SIDDHA PAPERS
A PEER REVIEWED ONLINE JOURNAL
ISSN: 0974-2522

Siddha Papers 2017 (12) (2)
RESEARCH ARTICLE
www.siddhapapers.org

SCIENTIFIC ASSESSMENT OF MALLIMATHURA DECOCTION

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ABSTRACT

In Siddha System of Medicine Asthma is defined as Eraipunoi or swasakasam, which was characterized by tightness of chest, difficulty in breathing and cough without expectoration. It is classified into five catagories they are Vali eraippu, Iyya eraippu, Iyya vali eraippu, Mukkutra eraippu and Mel nokku eraippu. The herbal formulation was **given to the patient under proper anupanam and pathiyam** while inhaling the medicines the patients are advised to avoid dried fish, packed food, tinned food, and refrigerated food, bottle drinks etc. They are advised to take fresh foods and healthy food inorder to improve their immunity power.**Objective:** The main objective of the present investigation is to evaluate the phytochemical constituents, antioxidant potential and antibacterial activity of Siddha Cough Decoction prescribed by the Traditional Siddha Practitioner to ensure the effect of medicine. **Methods:** *Mallimathura Decoction* was exploredfor the presence of phytochemicals, antioxidant and antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris* oftheselected human pathogens.**Result:** *Mallimathura Decoction* is a polyherbal formulation prepared from 8 different herbs being used for the treatment of *kapha* (*in Tamil*) diseases. The antibacterial assay revealed that the extracts corroborate good inhibitory activity against all test pathogens. The unexplored area of *Mallimathura Decoction*towards their antioxidation effect in aqueous, silver nitrate and chloroform extracts indicated promising antioxidant activities in concentration dependent manner.**Conclusion:** The present investigation concluded that the Siddha herbal preparations of *Mallimathura Decoction* have great potential as antioxidant and antimicrobial agent against many enteric pathogens. Thus these herbal preparations can be used to control or prevent the enteric bacterial infection.

Key words: Polyherbal formulation, antioxidation, Qualitative analysis, *Mallimathura Decoction*, Kalanchi

1. Introduction

India has an eminent wealth of therapeutic agents for various ailments and diseases in our traditional system of medicine. Numerous sorts of diseases have been treated with herbal medications throughout the history of mankind. Major formulations used in Siddha are based on herbs. The medicinal herbs are used as decoctions, infusions, tinctures and powders [1]. The therapeutic value of medicinal plants depends upon the presence of one or more constituents possessing certain physiological and pharmacological activity. The main herbs are selected according to the disease and other herbs are used to enhance the effects of chief herb [2]. There is currently a large and ever expanding global population base that prefers the use of natural products in treating and preventing medical problems because herbal plants were proved to have a rich resource of medicinal properties [3]. *Mallimathura Decoction* is a polyherbal formulation with 8 different herbs composition (Tamil) *Malli, Athimathuram, Kadugurohini, Thippili, Cittrarathi, Amukkara, Veppampattai* and *Nilavembu* (Table:1).The liquid form of this Siddha Decoction is used to treat cough, cold, phlegmatic conditions, pneumonia and a wide range of kapha disease. Analyzing the phytochemicals and evaluating the antimicrobial properties of medicinal plants provides scientists with insight to know how they are medicinally effective whereas, understanding the chemical composition leads to the development of new medicines. *Mallimathura Decoction* was exploredfor the presence of phytochemicals, antioxidant and antibacterial activity against selected human pathogens.

Human existence on earth has been made possible because of the vital role played by the plant kingdom in sustaining his life. A number of phytochemical compounds have been recognized as taxonomic markers, based on these biogenetic classification were proposed. The following three classes are recognized as chemical compounds as taxonomic markers, primary basic constituents, secondary and miscellaneous substances. The living plant may be considered as a biosynthetic laboratory not only for the primary metabolites like sugars, aminoacid and fatty acids that are utilized as food by man but also for a multitude of secondary products of pharmaceutical such as glycosides, alkaloids, flavonoids, vegetable oils etc. Secondary metabolites are biosynthetically derived from primary metabolites but are more limited or usually being restricted to particular taxonomic groups. They may represent a chemical adaptation to environmental stresses serve as defensive, protective or offensive chemicals against micro-organisms, insects and higher herbivorous predators. In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. Thus it is anticipated that the phytochemicals with adequate antibacterial efficiency are used for the treatment of bacterial infections. They are also cheap, easily available and affordable. *Mallimathura decoction* is a liquefied form of Siddha formulation, which is widely prescribed to cure cough. Phytochemicals are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health. The quantitative analysis of phytochemicals showed definite physiological actions on the human body.

MATERIALS AND METHODS

2.1 Collection of plant materials

The available fresh plant materials were collected from hills and hillocks of our District and other ingredients were procured from commercial Siddha raw drug store. The required ingredients were a shade dried, powdered and stored in porcelain pots. The Siddha

formulation was prepared as prescribed in the written scripts, books and palm leaf parchments of my Grandpa and Forefathers - Traditional Vadiyars (Table -1).

Table: 1 Composition of *Mallimathura Decoction*

S.No	Siddha Name	Scientific Name	Quantity
1	Kottamalli	***Coriandrum sativum*Linn.**	**25 g**
2	Athimathuram	***Glycyrrhiza glabra* L.**	**25 g**
3	Kadugu Rohini	***Picrorhiza kurroa*Royal Ex Benth.**	**15 g**
4	Thippili	***Piper longumL.***	**15 g**
5	Chitraraththai	***Alpinia speciosa***(Wendl.) K.Schum.	**10 g**
6	Amukkara	***Withania somnifera (*L.) Dunal**	**5 g**
7	Veppampattai	***Azadirachta indica*A.Juss**	**15 g**
8	Nilavembu	***Andrographis paniculata*Wall.ex Nees**	**5 g**

2.2 Preparation of Extract

All the dried herbs and fresh leaves were triturated in household mortar and pestle without adding water. The triturated herbs were weighed (Kalanchi). Decoction is prepared by adding the ingredients and steam boiled (3:1 ratio) and filtered. The sampling decoction was subjected to maceration using different solvents aqueous, silver nitrate and chloroform for 48 hrs. The extracts were filtered and evaporated to dryness and kept for further studies.

2.3 Phytochemical Analysis of Mallimathura Decoction

The phytochemical analysis of *Mallimathura Decoction*, aqueous, silver nitrate and chloroform extract of Siddha medicine were carried out to analyse the presence of alkaloid, flavanoid, phenol, terpenoid, saponin, reducing sugar, tannin, steroid and glycosides [4 & 5]

2.4 Antioxidation Assay

2.4.1Nitric oxide Radical Scavenging

Nitric oxide radical scavenging capacity of *Mallimathura Decoction* and extracts with 0.1 ml of sodium nitroprusside (10Mm) in phosphate buffer (0.2M, pH 7.8) was mixed with different concentration was incubated at room temperature for 15 minutes. After incubation 0.2 ml of Griess reagent was added. The absorbance of the reaction mixture was read at 546nm against the blank. All the readings were taken in triplicate and Gallic acid was used as a standard [6]. The percentage of inhibition was calculated by the following equation

$$\% \text{ Nitric oxide radical scavenging capacity} = (A0-A1)/A0) \times 100$$

2.4.2 Reducing Power Activity

The reducing power activity of *Mallimathura Decoction* was determined by spectrophotometric method [7].The extract was mixed with 2.5 ml of 0.2 M Potassium phosphate buffer (pH 6.6) and 2.5 ml of Potassium ferricyanide. The mixture was incubated at 50^0C for 20 minutes was then rapidly cooled, mixed with 2.5 ml of trichloroacetic acid and centrifuged at 5000 rpm for 3 minutes. An aliquot of supernatant was diluted with distilled water and 0.5 ml of 1% ferric chloride was added and allowed to stand for 10 minutes. The absorbance was read spectrophotometrically at 700 nm. Increased absorbance indicates increased reducing power. Vitamin C was used as positive control.

2.5 Antibacterial Activity

2.5.1 Extract preparation

The sterile disks were soaked in *Mallimathura Decoction*, aqueous, silver nitrate and chloroform extracts of 100ml decoction in 200 ml solvent for 12 hours. The extracts were filtered using Whatman filter paper (125 mm) [8].

2.5.2 Growth and Maintenance of Test Microorganism for Antibacterial Studies

Bacterial cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris* were obtainedfrom the Medical College, Kulasekharam were used for antibacterial studies. Thebacteria were maintained on Nutrient Agar (NA) slants at4°C. For further study, cultures have been grown inNutrient Broth (NB) for 24 hrs as overnight cultures.

2.5.3 Disc diffusion Method

The antibacterial assay of *Mallimathura Decoction* and extracts was performed by Disk diffusion method [9].The Nutrient agar media (20ml) was poured into sterilized petridish and left to solidify at room temperature. The overnight bacterial cultures have been spread plated on these petridish using sterile L rod. The filter paper discs were placed equidistantly on inoculated media and diffusion of solution was allowed to occur for 30 minutes at room temperature. Plates were incubated at 37°C for 24 hours. The average zone of inhibition was recorded. The diameters of the inhibition zones were measured in mm.

3. Result and Discussion

Kashayam is the term used in Tamilnadu often by our ancestors to ***treat cold***, ***cough***, fever, head ache etc. It is easily prepared at home with some herbs. The present investigation was done to analyse the qualitative, quantitative, antioxidant and antimicrobial activity of Siddha Decoction prescribed to cure cough was assorted with aqueous, ethanol and chloroform extract to assess the effect of medicine. The qualitative and quantitative analysis were done to assess the presence of phytochemicals and to estimate the quantification of constituents. Antioxidant assay were done to analyse the compounds which act as radical scavengers. The clinical isolates of *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were used for the antibacterial activity. The obtained datas were tabulated and discussed.

3.1 Qualitative Analysis

Qualitative analysis of the Siddha Decoction revealed the presence of alkaloids, phenol, terpenoid, tannin, steroid and glycosides; aqueous extract of alkaloid, phenol, tannin and steroid; ethanolic extract of alkaloids, phenol and glycoside and chloroform extract of alkaloid, phenol and terpenoid (Table: 2).

Table – 2 Qualitative Analysis of *Mallimathura Decoction* and extracts

S.No	Phytochemicals	Mallimathura Decoction	Aqueous Extract	Ethanol Extract	Chloroform Extract
1	Alkaloids	+	+	+	+
2	Phenol	+	+	+	+
3	Terpenoid	+	-	-	+
4	Tannins	+	+	-	-
5	Steroids	+	+	-	-
6	Glycosides	+	-	+	-
7	Saponin	-	-	-	-
8	Reducing sugar	-	-	-	-
9	Flavanoid	-	-	-	-

+ Presence - Absence

3.2 Quantitative Analysis

Quantitative analysis of the *Mallimathura Decoction* and extracts is done to assess the quantity of phytochemical constituents. The major phytochemical constituents of Siddha *Mallimathura Decoction* (Kashayam) reported the constituents of Alkaloid 2.5± 0.000ml/l; Phenol 1.261± 0.000ml/l; Terpenoid 2.371 ± 0.002ml/l; Tannin 1.823±0.002ml/l; Steroid 2.962 ±0.000 ml/l and Glycosides 2.217 ± 0.001ml/l; aqueous extract of Alkaloid 0.341± 0.000 ml/l; Phenol 0.231± 0.001ml/l; steroid 1.371 ± 0.002 ml/l and Tannin 1.823 ± 0.001ml/l and ethanolic extract of alkaloid 0.004 ± 0.003ml/l; phenol 0.031 ± 0.001ml/l and glycosides 0.071± 0.002 ml/l and chloroform extract of alkaloid 0.104±0.001ml/l; phenol 0.053 ± 0.001 ml/l and terpenoid 0.172± 0.003 ml/l.

3.3 Antioxidation Activity

Antioxidants are compounds which act as radical scavengers, prevent the radical chain reaction of oxidation delay or inhibit the oxidation process. It has been established that oxidative stress is one among the major causative factors in the induction of many chronic and degenerative diseases. In the present investigation the antioxidation activity of nitric oxide scavenging and reducing power activity of Siddha Decoction, aqueous, ethanol and chloroform extract after the addition of chemical ingredients were measured at 523 nm using ELICO Spectrophotometer.

3.3.1 Nitric Oxide Radical ScavengingNitric oxide injuries take place for the most part through peroxynitrite route, directly oxidize low density lipoproteins, resulting in irreversible damage to the cell membrane. Nitric oxide radical scavenging of the *Mallimathura Decoction*varied from the minimum inhibition of 61.36 ± 0.020 % at low concentration (25µl) to the maximum inhibition of 76.36 ± 0.010 % at high concentration (100µl); aqueous extract varied from the minimum inhibition of 49.26 ± 0.010 % at low

concentration (25µl) to the maximum inhibition of 63.01 ± 0.011 % at high concentration (100µl); ethanolic extract varied from the minimum inhibition of 51.27 ± 0.020 % at low concentration (25µl) to the maximum inhibition of 62.67 ± 0.011 % at high concentration (100µl); chloroform extract varied from the minimum inhibition of 67.63 ± 0.011 % at low concentration (25µl) to the maximum inhibition of 71.06 ± 0.025 % at high concentration (100µl) and standard Gallic acid varied from the minimum scavenging of 63.92 ±0.020 % (25µl) to the maximum scavenging of 66.28 ±0.005 % (100 µl) (Table: 3).

Table: 3 Nitric oxide Radical Scavenging of *Mallimathura Decoction* and extracts

Concentration of Medicine and Extracts	Mallimathura Decoction	Aqueous Extract	Ethanol Extract	Chloroform Extract	Gallic acid (Standard)
25µl	61.36 ± 0.020	49.26 ±0.010	51.27±0.020	67.63±0.011	63.92±0.020
50µl	63.33 ±0.010	53.76 ±0.005	54.63±0.020	67.91±0.011	65.71±0.010
75µl	67.29 ± 0.011	57.22 ±0.015	8.26±0.011	68.06±0.025	66.27±0.020
100µl	76.36 ±0.010	63.01 ±0.011	62.67±0.011	71.06±0.025	66.28±0.005

3.3.2 Reducing Power Activity

Reducing power activity of the *Mallimathura Decoction*varied from the minimum of 56.28 ± 0.005 % at low concentration (25µl) to the maximum inhibition of 83.72 ± 0.011 % at high concentration (100µl); aqueous extract varied from the minimum inhibition of 63.26 ± 0.011 % at low concentration (25µl) to the maximum inhibition of 74.69 ± 0.000 % at high concentration (100µl); ethanolic extract varied from the minimum inhibition of 73.69 ± 0.010 % at low concentration (25µl) to the maximum inhibition of 81.96 ± 0.010 % at high concentration (100µl); chloroform extract varied from the minimum inhibition of 69.23 ± 0.020 % at low concentration (25µl) to the maximum inhibition of 75.31 ± 0.015 % at high concentration (100µl) andstandard Vitamin - C varied from the minimum inhibition of 71.74 ±0.015 % (25µl) to the maximum inhibition of 82.95 ± 0.010 % (100 µl) (Table:4).

Table: 4 Reducing Power Activity of *Mallimathura Decoction* and extracts

Concentration of Medicine and Extracts	Mallimathura Decoction	Aqueous Extract	Ethanol Extract	Chloroform Extract	Vitamin – C (Standard)
25µl	56.28±0.005	63.26±0.011	73.69±0.010	69.23±0.020	71.74±0.015
50µl	64.74±0.017	67.97±0.020	75.73±0.010	71.40±0.011	74.24±0.005
75µl	73.11±0.005	72.42±0.020	79.62±0.005	73.27±0.015	78.29±0.000
100µl	83.72±0.030	74.69±0.000	81.96±0.010	75.31±0.015	82.95±0.010

3.4Antibacterial Activity

The clinical isolates of *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were used for the antibacterial assay in nutrient agar media (20ml). The cough medicine *Mallimathura decoction* prescribed to cure the patient at the age before 18 yearsin the forenoonshowed the maximum zone of inhibition (8mm) against *Pseudomonas aeruginosa*, *Proteus vulgaris* (6mm); *Staphylococcus aureus* (5mm) and minimum zone of inhibition (2mm) against *Klebsiella pneumonia*. Aqueous extract showed the maximum zone of inhibition (3mm) against *Pseudomonas aeruginosa;Staphylococcus aureus* and *Proteus vulgaris* (2mm) *and no inhibition* against *Klebsiella pneumonia*. Ethanolic extract showed the maximum zone of inhibition (3 mm) against *Proteus vulgaris* and *Staphylococcus aureus* (2mm) whereas, minimum zone of inhibition (1mm) against *Pseudomonas aeruginosa* and *Klebsiella pneumonia* and chloroform extract showed the maximum zone of inhibition (4mm) against *Pseudomonas aeruginosa, Staphylococcus aureus* (3mm) whereas, minimum zone of inhibition(2mm) *against Proteus vulgaris* and *Klebsiella pneumonia* (Table:5).

Table: 5 Antibacterial activities of *Mallimathura Decoction* and extracts

	Inhibition Zone in diameter (mm)			
Microorganism	**Pa**	**Sa**	**Pv**	**Kp**
Decoction	8 ± 0.69	5±0.025	6±0.011	2±0.005
Ethanol	1±1.00	2±0.005	3±0.741	1±0.037
Aqueous	3±0.011	2±0.037	2±0.025	-
Chloroform	4 ±0.58	3±0.011	2±0.017	2±0.011
Amikacin	7±1.53	8±0.005	9±0.025	6±0.025

***Pseudomonas aeruginosa* (Pa)** ***Staphylococcus aureus* (Sa)**

***Proteus vulgaris* (Pv)** ***Klebsiella pneumonia* (Kp)**

Conclusion

It is obvious that the plant kingdom offers a better panorama of providing useful medicinal compounds for the treatment of numerous challenging diseases. Elucidating the chemical structure of active components of herbs also make extent for synthetic modification for better pharmacokinetic profiles. The polyherbal formulation of *Mallimathura Decoction* was evaluated for the phytochemical, antioxidation and antibacterial activity. The presence of secondary metabolites revealed that the *Mallimathura Decoction*was found to be an effective antioxidant, when it is compared to standard antioxidant compounds revealed good inhibitory activity against all test pathogens (Gallic acid and Vitamin C).

Conflict of interest statement

We declare that we have no conflict of interest**.**

Acknowledgements
Reverential thanks to my Grandpa's and Uncle, Traditional Siddha Practitioners for their treasure of knowledge in the field of Siddha Medicine. I express my whole hearted thanks and indebtedness to my Guide Dr. B. Christudhas Williams for his valuable guidance. Prayerful thanks to my Father S. Raja Rethna Dhas, Mother D. Mary Mercy Bai, Sister R. Mary Suji and Brother R. Sujin Sam for their warm support, encouragement and prayers.

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