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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL EVALUATION & ANTI-OXIDANT ACTIVITY STUDY OF CLASSICAL SIDDHA MEDICINE SEENTHIL CHOORNAM

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ABSTRACT

The Siddha medicine is one of the traditional systems of Indian medicine which was introduced and propagated by SIDDHARS the saints who lived in the Southern part of Tamilnadu, India. This system primarily gave more emphasis on treating various diseases with herbal medicines. Aim**: -** The aim of the study is the Qualitative and Quantitative phytochemical evaluation and Anti-oxidant activity study of aqueous extract of classical Siddha medicine -SEENTHIL CHOORNAM. Materials &methods: - It contains Seenthil (Tinospora cordifolia. sp.), Karisalai (Ecilpta prostata, sp.) and Poonagam (Eudrilus Eugenia, sp), which were collected from various parts of Kerala and Tamilnadu. All the drugs were purified and combined in prescribed ratio as per the classical literature. Qualitative and quantitative phytochemical evaluation and anti-oxidant activity study were conducted. Result:-The qualitative phytochemical analysis showed the presence of Alkaloids, Flavonoids, Saponin, Protein and Carbohydrate in more quantity. Quantitative analysis showed, Alkaloids- 185 ±0.81 mcg/g, Flavonoids- 114.6 ± 1.63, Carbohydrate- 50.6 ± 0.81, Protein – 57.3 ± 0.81, Saponin -38 ± 1.41. Also Seenthil Choornam has good free radical scavenging activity against the free radical DPPH and have good antioxidant activity.

Key words: - Alkaloids, Flavonoids, Phytochemicals, Saponin, Seenthil Choornam, Antioxidant activity, DPPH

1. INTRODUCTION

The Siddha system of medicine is one of the traditional Indian systems of medicine, created and propagated by Siddhars –the saints who were lived ten thousand years back in Southern part of India, Tamilnadu. They had introduced a treatment methodology which is purely in harmony with nature. They had used the raw drugs which were bestowed by Mother Nature .It includes, Plants, Animal products, metals and minerals. Exploring more about those classical medicines through modern evaluation techniques are essential to know the complete therapeutic efficacy of such medicines.

Qualitative and quantitave phytochemical evaluation helps to find out the active molecules present in the drugs, which makes them therapeutically efficient. Phyto chemicals are non nutritive, secondary metabolites which possess strong antimicrobial, and antioxidant, anticancer and anti-inflammatory properties. [1]. And what is the role of these phytochemicals in human life? Due to the food habits, smoking, Alcoholism, daily activities and genetic causes, man is suffering from various diseases – Metabolic diseases, degenerative diseases, Autoimmune diseases, Cancer, Cardiac diseases Etc. Prevention and cure of these ailments through natural ways can be done by intake of specific phytochemicals in whole or modified forms. Also these phytochemicals are good free radical scavenging agents, which results in keeping the body safe from recurrent ailments.

This study deals with the qualitative and quantitative phytochemical analysis and Antioxidant activity study of aqueous extract of classical Siddha medicine Seenthil Choornam .The Seenthil Choornam is a Herbo-Animal medicine, mentioned in classical Siddha medical literature, Agathiyar Paripooranam-400 [2]. It contains *Seenthil (Tinospora cordifolia* sp.Menispermaceae family), *Karisalai (Ecilpta Alba* sp. Asteraceae Family) and *Poonagam (Eudrilus eugeniae* sp .Eudrilidae family) .The raw drugs were collected from Kerala And Tamilnadu. The identification and authentication of plant raw drugs were obtained from Department of Botany, Govt.Siddha Medical College and Hospital, Palayamkottai, Tirunelveli. The identification and authentication of *Poonagam*-Earthworm was obtained from, Advanced Centre of Environmental Studies and Sustainable Development-Inter University Centre Mahatma Gandhi University, Kottayam. All the drugs were purified, powdered and combined as per the literature. The phytochemical evaluation of aqueous extract of medicine shows the presence of Secondary metabolites Alkaloids, Saponin, Flavonoids and primary metabolites –Carbohydrates and Proteins. The presence of these phytochemicals makes this medicine more effective against inflammatory diseases, degenerating diseases and other chronic ailments. The antioxidant activity of sample Seenthil Choornam (SEC) makes it more effective in treating above said diseases.

3. MORPHOLOGY OF RAW DRUGS

3.1. *SEENTHIL (Tinospora cordifolia. Sp*)

Also, called as Heart leaved moonseed, Amrita, Guduchi, &Giloy. *Tinospora cordifolia* is a succulent, glabrous, deciduous climber, with corky and lenticelled bark.Leaves ovate-cordate. Flower yellow, in axillary racemes. Fruit globose, red when ripe. Flowering and fruiting during months December -June .Whole plant have medicinal properties. It has bitter taste [3]

Fig:-1 *Seenthil (Tinospora cordifolia)*

2. *KARISALAI (Ecilpta alba)*

It is an annual, erect or prostrate entirely pubescent herb, often rooting at nodes with opposite, sessile, usually oblong, 2.5 - 7.5 cm long leaves with white appressed hairs. Floral heads 6-8 mm in diameter, solitary, white, achene compressed and narrowly winged. It grows commonly in moist places as a weed all over the plains of India. [4]

Fig:-2 *Karisalai (Ecilpta alba)*

3. *POONAGAM (Eudrilus eugeniae*)

Body length: Complete matures, 90–185 mm or up to 250–400 mm under optimal culture conditions posterior tapering, becoming thinly flattened in terminal 'zone of growth' .Width: Approximately 4–8 mm. Mass: Mean per adult ca. 1.0 g (pers. obs.) or optimal maximum 5.0–6.0 segments: 161–211 (pers. obs. and Gates 1972) or 250–300, suggesting that larger worms add segments); constriction of 40–46 seen in several

Qld specimens may be artefactual.Colour: Red-brown dorsum fading posteriorly; anterior with bright blue/green iridescent sheen from cuticle

diffraction, ventrum beige, clitellum darker (sometimes lighter) than Surroundings. Prostomium: Small, open epilobous.Dorsal pores: None. Setae: Eight per segment from 2, closely paired; from 3/4 in c lines or slightly more median (sometimes in d lines).Clitellum: 13, 14, 15–18, usually 13, 14–18 and interrupted ventrally. Male pores: In 17 on tips of longitudinally grooved, tapering, eversible penes in large ventral chambers, retracted as lateral slits with wrinkled lips just anterior to 17/18 in line with b setae.

setae a–b on 17 absent (dehisced); ratio of aa: ab: bc:cd:dd:U on 7 = 6:1:5:1:10:0.5. Penile/genital setae absent.Nephropores: Just behind anterior furrow of each segment (longitudinal slits) Female pores: Combined with modified 'spermathecal pores' lateral, presetal in 14 as raised intrasegmental Openings just anterior to c setae. Gates (1972: 51) calls these "vaginal apertures".

Fig: 4 *Poonagam (Eudrilus eugeniae)*

4. MATERIALS AND METHODS

4.1. *Seenthil (Tinospora cordifolia)*-The raw drug was collected from Kottarakkara, Kollam District, Kerala. The matured stem of the plants were taken and are cleaned by washing them with water for 21 times and then soaking in butter milk, as per the literature. The purified raw drugs were dried in sunlight.

4.2*Karisalai* (*Ecilpta Alba*): The raw drug was collected from Tirunelveli District; Tamilnadu .The whole plants were cleaned by washing with water and dried in sunlight.

The identification and authentication of the plant raw drugs were obtained from Department of Botany, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamilnadu, India...

4.3. *Poonagam* (*Eudrilus eugeniae*)

The earthworm was collected from Vadipatti, Madurai District, and Tamilnadu. The worms were washed thoroughly with water and were soaked in milk for 3 hours to remove engulfed soil particles from body of earthworm. After that lime water was sprinkled over the earth worm. Then it was placed in sunlight until it was dried well. The identification and authentication of the earth worm were obtained from, Advanced Centre of Environmental Studies and Sustainable Development-Inter University Centre – Mahatma Gandhi University, Kottayam.

All the above medicines were finely powered and filtered. All the powered medicines were kept in air tight containers. Above three medicines were mixed in a ratio of 3:3:1 as per the literature

5. PHYTO CHEMICAL EVALUATION

5.1. QUALITATIVE PHYTOCHEMICAL ANALYSIS OF SEENTHIL CHOORNAM

TABLE NO -1 Tests for phytochemicals and the phytochemicals present in aqueous extract of Seenthil Choornam (SEC)

	TEST FOR PHYTOCHEMICAL	PHYTOCHEMICAL PRESENT
1.	**CHO**-Fehling's test [6]	**present**
2.	**Protein**- Biuret Test [7]	**present**
3.	**Glycoside**-Killiani test **[**8**]**	**Absent**
4	**Steroid**-Salkowski test[9]	**Absent**
5	**Alkaloids**- Mayer's test [10]	**Present**
6.	**Flavonoids**-Neutral $FeCl_2$ **[11]**	**Present**
7.	**Tannin**-Lead Acetate test [12]	**Absent**
8.	**Saponin**-Foam Test. [13]	**Present**
9.	Phenol-Ferric chloride test **[**14]	**Absent**
10.	**Triterpinoids**-$AlCl_2$ [15]	**Absent.**

Fig no: 4, Qualitative Phytochemical Analyses of trial drug – Test tubes with test sol. and reagents.

Qualitative phytochemical analysis of aqueous extract of sample Seenthil Choornam (SEC) (TABLE. No: 1, Fig: 4)

The qualitative analysis for phytochemicals present in the sample (Aq.SEC), through various chemical analyses shows the presence of following phytochemicals:-

1. Alkaloids
2. Flavonoids

3. Saponin
4. Carbohydrate
5. Protein

5.2 QUANTITATIVE PHYTOCHEMICAL ANALYSES

PPROCEDURE

5.2.1. ESTIMATION OF FLAVANOIDS

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample (Aq.SEC) and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometric ally. Results were expressed as catechin equivalents (mcg catechin /g dried extract).[16]

(Aq.SEC) was mixed with 0.5 ml of 0.1 N sodium hydroxide and 5 ml of alkaline copper reagent. The mixture was incubated in room temperature for 30 minutes. Folin– Ciocaiteau reagent, 0.5 ml was added and incubated again for 10 minutes at room temperature. The absorbance was read at 660 nm against a reagent blank. Bovine Serum Albumin (BSA) was used as a standard material and the results were expressed as BSA equivalents (mcg BSA /g dried extract). The estimation was done in triplicates and the results were expressed mcg of BSA/g sample.[18]

5.2.4 ESTIMATION OF CARBOHYDRATE

5.2.5. ESTIMATION OF SAPONNINS

5.2.2 ESTIMATION OF ALKALOIDS

To 1ml of sample (Aq.SEC), add 5 ml phosphate Buffer at PH 4.7 and 5 ml BCG solution then shake the mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents. Results were expressed as Atropine equivalents (mcg atropine/g dried extract).[17]

5.2.3 ESTIMATION OF PROTEINS

Protein content was estimated by the method of Lowry *et al* (1957**).** 1 ml of sample

The total sugar content was estimated by Anthrone method (Roe, 1955). A known amount of the sample (Aq.SEC) was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm.From the supernatant, 0.5 ml was taken and 5 ml of Anthrone reagent was added. The tubes were kept in a boiling water bath for 15 min. After that, they were kept in a dark room for another 15 minutes. The colour intensity developed was read in a spectrophotometer at 650 nm. Glucose was used as a standard material and the results were expressed as Glucose equivalents (mcg Glucose /g dried extract).[19]

Take 1ml of sample (Aq.SEC) add 2ml of 8 % Vanillin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60^0c for 10min. After 10 minutes the tubes were cooled. Absorbance was measured at 544nm against reagent blank. Diosgenin was used as a standard material and compared the assay with Diosgenin equivalents. Results were expressed as Diosgenin equivalents (mcg Diosgenin /g dried extract). [20]

6. ANTI OXIDANT ACTIVITY ASSAY

6.1. MATERIALS AND METHOD

DPPH assay –Blois, 1958

DPPH (1, 1 diphenyl-2-picryl hydrazyl) is a stable free radicals. DPPH with methanol appears deep violet colour and which can be measured at 520nm. If sample has an anti-oxidant activity deep violet becomes pale violet.

DPPH preparation:

Stock reagent DPPH was prepared. From which, working solution was prepared by diluting with appropriate solvent to obtained the absorbance of 0.98 at 520nm.

Sample preparation:

1mg of aqueous SEC sample was dissolved in 1ml of water and extract was made using Soxhlet extraction method. Standard ascorbic acid was also prepared in a same way as positive control.

Procedure:

2ml of DPPH solution were added to both standard and test tubes.100µl of test sample and standard solution of various concentrations (10, 20, 40, 80 and 100 µg/ml) and were added to respective tubes. Then stand for 30mins incubation. After incubation, observe the colour change and measured the absorbance of each tubes at 520nm.

Calculate the % of inhibition of each concentration with the formula,

$$\% \text{ of inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} X100$$

7. RESULT

7.1. QUALITATIVE ANALYSIS OF PHYTOCHEMICALS:

The qualitative analysis for phytochemicals present in the sample (Aq.SEC), through various chemical analyses shows the presence of following phytochemicals,

1. Alkaloids
2. Flavonoids
3. Saponin
4. Carbohydrate
5. Protein

7.2. QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

The quantitative analyses of phytochemicals which were identified in qualitative analyses of sample (Aq.SEC) showed the following estimations

Table no: 2 –Quantitative estimation of phytochemicals present in the Sample (SEC)

Test	Result (mcg/ G)
Alkaloid	185.3 ± 0.81
Flavonoid	114.6 ± 1.63
Carbohydrate	50.6 ± 0.81
Protein	57.3 ± 0.81
Saponin	38 1.41

The above table (TABLE.No:2) shows that the sample (Aq.SEC) has more alkaloids, followed by Flavonoids, then Carbohydrate and proteins. The Saponin is present in least quantity.

7.3-ANTIOXIDANT ACTIVITY ASSAY

OD value at 517 nm
Control Mean OD value: 0.935

Table .No.3 .Optical Density of sample –SEC at 517 nm

Concentration of sample SEC	OD value at 517nm (in triplicates)		
10µg/ml	0.880	0.885	0.887
20 µg /ml	0.804	0.809	0.811
40 µg /ml	0.656	0.659	0.662
80 µg /ml	0.423	0.428	0.425
100 µg /ml	0.334	0.337	0.339

Table .No.4 OD value of Standard-Ascorbic acid at 517 nm

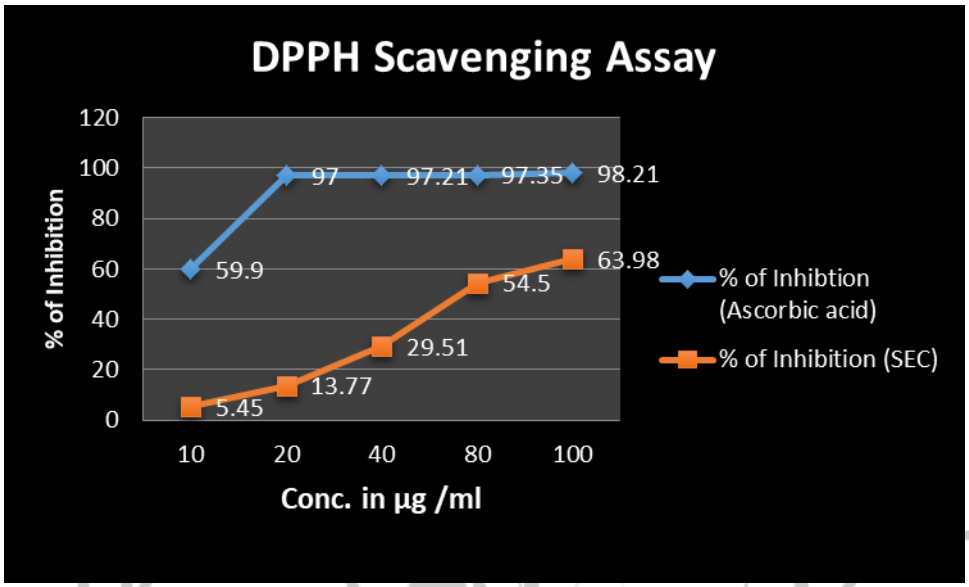
Concentration of Ascorbic acid	OD value at 517nm (in triplicates)		
10 µg /ml	0.371	0.375	0.378
20 µg /ml	0.026	0.028	0.030
40 µg /ml	0.024	0.026	0.028
80 µg /ml	0.022	0.025	0.027
100 µg /ml	0.015	0.018	0.017

Table.No.5 Percentage of inhibition of sample -SEC

Concentration of the sample SEC	Percentage of inhibition (in triplicates)			Mean Value (%)
10 µg /ml	5.88	5.34	5.13	5.45 ± 0.27
20 µg /ml	14.01	14.05	13.26	13.77 ± 0.31
40 µg /ml	29.83	29.51	29.19	29.51 ± 0.22
80 µg /ml	54.75	54.22	54.54	54.50 ± 0.18
100 µg /ml	64.27	63.95	63.74	63.98 ± 0.18

Table .No.6 Percentage of inhibition

Concentration of Ascorbic acid	Percentage of inhibition (in triplicates)			Mean Value (%)
10 µg /ml	60.32	59.89	59.51	59.90 ± 0.28
20 µg /ml	97.21	97.00	96.79	97.00 ± 0.14
40 µg /ml	97.43	97.21	97.00	97.21 ± 0.15
80 µg /ml	97.64	97.32	97.11	97.35 ± 0.18
100 µg /ml	98.39	98.07	98.18	98.21 ± 0.11



The result showed that the aqueous extract of mature stem of Seenthil (Tinospora cordifolia) possess, good antioxidant activity property against the DPPH.

8. CONCLUSION

Thus the Phytochemical evaluation – Qualitative and Quantitative, of classical Siddha medicine, Seenthil Choornam, through various chemical analyses and of *Karisalai* (*Ecilpta Alba*) is also helpful in treating, the subsequent ailments which are present in chronic diseases like autoimmune inflammatory disease, Liver diseases, and skin diseases. Presence of these bioactive molecules in this medicine, gives the advantage of using this drug to treat diseases which are through Spectrometry revealed that the Aq.extract of medicine (SEC), contains, Alkaloid=185.3 ± 0.81, Flavonoid =114.6 ± 1.63, Carbohydrate=50.6 ± 0.81, Protein-57.3± 0.81, Saponin= 38 ± 1.41.The alkaloidal and Flavanoid content of *Seenthil* (*Tinospora cordifolia* Sp), makes it more efficient in treating inflammatory diseases, Infectious disease and other systemic diseases. Like that, the phytochemical contents having multi system involvement. Further, isolation of individual molecules and more bio active components will improve the specificity in implementation of this medicine in systemic diseases and other more complex and chronic diseases. The antioxidant activity assay shows

Aqueous extract of Seenthil Choornam has good free radical scavenging activity against DPPH. So this drug can be used to treat inflammatory and degenerative diseases

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REFERENCE

1. BhanuPrakash, **Functional** and Preservative properties of Phytochemicals, Charlotte Cockle, 2020.

2. R.C .Mohan, Agathiyar Paripooranam-400, 213, 7, N.G.O. Colony, Vadapazhani, Chennai 26. Thamarai Noolagam.2012 January.

3. Dinesh Jadhav, Tinospora cordifolia (Wild.)Miers.ex Hk.f.&Th, Medicinal Plants of India,
A guide to Ayurvedic &Ethnomedicinal uses of plants, Volume-1,Scientific Publishers (India), 5-A,New Pali Road ,P.O.Box 91,Jodhpur-342 001(Raj.),2008,229-230, www.scientificpub.com

4. Rajendra Sharma, Tarun Gupta, Ecilpta alba, Encyclopedia of Medicinal Plants, Volume-3,
Campus Books International, 4831/24, Prahlad Street, Ansari Road, Darya Ganj, New Delhi-110002, 2009, 610-612.

5.Rober.J.Blackmore, Eco-taxonomic aspect of an iconic vermicomposter - African night crawler, earthworm Eudrilus eugeniae (Kinberg 1867), African invertebrates, 2015;56(3):527-548.

6. Brain KR, Jones BE, Turner TD, Application of densitometry to the qualitative and quantitative evaluation of pharmaceuticals colorants, Journal of chromatography A, 1975, 109 (2), 383-388.

7. S. H., Essentials of pharmacognacy, First edition, Birla publications, New Delhi.pp 2006 . 357- 359, 588-590.

8.Ansari.S.H,Essentials of Pharmacognacy, Chapter.4,Glycoside, First edition, Birla Publications, New Delhi,2006

9. Priyanka Mishra ,Preya Jamdar ,Sharav Desai ,Dhara Patel , Dhananjay Meshram, Phytochemical Analysis and In Vitro Antibacterial activity of *Tinospora cordifolia* Int.J, Curr, Microbiology, App, Sci, (2014), 3(3): 224-234.

10. Ansari.S.H, Essential of Pharmacognacy, First edition, Birla Publications, New Delhi.pp 2006, 357-359,588-590.

11. Harborne JB. Phytochemical methods, 2nd edition, Chapman and Hall

publications, London, New York, 1984, 288.
12. Mukherjee, P.K.2002.Quality control of herbal drugs, business horizons Pharmaceutical publishers, New Delhi.356-358.

Pharmaceutical publishers, New Delhi.356-358.
15. Haborne, J.B., In: Phytochemical methods, 2nd edition, Chapter 3, The terpinoids, Chapman and Hall, New York, 1984,

16. Naima Saeed, Muhammad R Khan, Maria Shabbier. Antioxidant activity, total Phenolic and Total Flavonoids contents of whole plant extract *Torilis leptophyllous* L.BMC Complementary and Alternative medicine, 2012; 12:221
17. Tab Sum, Shazia & Khare, S. & Jain, and Kirti. (2016). Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of *Abrus precatorius* L seeds. Asian Journal of Pharmaceutical and Clinical Research .9.371-374.

13. Ansari.S.H, Essentials of Pharmacognacy, First edition, Birla Publications, New Delhi p
2006, 357-359,588-590.
14. Mukherjee, P.K.2002.Quality control of herbal drugs, business horizons

18. Lowry OH, Roseeobrough NJ, Farr AL, Randall RJ. Protein measurements with the Folin's Phenol reagent .J Biol Chem.1957; 193: 265-75.

19. Roe, J.H, 1995, "The determination of sugar in blood and spinal fluid with Anthrone reagent "Ibid. ill: 335-343.

20. Sim, E.E.W.E.I.2011, Isolation and determination of anti-nutritional compounds from root and shells of peanuts (*Arachis Hypogea*).A project report of Department of Chemical Science Faculty of Science University Tunku Abdul Rahman, 34-35.