. The anti hyperlipidemic potential of leaves of *Bambusa bambos* was studied by Kaikini.[64]

Cinnamomum zeylanicum

Almost every part of the cinnamon tree including the bark, leaves, flowers, fruits and roots, has some medicinal or culinary use. The volatile oils obtained from the bark, leaf, and root barks vary significantly in chemical composition, which suggests that they might vary in their pharmacological effects as well.[65] Ranasinghe have reviewed the medicinal properties of Cinnamon.[66] Jayaprakasha have also critically reviewed about the biological activities of cinnamon.[67] Elumalai have studied the antimicrobial activities of oil from cinnamon bark.[68]

Elettaria cardamomum

Cardamom is another important culinary ingredient used for its characteristic aroma. Apart from the aroma it has medicinal value. Verma have reported blood pressure lowering, fibrinolysis enhancing and antioxidant activities of Cardamom.[69] Khan have shown the pharmacological basis of cardamom as medicine for asthma.[70]

The present study deals with the antioxidant assay and GC MS analysis of Talisapatradi churnam to have a better insight into its various medicinal roles as claimed by Ayurveda. This knowledge can lead to the correlation of the data with those of individual constituent's plants to validate the efficacy of this medicine.

MATERIALS AND METHODS

Talisapatradi churnam was procured from standard Sidha medical pharmacy at Chennai and was used for the study.

Antioxidant study

Antioxidant study was performed by DPPH Assay, FRAP Assay and Hydrogen Peroxide Scavenging Activity assay.

DPPH Assay (1,1-diphenyl-2-picrylhydrazyl) (Blios, 1958)[71]

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)[72]

Sample of Talisapatradi churnam 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)[73]

Sample of Talisapatradi churnam 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 Talisapatradi Churnam.

The medicine, Talisapatradi churnam 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

The antioxidant study

DPPH Assay results are shown in Table No. 1.

From the results it shows that IC50/ml was lowest value (131.966) indicating highest activity in Water solution as compared to ethanol and methanol solutions.

FRAP test Results are mentioned in Table No.2.

From the Table no. 2 it is clear that methanol solution Talisapatradi churnam indicated antioxidant activity.

Hydrogen peroxide scavenging assay results of Talisapatradi churnam is shown in Table No.3

From the results it is clear that the Methanolic solution of Talisapatradi churnam indicated more antioxidant activity (4.48) when compared with that of water (2.39).

GC MS Analysis results of Talisapatradi Churnam are shown in Figure No. 1 and Table No. 4.

Table no. 4 indicates more quantities of some biomolecules like n-Hexadecanoic acid, Octadecanoic acid, piperine, Phenol, 2, 4-bis (1, 1-dimethylethyl), Glycerol 1-palmitate, 7, 9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Eicosane, 2-methyl- derivative, Octadecanoic acid esters, 2-(3,4-Methylenedioxyphenyl)cyclohexanone and 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)]decan-7-one.

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

S. No	Solution	Conc.	OD	Neg.Control	% Activity	m value	C value	IC50	IC50/ml
1	Ethanol	100	1.064	1.295	18.147	0.067	5.343	666.5224	222.1741
2		200	1.029	1.295	19.922				
3		300	0.887	1.295	26.640				
4		400	0.840	1.295	29.498				
5	Methanol	100	1.004	1.295	17.8378	0.083	4.216	581.6145	183.8715
6		200	1.029	1.295	20.5405				
7		300	0.887	1.295	31.5057				
8		400	0.840	1.295	35.1351				
9	Water	100	0.948	1.295	27.7953	0.108	7.243	395.8981	131.966
10		200	0.879	1.295	32.1235				
11		300	0.820	1.295	36.6753				
12		400	0.654	1.295	49.4980				
13				1.295					

Table 2: Indicates the FRAP assay patterns of Talsapatradi churnam in Methanol solution.

Methanol	100	0.178	0.0274	0.1086	2.532847	25.32846715		
	100	0.167	0.0274	0.1086	2.131387	21.31386861		
	100	0.156	0.0274	0.1086	1.729927	17.29927007	21.31	4.01

Table 3: Indicates the Hydrogen Peroxide Assay results of Talisapatradi Churnam.

Methanol	100	0.487	0.748	0.34893	34.89304813		
	100	0.487	0.748	0.34893	34.89304813		
	100	0.429	0.748	0.426471	42.64705882	37.48	4.48
Water	100	0.658	0.748	0.120321	12.03208556		
	100	0.647	0.748	0.135027	13.5026738		

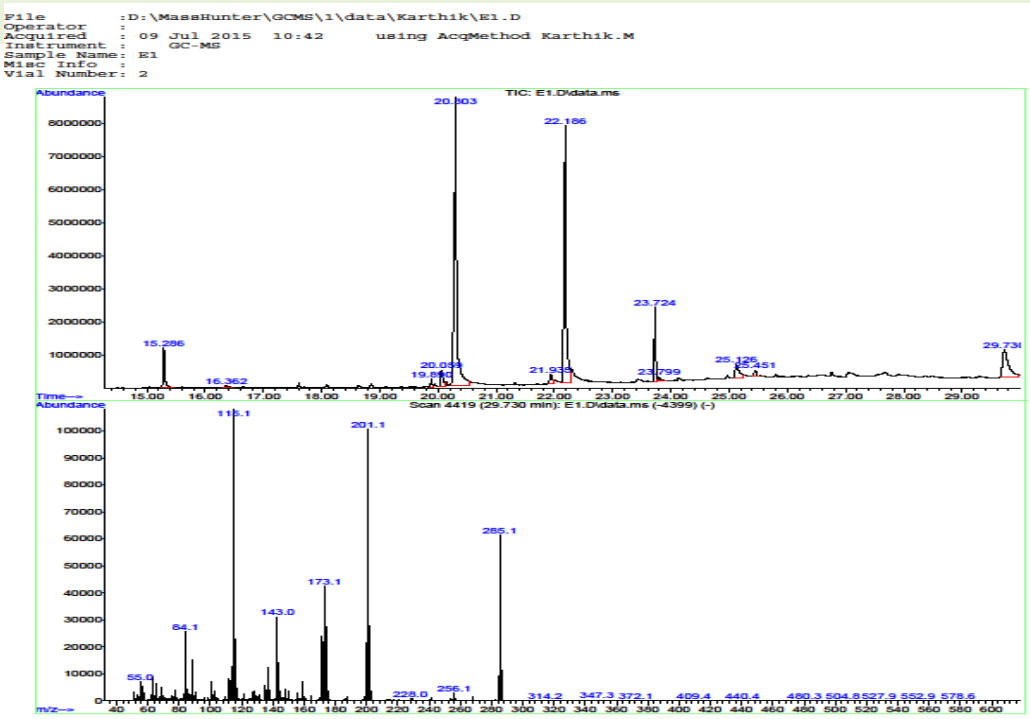

Figure 1: shows the GCMS graphs of Talisapatradi Churnam.

Table 4: Depicts the retention time, names of the compounds, molecular formula, molecular weights and % peak values.

S. No.	Retention Time (Min)	Name of the Compound	Molecular Formula	Molecular Weight	Peak %
1.	15.036	Heptadecane	C17H36	240	0.131
2.	15.286	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	3.927
3.	15.605	Hexadecane, 2,6,11,15-tetramethyl-	C20H42	282	0.100
4.	16.362	Diethyl Phthalate	C12H14O4	222	0.442
5.	16.637	β-Asarone	C12H16O3	208	0.113
6.	17.144	geranyl-α-terpinene	C20H32	272	0.046
7.	17.613	Hexadecane, 2,6,11,15-tetramethyl-	C20H42	282	0.355
8.	17.707	Heptadecane, 9-hexyl-	C23H48	324	0.045
9.	17.763	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	0.041
10.	18.088	Eicosane, 2-methyl-	C21H44	296	0.186
11.	18.639	3-Hexadecanol	C16H34O	242	0.232
12.	18.851	Isopropyl myristate	C17H34O2	270	0.345
13.	19.377	Phthalic acid, hept-4-yl isobutyl ester	C19H28O4	320	0.041
14.	19.890	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	296	0.590
15.	19.940	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	276	2.238
16.	20.059	5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)]decan-7-one	C14H22O2	222	2.137
17.	20.303	n-Hexadecanoic acid	C16H32O2	256	40.287
18.	21.329	1-Methyl(tetramethylene)silyloxyundec-2-ene	: C16H32OSi	268	0.269
19.	21.935	Eicosane, 2-methyl-	C16H32OSi	296	1.174
20.	22.186	Octadecanoic acid	C18H36O2	284	30.948
21.	23.724	2-(3,4-Methylenedioxyphenyl)cyclohexanone	C13H14O3	218	5.449
22.	23.799	Heneicosane	C21H44	296	0.448
23.	24.969	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	0.231
24.	25.126	Glycerol 1-palmitate	C19H38O4	330	2.736
25.	25.451	1,3-Benzodioxole, 5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1α,3aα,4β,6aα)]-	C20H18O6	354	0.660
26.	29.736	Piperine	C17H19NO3	285	8.832

There are some bio molecules which were present in small quantities like Heptadecane, 1,3-Benzodioxole, 5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1α,3aα,4β,6aα)]-, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Heneicosane, Heptadecane, 2,6,10,15-tetramethyl-, Isopropyl myristate, Hexadecane, 2,6,11,15-tetramethyl-, Diethyl Phthalate, β-Asarone and geranyl-α-terpinene.

The medicinal role of some of the bio molecules are discussed to find any correlation between the medicinal activity of Talisapatradi choornam and those of the bio molecules present as was found in the GC MS analysis.

1. n- Hexadecanoic Acid

n- Hexadecanoic acid is reported to have activities like antioxidant, hypocholesterolemic, nematicide, anti androgenic, as flavoring agents, hemolytic, antibacterial and cytotoxic and as 5-alpha reductase inhibitor.[74-76]

2. Octadecanoic acid

Octadecanoic acid is Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, antipsychotic and insectifuge.[77]

3. Piperine

Piperine has diverse biological and supportive therapeutic activities like radioprotective, immunomodulatory and anti tumor activities, antidepressant, anticonvulsant, antinociceptive, and anti-arthritic.[52,78-83] 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.[84] 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.[85] Dendrite elongation inhibition activity was reported by Rao.[86]

4. Phenol, 2, 4-bis (1, 1-dimethylethyl) – derivative is present in various plants and is known for its antibacterial and anti-inflammatory activities.[87]

5. Glycerol 1-palmitate: This molecule functions as soap or detergent.

6. 7, 9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione. This is a steroidal anti-mineralocorticoid agent. It functions as anti-androgen and weak progestogen properties, as well as some indirect estrogen and glucocorticoid effects, which is used primarily as a diuretic and antihypertensive. used primarily to treat heart failure, ascites in patients with liver disease, lowering hypertension, hypokalemia, secondary hyperaldosteronism (such as occurs with hepatic cirrhosis), and Conn's syndrome (primary hyperaldosteronism), frequently used to treat a variety of cosmetic conditions including hirsutism, androgenic alopecia, acne, and seborrhea in females and male pattern baldness.[88]

7. Eicosane, 2-methyl- derivative is a good antioxidant.

8. Octadecanoic acid esters are reported to be antiviral, antibacterial and antioxidant activities.[89]

9. Isopropyl myristate. Isopropyl myristate is a known compound used as skin care lotion and emmolient.[90]

10. Diethyl Phtalate is used in cosmetics but higher concentration may result is teratogenic and endocrine disrupting activity.[91]

11. β- Asarone is a known antifungal.[92]

12. Heptadecane and Octadecane, 3-ethyl-5-(2-ethylbutyl)- are known as antimicrobial and antifungal agents.[93]

The activities of Geranyl-α-terpinene, 1, 3-Benzodioxole, 5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1α,3aα,4β,6aα)]-, Heneicosane, 2-(3,4-Methylenedioxyphenyl)cyclohexanone, 1-Methyl(tetramethylene)silyloxyundec-2-ene.

Heptadecane, 2,6,10,15-tetramethyl-, Phthalic acid, hept-4-yl isobutyl ester, 3-Hexadecanol, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Heptadecane, 9-hexyl-, Hexadecane, 2,6,11,15-tetramethyl-, Heptadecane, 2-(3,4-Methylenedioxyphenyl)cyclohexanone- 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)]decan-7-one- etc are not clearly known. Further work is in process to identify their medicinal role as well.

CONCLUSION

From the above results and discussion it is possible to conclude that the various biomolecules present have antibacterial, antifungal and antioxidant properties which is reflected in the use of Talisapatradi churnam for the treatment of respiratory diseases. Further work towards identifying the exact molecules responsible for their role as medicine is under way.

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