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Bioactive Constituent Profiling of Poly Herbal Distillate Thribhaladi Dravagam and Its Therapeutic Role

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ABSTRACT

Introduction: Hydro distillation in Siddha medicine is the technique of extracting valuable principles from a pre-processed raw drug through the traditional process of distillation under a controlled temperature setting. *Thribhaladi Dravagam* (TD) is one of the polyherbal distillate formulation stated in the Tamil medical text specified for phlegmatic affections. The drug was subjected to GC-MS studies to screen its bioactive sketch. **Methods**: Two sets of raw drug samples from different locations were collected. The raw drugs were purified and pre-processed as per the classical literature and distilled in a traditional apparatus. Two distillate samples (TD1 & TD2) were prepared from the set of ingredients and were screened for its constituents. **Results**: The Gas chromatogram of TD 1 revealed 4 prominent peaks with retention time ranging from 4.84 to 8.27. TD 2 reported 8 prominent peaks with retention time ranging from 3.35 to 7.88. The study testified the presence of active volatile metabolites like aldehydes and oxygenated monoterpenes like citral and borneol, monocyclic alcoholic compounds like terpineol and organic acids like caprylic acid from both the samples. TD 2 reported more pharmacologically valuable lead molecules than TD1, which may be due to the variation in diversity of the two sets of raw drug samples. **Conclusion**: The bio constituents of TD was found to be pharmacologically significant from the review of various reputed research works supporting the traditional claim on its role in the respiratory system and for maintaining good health. Through this review of *Thribhaladi Dravagam* and its analytical part performed through GC-MS, the medical applications of TD in multiple clinical associations and as a perfect health promoter has been justified for promoting its traditional value.

KEYWORDS- Citral, Caprylic acid, GC-MS, Isoborneol, Siddha medicine, Theeneer, Thribhaladi Dravagam, Terpineol.

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INTRODUCTION

Siddha system has glorified antiquity as the original native wealth of south India and other Tamil speaking regions across Srilanka.[1] The system comprehends all the values essential for the society focusing on spiritual, moral, social, cultural, and healthcare aspects.[1] The vast materia medica, the exquisite formulary collections have been well written and documented as a consistent source of remedies for all the 4448 ailments affecting the humankind.[2-4] Herbal distillates (*Theeneer*) are one of the special classes of Siddha medicine with notable benefits to health.[5] A vast number of single herbal and polyherbal combinations, which are presented through the Siddha classical literatures, are still not in medical practice. *Thribhaladi Dravagam* is one of the formulations mentioned in the classical Siddha medical texts of Siddhar Yakob indicated for phlegmatic afflictions. So farNo studies are available on this effective distillate medicine. Therefore, an initial attempt was done to screen TD through Gas Chromatography Mass Spectrometry (GC-MS) with an aim to profile its active bio constituents.

METHODOLOGY

Ingredient Details

Thiribhaladi dravagam is a blend of six herbal materials under the class of Thiribhalai and Thirikadugu.[7] (Table 1) for this studies two different sets of raw drug samples were procured, one collected from country merchants of South India and the other from North East Indian market. (Fig.1)

Table 1: Ingredients of *Thiribhaladi Dravagam*

S.No	Ingredient	Botanical Name	Part Used	Quantity
Thiribhalai				
1	Kadukkai	*Terminalia chebula* Retz.	Dry fruit (seed removed)	100 g
2	Nellikkai	*Phyllanthus emblica* L.	Dry fruit (seed removed)	100 g
3	Tantrikkai	*Terminalia bellirica* (Gaertn.)Roxb.	Dry fruit (seed removed)	100 g
Thirikadugu				
4	Chukku	*Zingiber officinale* Roscoe.	Dry Rhizome (Outer skin removed)	100 g
5	Milagu	*Piper nigrum* L.	Dry fruit	100 g
6	Thippili	*Piper longum* L.	Dry Berry	100 g
7	Water			6 Litres

Figure 1: Ingredients of *Thiribhaladi Dravagam*

Method of Preparation of Distillate Sample

All the six ingredients were purified as per the classical texts, dried, coarsely powdered and soaked in water for a period of 3 days.[5-6] On the 4th day, the distillate was prepared in a traditional still and preserved for GC-MS studies. Two sets of distillates were prepared from the different samples for observing variation in analysis.

Gas Chromatography- Mass Spectrometry (GC- MS)

GC-MS is a reputed analytical tool for the identification and quantitation of bioactive principles (organic and volatile) within a hydro distillate.[8] This instrument is very reliable for screening distillate preparations like Theeneer. The study was executed with Agilent 7890B GC coupled to 5977A MSD besides NIST Ver.2.1 MS data library Specification.

RESULTS

Organoleptic characters

Both the samples of TD were colourless having a volatile tinge with clarity implied. The sample distillate TD 2 was having a more pleasant aroma and a slightly pungent taste than TD 1.

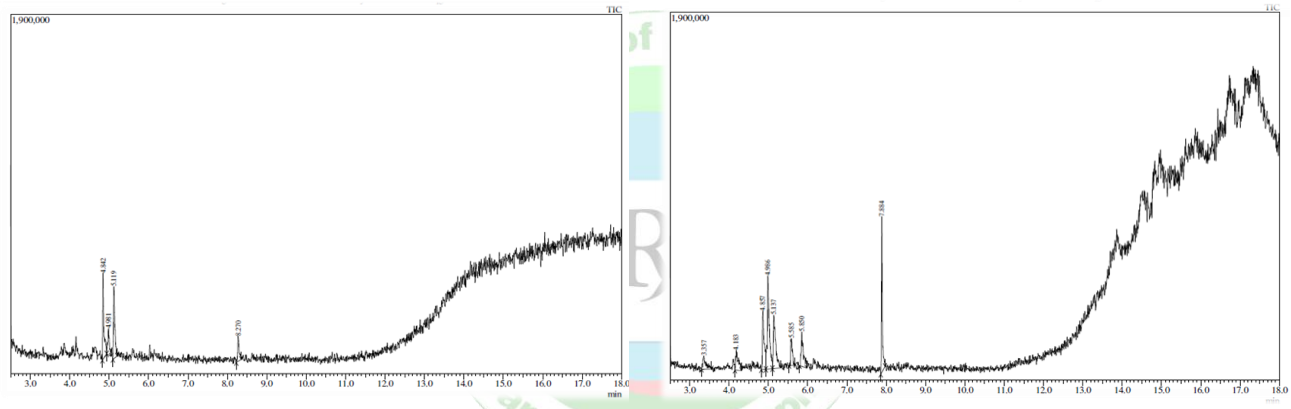
GC- MS reports of TD1 & TD2

The Gas chromatogram of TD 1 revealed 4 prominent peaks with retention time ranging from 4.84 to 8.27. TD 2 reported 8 prominent peaks with retention time ranging from 3.35 to 7.88. The detailed report of the compounds

retention time (RT), Peak intensity rank, Molecular weight, chemical formula, and chemical structure are presented in Table 2 and Fig 2-3.

Table 2: Gas Chromatography report of *Thiribhaladi Dravagam* (TD1 & TD2)

Peak no	Retention Time	%Peak Area	Peak Intensity Rank	Mol. Wt.	Name of the Compound	Chemical Formula
TD 1						
1	4.84	35.09	2	154	*Isoborneol*	$C_{10}H_{18}O$
2	4.98	15.33	3	144	*Octanoic Acid*	$C_8H_{16}O_2$
3	5.11	35.79	1	154	*p-menth-1-en-8-ol (Alpha terpineol)*	$C_{10}H_{18}O$
4	8.27	13.79	4	208	*Asarone*	$C_{12}H_{16}O_3$
TD 2						
1	3.35	2.92	8	154	3-Terpinen-1-ol	$C_{10}H_{18}O$
2	4.18	4.37	7	298	Linoleic acid chloride	$C_{18}H_{31}O_{10}$
3	4.85	12.84	3	154	Isoborneol	$C_{10}H_{18}O$
4	4.98	20.44	2	154	p-menth-1-en-8-ol	$C_{10}H_{18}O$
5	5.13	11.72	4	154	3-Cyclohexene-1-methanol	$C_{10}H_{18}O$
6	5.58	5.89	6	168	Cyclopropanemethanol	$C_{11}H_{20}O$
7	5.85	7.56	5	152	Trans-Citral	$C_{10}H_{16}O$
8	7.88	34.26	1	208	Asarone	$C_{12}H_{16}O_3$



TD1 **TD2**

Figure 2: Gas Chromatography of *Thiribhaladi Dravagam*

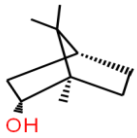
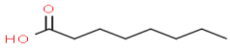
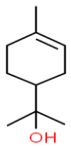
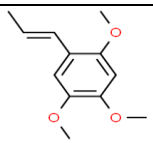
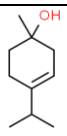
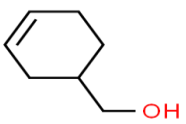
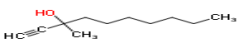
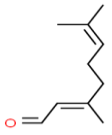
Isoborneol	**Octanoic Acid**	**p-menth-1-en-8-ol**
		
Asarone	**3-Terpinene-1-ol**	**Linoleic acid chloride**
		
3-Cyclohexene-1-methanol	**Cyclopropanemethanol**	**Trans-Citral**
		

Figure 3: Important Bioactive Compounds from TD 1 & TD 2

DISCUSSION

In the Indian system of medicine (ISM) like Siddha and Ayurveda, the herbal distillates are mentioned as *Theeneer* or *Arka*. [5, 9] The process of hydro distillation selectively extracts the valuable principles from a pre-processed raw drug under a controlled temperature setting.[10] Numerous traditional methods are adopted that enables the yield of standard quality distillate.[11] An herbal distillate, which satisfies all the parameters of traditional qualities like standard colour, taste, aroma and volatile tinge, is considered equal to elixirs.[10] Apart from its specific indication, herbal distillates particularly prepared from spice resources are meant for regularizing good digestion and appetite because of the presence of natural pungent principles. [12] The quality parameter of TD was in the standard track. The clarity specifies the purity of the distillate and the presence of volatile tinge is an indication of good extraction of essential oils from the ingredients. The pleasant typical aroma and the slightly pungent taste is the reflection of the attributes of the raw drugs used.[10]

The role of TD in managing phlegmatic diseases could be highlighted from the medicinal properties of raw drugs used in it and the traditional attributes supporting the claim. [2, 13] Muphalai or thiriphalai, the combination class of herbs constituted by 3 myrobalans (*Terminalia chebula* Retz, *Phyllanthus emblica* Linn and *Terminalia bellirica* (Gaertn.)Roxb) that is highly praised in ISM for its universal role in and as a good health promoter.[14]The combination has been therapeutically accepted for a broad spectrum of conditions like ulcers of the anorectal region, gastric ulcers, gastro intestinal ailments, skin diseases, bronchial asthma, cough, anaemia, leucorrhoea, leucoderma and liver diseases, ascites, hypertension, and bleeding disorders.[2, 13] It is an effective anti-ageing formula classified under rejuvenator (Kayakalpam) category.[15] Likewise, the combination class of three spices (Mukadugu- *Zingiber officinale* Roscoe, *Piper nigrum* Linn, and *Piper longum* Linn.) are considered as the perfect balancer of three humors, further indicated for indigestion, convulsions, toxic bites, head diseases, face diseases, ear diseases and rheumatic pain. All the ingredients of Muphalai and Mukadugu are traditionally used for phlegmatic ailments in traditional Siddha medicine.[2,13] These group of drugs are the key ingredients in many herbal distillates described in ancient literature works of ISM.

TD is an elite blend of Muphalai and Mukadugu indicated for phlegmatic disorders.[6] The distillate was subjected to preliminary GC-MS studies to support the traditional claim and to further evaluate other leading therapeutic potential of the drug. This may substantiate the usage of TD in a wide range of diseases apart from the indicated conditions.

GC-MS of both the samples of TD reported the presence of volatile metabolites like aldehydes and oxygenated monoterpenes (e.g. citral and borneol), monocyclic alcoholic compounds (e.g. Terpineol) and organic acids (e.g. Caprylic acid). TD 2 reported more pharmacologically valuable lead molecules than TD1, which may be due to the variation in diversity of the two sets of raw drug samples collected from two different locations.

Monoterpenes are considered as the best-documented principle constituent of essential oils from aromatic raw drugs.[16] The distillates prepared from most of the spices impart a fine pleasant aroma due to the presence of these natural scented compounds. The typical lemony odour of the ginger distillate is due to the presence of Alpha terpineol and citral, and the characteristic pepper odour of the distillate from the *Piper nigrum* Linn is due to the presence of the monoterpene isoborneol.[17-18] We may infer that the pungent and pleasant aroma of TD is may be due to these two principles.

Citral an important bioactive monoterpene aldehyde and a pharmacologically important molecule reported in *Zingiber officinale* Roscoe and *Piper nigrum* Linn.[17-18]The therapeutic potential of the ginger rhizome as a bronchodilator is well supported by the presence of this active component citral. In one of the study, it significantly blocked the rat tracheal chain contraction induced by carbachol (CCh).[19] Another study described its action in the central nervous system as an anticonvulsant and anxiolytic. The studies were piloted in important animal models of epilepsy by the induction of MES (Maximal electroshock test) and through the administration of PTZ (Pentylenetetrazole).[20] Citral has also been verified for its inhibitory effects on transient receptor potential (TRP) channels in dorsal root ganglion neurons. This activity of sensory inhibition is considered as a medical miracle for the cure of numerous symptoms like pain and itching that involves the superficial sensory nerves and skin.[21] Many reports show that citral shows a vital part in the deterrence of threatening cancer development especially hepatocarcinogenesis.[22] Diet -induced obesity studies in the rat model demonstrated the role of citral in the reduction of accumulated abdominal fat, weight gain to high calorie diet and hyperinsulinemia than the control group. The glucose tolerance was also improved noticeably. This infers the role of this wonderful molecule in dealing with the burden of lifestyle diseases. [23] Here the presence of Trans-citral (Geranial) has been reported in the GC-MS study, which is one of the principle isomer of citral.

The terpineol compounds are natural monocyclic monoterpene tertiary alcohols with a comprehensive array of biological properties. They are found abundantly in herbal sources particularly in the essential oil of *Zingiber officinale* Roscoe, *Piper nigrum* Linn and *Piper longum* Linn.(24) GC-MS of TD reported the presence of two compounds α-terpineol and its isomer 3-Terpinen-1-ol, an aromatic volatile alcohol that is one of the important isomer of terpineol holding vast no of reputed biological activities.[24] Many of the studies prove its role as an antiproliferative on human erythroleukemic cell lines. (K562 cells). [25]The activity of alpha terpineol in blocking calcium ion entry in voltage dependent calcium channels,

consecutively ensuing in vasorelaxation is well recognized in one investigation.[26]

The monoterpene Isoborneol is actively reported in raw drugs like *Zingiber officinale* Roscoe, *Piper nigrum* Linn and, *Piper longum* Linn.[27] It is one of the active principles spotted in both the samples of TD. The cytoprotective role of isoborneol has been investigated in human neuroblastoma cell lines (SY5Y cells) against the OHDA neurotoxin induced apoptosis. The treatment with isoborneol significantly declined the reactive oxygen species (ROS) generated by the neurotoxin. Furthermore, the apoptosis was reversed. This indicated the super potential of the lead molecule Isoborneol for tackling neurodegenerative conditions like Parkinson's disease associated with oxidative stress.[28]

Isoborneol, terpineol and, isocitral have the caring role in the respiratory system as antispasmodics, bronchorelaxant, antiallergic, antihistaminic, expectorant and mucolytic.[27] These molecules were found to be beneficial in respiratory diseases like cough, bronchitis and bronchial asthma. The effectiveness of borneol and terpineol in blocking histamine-induced bronchoconstriction (Guinea pig tracheal chain method) has been reported in one study.[29]Caprylic acid (octanoic acid) is a saturated medium chain fatty acid normally found in most of the plant seeds and kernels. [27] Its existence is reported in *Piper nigrum* Linn, *Terminalia chebula* Retz, and *Terminalia bellirica* (Gaertn.) Roxb.[30-32] The active compound asarone is most commonly found in ginger species. Almost all compounds spotted in TD have reputed biological activities as illustrated in Table 4. [27, 33.-37.]

Table 4: Pharmacologically essential compounds spotted in *Thiribhaladi dravagam*

Compounds	Proven Activity
Asarone	Anticonvulsant, Antipyretic, Anti-Spasmodic, Cardio-depressant, CNS-Depressant, Emetic, Fungicide, Hypothalamic Depressant, Myo relaxant, Neuroprotective, Sedative, Tranquilizer
Isoborneol	Anti-bacterial, Anticonvulsant, Anti-Herpetic, Anti-inflammatory, Anti-proliferant, Antiseptic, Antiviral, Antiasthmatic. Analgesic, Hypnotic, Immunostimulant, Motor- stimulant, Neuroprotective, Sedative
Terpineol	Analgesic, Anesthetic, Antiallergenic, Antiasthmatic, Anti-bacterial, Anticancer, Antihypertensive, Anti-inflammatory, Antioxidant, Anti-proliferant, Antispasmodic, Antitussive, Cholagogue, Expectorant
Trans Citral	Antiallergic, Antianaphylatic, Anti-bacterial, Antispasmodic, Anticancer, Anti-Clastogenic, Anti-helicobacteric, Anti-herpetic, Antihistaminic, Anti-inflammatory, Antileishmanic, Antioxidant, Antiseptic, Analgesic, Antispasmodic, Antiulcer, Antiviral, Antipyretic, Antiadipogenic, Anti parasitic, Bronchorelaxant, Calcium antagonist, Carminative, Digestant, Diuretic, Expectorant, Fungicide, Larvicidal, Myorelaxant, Nematicide, Sedative.
Octanoic acid (Caprylic acid)	Antiseptic, Candidicide, Fungicide

CONCLUSION

Through this review of the traditional claim of *Thiribhaladi Dravagam* and its analytical part performed through GC-MS, we may consider its medical applications in multiple clinical associations and as a perfect health promoter. The distillate may be considered as an elixir with antioxidant and tonic properties (Kalpa dravagam) due to the presence of these active bio principles. Apart from its therapeutic role in phlegmatic respiratory diseases, the distillate may be taken on a regular basis for improving digestion and metabolism, for controlling diabetes, for prevention of cardio vascular diseases and hyperlipidaemia associated with obesity, for neurodegenerative conditions, and as a supportive health supplement for cancers. As GC-MS is only one of the analytic methodical steps for understanding the bio picture of an herbal distillate, further clinical trials are needed on the side to validate its unmapped efficacy for endorsing its scientific recognition.

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