International Journal of Reverse Pharmacology and Health Research (IJRPHR)

Research article

Estimation of Phytochemical compounds and Antioxidant potential of Siddha drug *"Thiripalai Mathirai"*

[1*]Maheshwari.B, [2]Manoharan.A, [3]Komalavalli.T

[1*]PG Scholar, [2]Professor, Head of the Department, [3]Associate Professor, Department of Pothu Maruthuvam, GSMC, Palayamkottai,Tamilnadu, India

Abstract

Background: Thiripalai Mathirai (Tablet) (TPT) is a Siddha formulation, which consists of three fruits (Thiripala), namely Terminalia chebula (Retz.), Terminalia Belerica (Gaertn.)Roxb. & Emblica officinalis (Gaertn.) used for the treatment of Iron deficiency anaemia (Pitha pandu), Hyperlipidemia, Hepato Tonic, Antioxidant and Antidiabetic activity (Nadkarni, 1976). It was evaluated for Quantitative, Qualitative estimation of Phytochemical constituents and antioxidant activity. The antioxidant activity is confirmed by total phenol estimation by using FollinCio-caiteau method. The test drug was referred in the classical siddha text "*Kadukkai Vallaraiyin Thani Maanbu*" (Hakkim.P.Mohammed Abdullah Sahib).

Objective: The objective of this study is to determine the antioxidant potential and phytochemicals in Thiripalai mathirai (TPT).

Methods: The qualitative phytochemical analysis were carried out for major compounds, such as alkaloid, phenols etc. The phytochemical analysis is detected by using Harborne and Onwukaemeand co-workers, 1999 methods. The Quantitative estimation were done through spectrophotometric methodology, detection of alkaloids and phenol estimation is using FolinCio-calteau methodolgy.

Results: The end of result concluded the presence of glycosides, alkaloids, flavonoids, phenols, terpenoids, steroids and tannins in the Thiripalai mathirai (TPT). The aqueous extract of alkaloid and total phenolic compound present in the sample was 3.2604mg and 0.52mg in 10mg respectively, which are expressed in caffeine units.

Conclusion: The presence of phytochemicals in quantitative estimation may scavenge the free radicals and it has synergetic activity as antioxidant which serves for the mankind.

Keywords: Thiripalai mathirai, Terminalia chebula, Siddha drug, Iron deficiency anaemia

Introduction

Thiripalai mathirai (TPT) is a Siddha formulation, which is prepared by equal ratio of three medicinal plants and *Annabedhi chenduram (Iron sulphate)*. *It* is used in Siddha medicine for treating various clinical conditions. Traditionally, *Thiripalai* have been used in treating Jaundice, Obesity, Cardiac diseases, UTI, Aneamia, Tuberculosis and Venereal disease (Yoga narasimhan, 2000).

Address for correspondence:

Maheshwari B

[1]Post Graduate Scholar, Department of Maruthuvam, GSMC, Palayamkottai, Tamilnadu, India

CODENJ : IJRPHR

To access this article online

Website : http://www.ijrphr.com/

DOI : 10.121/ijrphr/02.0203.310

Quick response code

How to cite this article:

[1*]Maheshwari.B, [2]Manoharan.A, [3]Komalavalli. T, Estimation of Phytochemical compounds and Antioxidant potential of Siddha drug *"Thiripalai Mathirai"* International Journal of Reverse Pharmacology and Health Research, 2019, 2(1), 25-30

Received: January, 2018.

Accepted: March, 2019.

Natural products play an important role in the field of new drugs research and development because of low toxicity, easy availability and cost effective manner. The Thiripalai composition contains chebulinc acid, tannic acid, gallic acid, resin, anthroquinone and sennoside (Nadkarni, 1976). It also contains Glycosides, Sugar, Triterpenoids and Steroids (Wealth of India, 1976). These compounds are exhibiting antibacterial (Ahmed I et al, 1998), anti fungal, anti oxidant (M.S.Blois, et al. 1958) properties. The Phenolic compounds have the scavenging activity to inhibit free radicals in the cell cycles. The production of powerful oxidants called reactive oxygen species (ROS) such as free radicals. Excessive production of free radicals, beyond the antioxidant capacity of the body can cause oxidative stress and damage to DNA.

Hence, the present study is designed to evaluate Qualitative and Quantitative estimation of phytochemical and assess the total antioxidant capacity of *Thiripalai Mathirai (TPT).*

MATERIALS AND METHODS

Collection of raw drugs

The raw drugs are collected from Nagarcoil, Tamilnadu. The collected drugs were identified and authenticated by the Medicinal Botanist at Govt. Siddha Medical College& Hospital, Palayamkottai, Tirunelveli (Table.1.)

Table 1. Ingredients of Thiripalai mathirai

Ingredients	Botanical name/ Family	Part Used	Quantity
Manjal Kadukkaithol	*Terminalia chebula (combretaceae)*	Outer covering of dried fruit	Equal ratio
Nellimulli	*Emblica officinalis (Euphorbiaceae)*	Dried fruit pulp	Equal ratio
Thaanrikkai Thol	*Terminalia bellirica (Combretaceae)*	Outer covering of dried fruit	Equal ratio
Annabedhi Chenduram	-	-	Equal ratio

Preparation of Thiripalai Mathirai

The combination of TPT mentioned in table 1 is taken in equal quantity and powdered (Choornam) mixed along with Annabedhi chenduram according to the SOP, mixed powder was ground with Vallarai juice and Nellikkai juice each 7 times, finally prepared as tablet form, size is kundrimani (130mg). The tablet was issued along with Karisalai Kudineer as adjuvant.

Extraction of Drug Thiripalai Mathirai

The aqueous extract of Thiripalai Mathirai was prepared at first. The tablet was powdered was dried at 40 ± 1 °C in incubator. Then, 25 g of the fine powder was dissolved in 250 mL of distilled water and kept in an airtight glass jar. This mixture was incubated at 37 ± 1 °C for 72 h in a Soxhlet extraction apparatus. The deep reddish brown extract of Thiripalai Mathirai was collected. Then this extract was dried in a vacuum desiccator to obtain a dry mass, stored in a refrigerator at 4 °C and used for the experiments.

Phytochemical Analysis

The following tests are used for the analysis of phytochemicals as described by Harborne and Onwukaemeand coworkers, 1999 were carried on aqueous extract of the sample. The following methods were used for this study (Table 2).

Table 2. Phytochemical analysis of Thiripalai Mathirai

Test	Method	Inference
Alkaloids	Dragandroff's test	Presence of alkaloids(+)
Flavanoids	Test for flavanoids	Presence of flavanoids (+)
Saponins	Test for saponins	Presence of Saponins(+)
Phenols	Ferric chloride test	Presence of phenols(+ +)
Steroids	Salkowski test	Presence of steroids(+)
Glycosides	Test for glycosides	Presence of glycosides (+)
Terpenoids	Test for terpenoids	Presence of terpenoids (+)
Tannins	Ferric chloride test	Presence of tannins(+)

Quantitative estimation of phytochemical constituents

Estimation of Alkaloids

The alkaloids estimation was performed by spectrophotometric method of Dragendoffs reagent as it was described by Sreevidya and Mehrotra (2003).

Stock Solution of Extracts:

10 mg of sample was accurately weighed and made upto 1ml with DMSO. The sample of Thiripalai Mathirai was centrifuged over 10 min (3000pm)to remove residual suspended particles and then 0.5ml sample were mixed with 1ml of HCl 0.1 N. Then 0.25ml of Dragendroffs reagent was added to the previous mixture for precipitation and the precipitate was centrifuged over 5 min (3000 pm).This precipitate was further washed with 0.25 ml of ethanol. The filtrate was discarded and the residue was then treated with 0.25ml of disodium solution (1%wlv)The brownish black precipitate formed was the centrifuged(5 min 3000 rpm).This residue was dissolved in 0.2 ml of concentrated nitric acid and 0.1 ml was then pipette out and mixed with 0.5 ml of thiourea solution (3% w/ v).The absorbance of this solution was measured at 435 nm against a blank containing 0.1 ml of concentrated nitric acid and 0.25ml of thiourea solution (3% w/v)

Estimation of Total Phenolic Content by Folin Cio-calteau method

Preparation of reagents

a**).** FolinCio-calteau reagent (2N): It was diluted to 1:10 ml with distilled water.

b). Sodium carbonate solution: 7.5 % solution of sodium carbonate (anhydrous) was made with distilled water

Preparation of sample solution

100µl was pipetted out from sample; *Thiripalai Mathirai* and 5ml of FolinCio-calteau reagent was added. After 5 minutes, 4ml of sodium carbonate solution was added and incubated at room temperature for 2 hours. Then, absorbance was measured at 750nm and the values obtained were interpreted in the standard graph of Gallic acid to get the milligram equivalents of Gallic acid. The Concentration of Stock value is 10mg/ml.

Preparation of Standard graph of Gallic acid

10mg Gallic acid was weighed and made up to 1ml with methanol in a 10ml standard flask. From the stock solution (10mg/ml stock solution), solutions of concentration was 100, 200, 400, 800 and 1000µg/ml respectively. 5ml of FolinCio-calteau reagent was added and 4ml of 7.5% sodium carbonate solution was added after 5 minutes. It was stirred and incubated at room temperature for 2 hours. After 2 hours, absorbances of the solutions were measured at 750nm using UV-VISIBLE spectrophotometer (Agilent, Cary 60). The absorbance values were plotted against concentration and standard graph was obtained.

Total Antioxidant Activity

Different concentrations of extracts 125-2000µg/ml from a stock concentration of 10mg/ml was obtained with 3ml of reagent solution (0.6ml H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate)..The tube containing the reaction solutions were incubated at 95c for 90 minutes. The absorbance of the solution was measured at 695nm against blank after cooling to room temperature (Methanol 0.3ml) in the place of extract was used as blank. The antioxidant activity is expressed as number of gram equivalent of ascorbic acid.

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

DISCUSSION AND RESULTS

The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [Edeogal et.al, 2005]. The Phytochemical screening of the aqueous extract of TPT was resulted in the presence of glycosides, alkaloids, flavonoids, saponins, phenols, steroids, terpenoids and tannins (Table 2).

The presence of enough amount of alkaloid was shown in quantitative estimation of alkaloids were tabulated Table 3 & 4 and graphed in figure 1.

Table 3. Estimation of Alkaloids calibrated with Caffeine units

Concentration	Absorbance
200	0.2497
400	0.4794
600	0.7415
800	0.9412
1000	1.1531

Figure 1. Standard chart for Alkaloid estimation

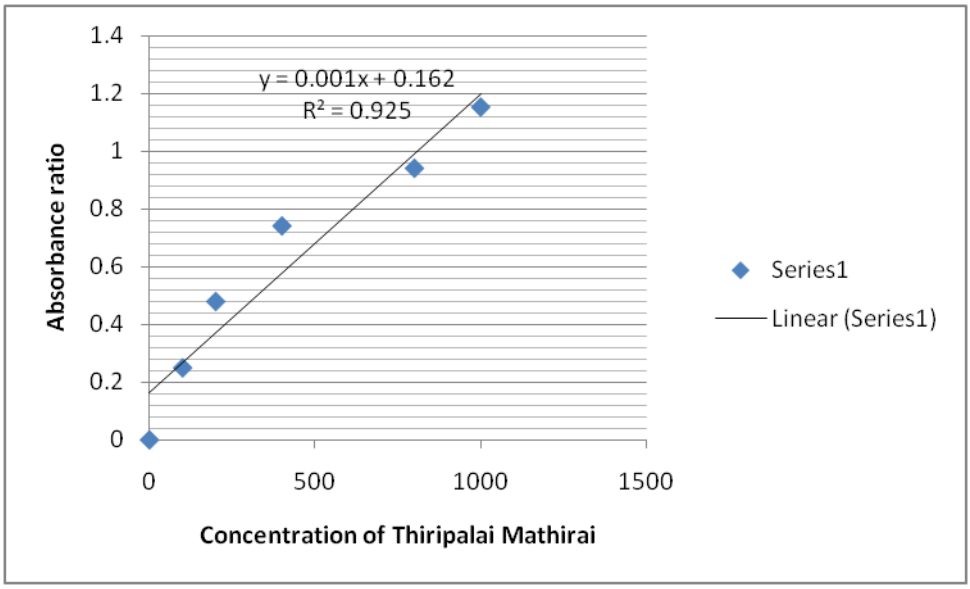

Figure 2. Standard graph of Phenol

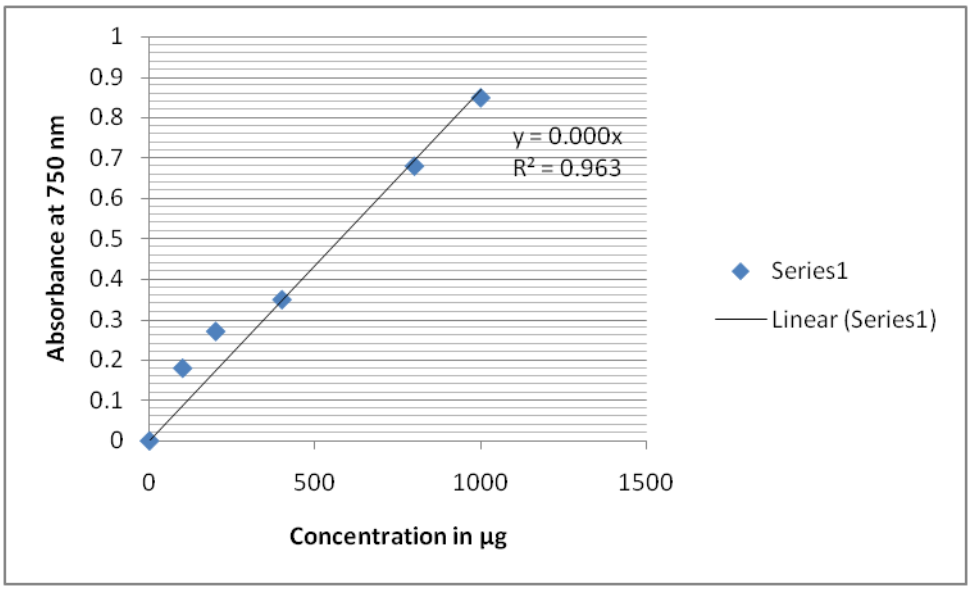

Table 4. Alkaloid estimation in Thiripalai Mathirai

Sample name	Absorbance	Amount of alkaloid in terms of caf-
Aqueous extract of TPT	0.8151	3.2604mg in 10mg sample

In figure no.1, the R value (0.92) lies within the limit of 1, it was presented with non deviation of compound estimation for Thiripalai Mathirai (TPT). According to Table no 5 & 6, Figure 2 showed the aqueous extract of Thiripalai Mathirai has 0.52mg of Phenols in 10mg sample.

The R value (0.96) lies within the limit of 1 and showed the non deviation of compound estimation in Thiripalai Mathirai.

Table 5. Phenolic compound estimation with standard Gallic Acid

Concentration(µg/mL)	Mean OD(750nm)
100	0.1801
200	0.2712
400	0.3500
800	0.6806
1000	0.8500

ISSN 2589 3343

Figure 3. The graphical representation of dose dependency and its antioxidant potential of Thiripalai Mathirai

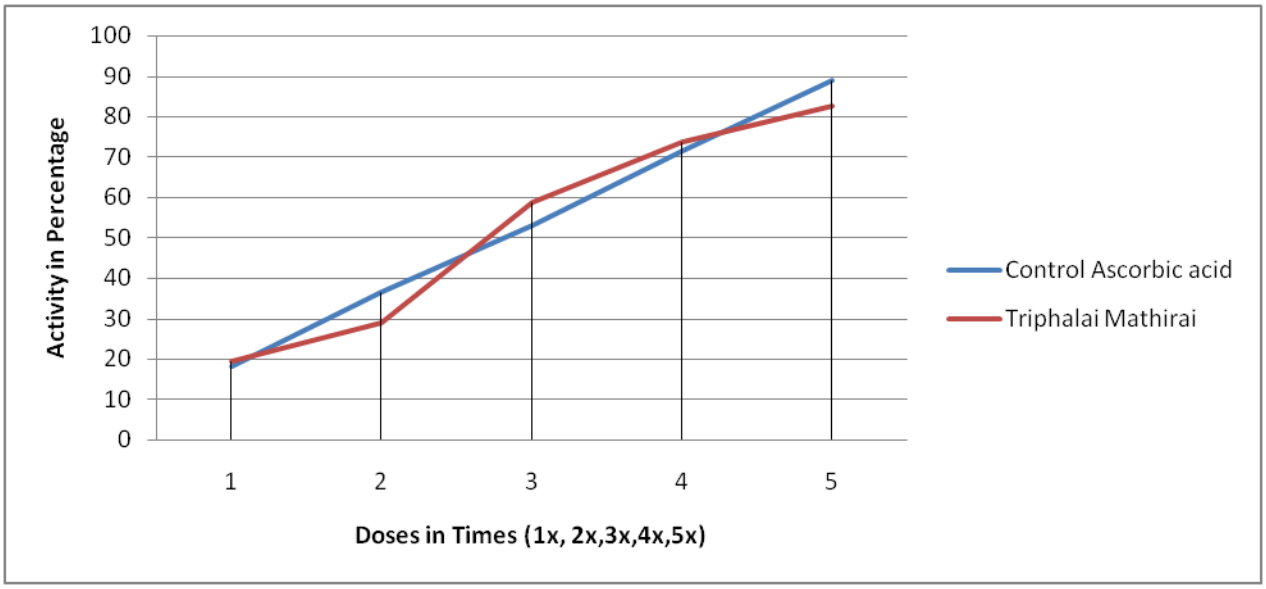

Table 6. Estimation of Total Phenolic content in Thiripalai Mathirai.

Sample Code	O D (750nm)	Concentrations
Aqueous extract of *TPT*	0.3439	0.516056423mg in 10mg sample

According to Table 7 and Figure 3, Total antioxidant capacity of Thiripalai Mathirai with Ascorbic acid as a Standard and IC 50 Value – 692.452µg/mL (Calculated using ED50 PLUS V1.0 Software).

Table 7. Total antioxidant activity of *Thiripalai Mathirai* with Ascorbic acid standard

Concentrations(µg/ml)	Absorbance	Percentage of inhibition
Control : 0.1153 Ascorbic acid		
125	0.0985	18.02575
250	0.0813	36.48069
500	0.0659	52.95064
1000	0.0489	71.24464
2000	0.0325	88.8412

Concentrations(µg/ml)	Absorbance	Percentage of inhibition
Thiripalai Mathirai		
125	0.0904	19.36
250	0.0796	28.99
500	0.0463	58.70
1000	0.0297	73.51
2000	0.0196	82.52

CONCLUSION

The Phytochemical screening may be helpful in new drug discovery and clinical practices. Its quantitative estimation may scavenge the free radicals and acts as strong antioxidant which serves for the mankind. However further studies are required to study its comprehensive analysis including quantitative analysis, characterize its chemical structure and assess its biological activities.

Source of Support

Nil

Conflict of Interest

None declared

References

1. Hakkim.P.Mohammed Abdullah Sahib, Kadukkai Vallaraiyin Thani Maanbu, Edition 1992, page no-81.
2. Dr.K.S.Murugesamudaliar, Gunapadam Mooligai Vaguppu-I Part, Edition 2008.
3. Dr.R.Thiyagarajan, Gunapadam Thathu Vaguppu-II Part, Edition 2009.
4. Dr.K.M.Nadkarni, Indian Materia Medica, Vol:1, Publisher: Popular Prakash, Mumbai, India.
5. H.P.S. Makkar, T. Norvsambuu, S. Lkhagvatseren, K. Becker. Plant Secondary Metabolites in some Medicinal Plants of Mongolia Used for Enhancing Animal Health and Production. Tropicultura. 2009; 27 (3):159-167.
6. Fu G, Pang H, Wong YH. Naturally occurring phenylethanoid glycosides: potential leads for new therapeutics. Curr Med Chem. 2008; 15 (25):2592-613.
7. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol. 1998 Sep; 62(2):183-93.
8. Sato Y, Oketani H, Singyouchi K, Ohtsubo T, Kihara M, Shibata H, Higuti T. Extraction and purification of effective antimicrobial constituents of Terminalia chebula RETS. against methicillin-resistant Staphylococcus aureus. Biol Pharm Bull. 1997 Apr; 20(4):401 -4.
9. Jain S K, Medicinal plants, National book trust, India, 1999, 178.
10. Anonymous, Wealth of India, Volume X (Combretaceae), Plant Systematic and Evolution, (Rh-Z) New Delhi Directorate CSIR, 1976, 177.
11. M. S. Blois, "Antioxidant determinations by the use of a stable free radical," Nature, vol. 181, no. 4617, 1958, 1199– 1200.
12. C. Alasalvar, M. Karamac, R. Amarowicz, F. Shahidi, Antioxidant and antiradical activities in extracts of hazelnut kernel (Corylus avellana L.) and hazelnut green leafy cover.
13. World Health Organization. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 28, 1993, 13-14.
14. Ayyanara M, Ignacimuthu S. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western