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Antioxidant Potential of Paavu Chooranam Prescribed to Cure Breast Cancer by the Cheruthikonam Traditional Siddha Medicinal Practitioner of Kanyakumari District, India



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Abstract

Paavu Chooranam is a Siddha polyherbal formulation comprised of 14 traditionally used herbs explore for the treatment of cancer. The present investigation was mainly focused on the antioxidation activities since, there is no information regarding pharmaceutical activities of antioxidants Hydroxyl radical scavenging, DPPH, Nitric oxide radical scavenging, Hydrogen per oxide radical scavenging and Reducing power activity. The unexplored area of Paavu Chooranam towards their antioxidation effect in aqueous, silver nitrate and ethanol extracts indicated promising antioxidant activities of crude extract.

Key words: Polyherbal formulation; Antioxidation; Paavu chooranam; Kalanchi; Samoolam Siddha, Breast Cancer, Traditional

Introduction

Cancer is a global problem of serious nature and it is the second leading cause of death next to Cardiac diseases throughout the world. The ancient text of Siddha Medicine, reported that the Siddhas, Saints and Seers had given details about "Puttru Noi" and its treatments. Scientific documentation of traditional system of medicine is increasing and need for preparing it for Siddha formulation has become the need of the hour. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects [1]. The ancient use of plants for healing purposes forms the origin of lot of modern medicine. Many traditional drugs originate from plant sources: a century ago, most of the effective drugs were plant based [2]. This polyherbal formulation is a composition of 14 different herbs viz., (In Tamil) Athimathuram, Jathikkai, Chukku, Milagu, Thippili, Seeragam, Karumseeragam, Lavangapattai, Kostam, Kadukkai, Thandrikkai, Parangipattai, Vasambu, Yanai Thippili and Indhuppu (natural mineral). The powder form of this Siddha Chooranam is used to treat Cancer. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Low levels of antioxidants or inhibition of the antioxidants enzymes, cause oxidative stress and may damage or kill cells. The medicinal qualities of plants are of course due to chemical constituents. Plants synthesize many compounds called primary metabolites that are critical to their existence. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent, produce free radicals. In turn, these radicals start chain reactions in a cell may cause damage or death. Antioxidants terminate these chain reactions by removing free radical intermediates inhibit other oxidation reactions [3].

Materials and Methods

Collection of plant materials

The plant materials required for the formulation of Siddha medicines were collected from the hills and hill locks of Molliadi of Kanyakumari District and other ingredients were procured from commercial Siddha raw drug stores. All the ingredients were shade dried, powdered and sieved was formulated into medicines and stored in porcelain pots for further use. The Siddha formulation were prepared as prescribed in the written scripts, books and palm leaf parchments of My Grandpa and Forefathers - Traditional Vadiyars (Table 1 & Figure 1).

Table 1: Composition of Paavu Chooranam.

S.No	Siddha Name	Scientific Name	Quantity
1	Athimathuram	Glycyrrhiza glabra	5 Kalanchi
2	Jathikkai	Myristica fragrans	3 Kalanchi
3	Chukku	Zingiber officinale	3 Kalanchi
4	Milagu	Piper nigrum	3 Kalanchi
5	Thippili	Piper longum	2 Kalanchi
6	Seeragam Samoolam	Cuminum cyminum	1Kalanchi
7	Karumseeragam	Nigella sativa	3 Kalanchi
8	Lavangapattai	Cinnamomum tamala	3 Kalanchi
9	Kostam	Saussurea lapa	3 Kalanchi
10	Kadukkai	Terminalia chebula	3 Kalanchi
11	Thandrikkai	Terminalia belerica	3 Kalanchi
12	Parangipattai	Smilax china	2Kalanchi
13	Vasambu	Acorus calamus	3 Kalanchi
14	Yanai Thippili	Balanophora fungosa	3 Kalanchi
15	Indhuppu	Sodium chloride	5gm

Preparation of extract

All the dried herbs were finely powdered and fresh leaves were triturated in household mortar and pestle without adding water. The powdered herbs were weighed (Kalanchi). The sampling Chooranam was subjected to maceration using different solvents aqueous, silver nitrate and ethanol for 48 hrs. The extracts were filtered and evaporated to dryness and kept for further studies.

Antioxidation assay of paavu chooranam

Antioxidation assay of the Paavu Chooranam and extracts were conceded using hydroxyl, DPPH, nitric oxide, hydrogen per oxide and reducing power activity [4,5].

Result and Discussion

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the Paavu Chooranam revealed that the inhibition varied from the minimum of

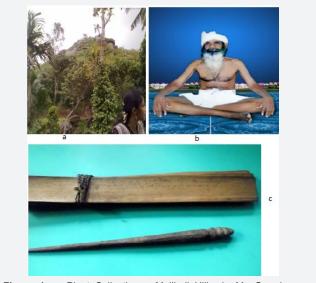


Figure 1: a. Plant Collection – Molliadi Hills, b. My Grandpa - Traditional Siddha Practitioner, c. Palm Leaf Parchment of my Forefather – Traditional Vadiyar.

 54.30 ± 0.005 % (25μ l) to the maximum of 83.02 ± 0.015 % (100µl). On the other hand, aqueous extract of the Chooranam varied from the minimum inhibition of 46.20±0.010 % (25µl) to the maximum inhibition of 61.72±0.011 % (100µl). Meanwhile, ethanolic extract of the Chooranam varied from the minimum inhibition of 49.61±0.015 % (25 μ l) to the maximum inhibition of 62.20±0.000 % (100µl). The silver nitrate assorted in the chooranam varied from the minimum inhibition of 55.21±0.005 % (25 μ l) to the maximum inhibition of 67.42 \pm 0.005 % (100 μ l). The antioxidant potential of standard antioxidant L-ascorbic acid varied from the minimum inhibition of 59.27±0.000 % (25µl) to the maximum inhibition of 85.29±0.000 % (100µl). In general, hydroxyl radical scavenging of the Paavu Chooranam and extracts varied from the minimum inhibition 46.20±0.010 % (25µl) of aqueous extract to the maximum inhibition 85.29±0.000 % (100µl) of L-ascorbic acid (Table 2 & Figure 2).

Table 2: Hydroxyl Radical Scavenging of Paavu Chooranam and Extracts.

Concentration of medicine and extracts	Paavu Chooranam	Aqueous Extract	Ethanol Extract	Silver nitrate (Nanoparticle)	L-Ascorbic acid
25μl	54.30±0.005	46.20±0.010	49.61±0.015	55.21±0.005	59.27±0.000
50μl	65.26±0.000	54.39±0.011	53.23 ±0.011	61.67±0.011	61.93±0.020
75µl	76.72±0.011	59.62±0.015	57.61±0.000	63.70±0.010	72.61±0.000
100μl	83.02±0.015	61.72±0.011	62.20±0.000	67.42±0.005	85.29±0.000

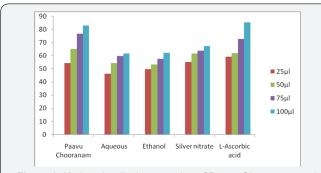


Figure 2: Hydroxyl radical scavenging of Paavu Chooranam and

DPPH radical scavenging assay

DPPH radical scavenging activity of the Paavu Chooranam varied from the minimum inhibition of 61.72±0.011 % (25µl)

Table 3: DPPH Radical Scavenging of Paavu Chooranam and Extracts.

Concentration of medicine and extracts	Paavu Chooranam	Aqueous Extract	Ethanol Extract	Silver nitrate (Nanoparticle)	L-Ascorbic acid (Standard)
25μl	61.72±0.011	47.01 ±0.010	59.62 ±0.010	66.71 ±0.010	62.71 ±0.000
50μl	69.36 ±0.000	49.62 ±0.000	67.29 ±0.005	74.62 ±0.020	75.36 ±0.000
75μl	77.21 ±0.011	54.79 ±0.010	76.34 ±0.011	77.91 ±0.000	79.24 ±0.000
100μl	85.62 ±0.005	63.71 ±0.005	81.26 ±0.011	79.20 ±0.005	86.39 ±0.010

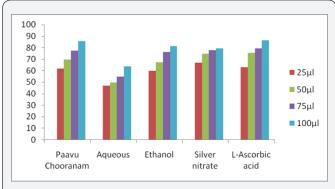


Figure 3: DPPH Radical Scavenging of Paavu Chooranam and Extracts.

Nitric oxide radical scavenging assay

Nitric oxide injuries take place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize low density lipoproteins, resulting in irreversible damage to the cell membrane. Inhibition increased with increasing concentration of the extract, the present investigation revealed that the Paavu Chooranam and extracts showed nitric oxide scavenging activity. The nitric oxide radical scavenging of Paavu Chooranam, aqueous, silver nitrate and ethanolic extracts increased gradually in concentration dependent manner. In general, among the medicine and extracts maximum reduction

to the maximum inhibition of 85.62±0.005 % (100µl). On the other hand, aqueous extract of the Chooranam varied from the minimum inhibition of 47.01±0.010 % (25µl) to the maximum inhibition of 63.71±0.005 % (100µl). Meanwhile, ethanolic extract of the Chooranam varied from the minimum inhibition of $59.62 \pm 0.010 \% (25 \mu l)$ to the maximum inhibition of 81.26 ± 0.011 % (100 µl). The silver nitrate assorted in the Chooranam varied from the minimum inhibition of 66.71 ± 0.010 % (25µl) to the maximum inhibition of 79.20±0.005 % (100µl). The antioxidant potential of standard antioxidant L-ascorbic acid varied from the minimum inhibition of 62.71 ± 0.000 % ($25\mu l$) to the maximum inhibition of 86.39±0.010 % (100µl). In general, DPPH scavenging assay of the Paavu Chooranam and extracts varied from the minimum inhibition 47.01±0.010 % (25µl) of aqueous extract to the maximum inhibition 86.39±0.010 % (100µl) of L-ascorbic acid (Table 3 & Figure 3).

was noticed in Paavu Chooranam 79.69±1.55 (100µl) whereas, minimum reduction was noticed in silver nitrate extract 61±1.69 (100µl). Tremendous result was observed in Paavu Chooranam with scavenging ranges 79.69±1.55 at 100μl than the extracts compared with 78.9±1.70 at 100µl for Gallic acid which served as positive control. The increasing evidences suggest that the nitric oxide and its derivatives produce activated phagocytes may have genotoxic effect and may contribute in the multistage carcinogenesis process [6]. The antioxidative defense systems and production of these reactive species in a healthy organism is approximately balanced. Antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals [7] (Table: 4 & Figure: 4).

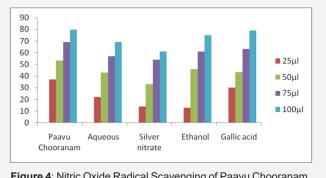


Figure 4: Nitric Oxide Radical Scavenging of Paavu Chooranam.

Table 4: Nitric Oxide Radical Scavenging of Paavu Chooranam.

Concentration of Medicine	Paavu Chooranam	Aqueous Extract	Silver nitrate	Ethanol Extract	Gallic acid
25μl	37.11±0.97	22±2.14	14±1.10	13±1.50	30.2±1.50
50μl	53.39±1.35	43±3.00	33±1.46	46±1.80	43.5±2.40
75µl	69.00±1.11	57±2.30	54±1.20	61±0.56	63.2±1.90
100μl	79.69±1.55	69±1.95	61±1.69	75±0.48	78.9±1.70

Hydrogen per oxide radical scavenging assay

Hydrogen per oxide radical scavenging activity of the Paavu Chooranam varied from the minimum inhibition of 71.39±0.020 % (25 μ l) to the maximum inhibition of 87.21 \pm 0.005 % (100 μ l). On the other hand, aqueous extract of the Chooranam varied from the minimum inhibition of 44.73 ± 0.011 % (25µl) to the maximum inhibition of 69.30±0.011 % (100µl). Meanwhile, ethanolic extract of the Chooranam varied from the minimum inhibition of 48.71 ± 0.015 % (25μ l) to the maximum inhibition of 71.62±0.010 % (100µl). The silver nitrate assorted in the Chooranam varied from the minimum inhibition of 57.61±0.015 % (25 μ l) to the maximum inhibition of 74.98 \pm 0.010 % (100 μ l). The antioxidant potential of standard antioxidant L-ascorbic acid varied from the minimum inhibition of 68.42±0.015 % (25 μ l) to the maximum inhibition of 85.26 \pm 0.015 % (100 μ l). In general, hydrogen per oxide scavenging of the Paavu Chooranam and extracts varied from the minimum inhibition 44.73±0.011 % $(25\mu l)$ of aqueous extract to the maximum inhibition 87.21 ± 0.005 % (100µl) of Paavu Chooranam (Table: 5 & Figure: 5). Reducing power activity

The reducing power assay exhibited the presence of antioxidants in the extract, which resulted in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The maximum reducing property was found at 100μ l (Table 5). The reducing power

activity of Paavu Chooranam, aqueous, (Table: 6) silver nitrate and ethanolic extracts increased gradually in concentration dependent manner. In general, among the medicine and extracts maximum reduction was noticed in Paavu Chooranam $84.29\pm2.05~(100\mu\text{l})$ whereas, minimum reduction was noticed in silver nitrate extract $53.21\pm1.20~(100\mu\text{l})$ (Table 6 & Figure 6). The phenolic antioxidants usually scavenge free radicals by an electron transfer mechanism8. The reducing power capacity of extract may serve as a significant indicator of its potential antioxidant activity.

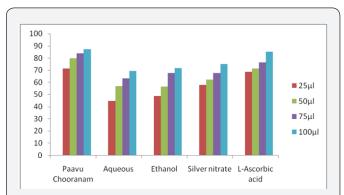


Figure5:Hydrogen peroxide Radical Scavenging of Paavu Chooranam and Extract.

 Table 5:Hydrogen peroxide Radical Scavenging of Paavu Chooranam and Extracts.

Concentration of medicine and extracts	Paavu Chooranam	Aqueous Extract	Ethanol Extract	Silver nitrate (Nanoparticle)	L-Ascorbic acid (Standard)
25µl	71.39±0.020	44.73 ±0.011	48.71 ±0.015	57.61 ±0.015	68.42 ±0.015
50μl	79.62 ±0.000	56.61 ±0.000	56.32 ±0.020	62.31 ±0.000	71.32 ±0.010
75µl	83.71 ±0.005	63.21 ±0.015	67.49 ±0.000	67.61 ±0.020	76.29 ±0.000
100μl	87.21 ±0.005	69.30 ±0.011	71.62 ±0.010	74.98 ±0.010	85.26 ±0.015

Table 6: Reducing Power Activity of Paavu Chooranam.

Concentration of Medicine	Paavu Chooranam	Aqueous Extract	Silver nitrate Extract	Ethanol Extract	Vitamin C
25μl	51.24±2.49	27.2±1.63	31.20±1.24	34.4±1.5	32.08±0.89
50μl	68.22±0.71	38.3±0.86	35.1±2.08	43.30±1.2	47.05±1.06
75µl	74.91±1.69	46.98±1.69	45.0±1.28	53.5±1.7	66.25±0.79
100μl	84.29±2.05	68.12±0.83	53.21±1.20	59.7±1.05	81.69±1.08

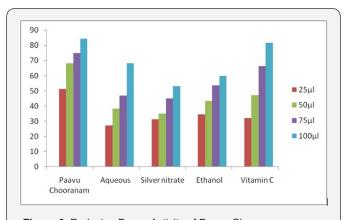


Figure 6: Reducing Power Activity of Paavu Chooranam.

Conclusion

It is flagrant that the plant kingdom offers a better prospect 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 Paavu Chooranam was evaluated for the antioxidant potential showed that the Paavu Chooranam was found to be an effective antioxidant, when it is compared to standard antioxidant compounds (L- Ascorbic acid, Gallic acid and Vitamin C).

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