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Invitro Antioxidant Activity of Siddha Herbomineral Formulation Muthu Parpam

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ABSTRACT:
Traditional medicines are recently gaining worldwide acceptance due to its health promotive and rejuvenating approach, less side effects, cost effectiveness and easy availability. *Muthu parpam* is a Siddha Herbo mineral formulation derived from marine mollusc pinctada. The traditional way of describing an antioxidant is a holistic medicine which has the ability to arrest/ delay ageing and age-related disorders and rejuvenating or revitalizing the functional body systems as a whole. *Muthu Parpam* is one such formulation in traditional Siddha text Theran tharu and has also been indicated in Siddha formulary of India-Part-1 a compendium of Siddha formulations The gem pearl (*Muthu*) has been indicated for a variety of acute and chronic diseases like otorrhoea, otitis media, asthma, diabetes, eye diseases, piles and urinary diseases and various neuro muscular diseases. Since most diseases and its complications are free radicals mediated and various scientific papers confirm the fact that administration of pearl powder causes substantial increase in antioxidant levels and suppresses the lipid peroxidation products that causes diseases, we have evaluated the antioxidant activity of *Muthu parpam*, a calcined ash of pearl gem through our present study.

1. Introduction

Pearls are produced by the living bivalve mollusks of both fresh and salt water. The Quality of the Pearl varies and it is categorized by its Colour, Size, Shape, Appearance and its Nature. Pearls are mainly made up of Calcium Carbonate ($CaCO_3$), Conchiolin and Orthorhombic in structure. [1]. Nowadays, Pearls are commonly used as ornaments since it has a long history for its usage by our ancestors. The pearl has its signature in the Ancient Tamil Epics for its specific usage as an ornamental jewel as well as one of the most precious gems.

In traditional medicine like Siddha, Ayurvedha medicinal formulations contain calcinated ash of pearl powder known as *parpam* (Tamil) or *Bhasma* (Sanskrit). Since time immemorial, it is widely used for various human ailments since the beginning of mankind. The

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Siddha classical text Theran Tharu written by sage Theraiyar has mentioned about the formulation, dosage and indication of *Muthu parpam*. The formulation has also been published in Siddha formulary of India-Part I, a compendum of Siddha medicines for Siddha practitioners [1].Though the medicines are Previously published studies on *Muthu parpam* Indicates the presence of the elements Calcium, Phosphorus, Carbon, Sodium, Magnesium, Iron, Potassium and the mineral Calcium is at its highest concentration of 960.785 mg/L.Further the preliminary analysis strongly indicate the absence of the heavy metals such as Arsenic, Mercury, Cadmium, Copper, Zinc, Lead and the element Sulphur denoting its safety for human consumption.[2] A preclinical study on instrumental analysis of *Muthu parpam* concludes the composition of *Muthu parpam* is mainly of Calcium Nano particles (XRD), with functional groups such as Hydroxy group. H bonded, Methylene(>CH2), Alkenyl, Carbonate ion, Alkyl substituted ether, Epoxy & oxirane rings, Methylene, Aliphatic iodo compounds, Polysulfides. Acute toxicity studies on *Muthu parpam* revealed that the LD 50 value is more than 2000mg/kg body weight and repeated oral 28days toxicity showed no significant changes in Hematological, Bio chemical parameters of experimental animals in all the three doses- Low dose - 300 mg/kg, Mid dose - 600mg/kg, High dose -1200 mg/kg body weight and no abnormal findings was seen in histopathology. Therefore, it can be concluded that the dosage level of *Muthu parpam* -260 mg as mentioned in the Siddha text, Theriyar Tharu is the safer dosage for oral administration.[3]. Traditionally pearl oyster has been regarded as demulcent, digestive, cardiac tonic, appetizer and an antacid. The gem pearl is indicated for otorrhoea, otitis media and chronic illness such as asthma, diabetes, eye diseases, piles and urinary diseases and various neuro muscular diseases [4] Till date there is very scarce scientific papers on the effectiveness of *Muthu parpam* (Calcined ash of Pearl gem) towards eye diseases. Hence an effort was made to evaluate the antioxidant activity of *Muthu parpam* which can contribute to its effectiveness towards reversal of eye diseases such as cataract that are formed due to generation of free radicals.

Antioxidant and Anti-Aging Applications

Lipid peroxidation can be induced by free radical oxidation and cause irreversible impairment of cellular macromolecules such as membrane lipids, proteins, and nucleic acids via reactive oxygen species (ROS) [5]. In brief, excessive production of ROS will lead to cellular injury and expedite the process of aging. An imbalance between ROS and antioxidant causes oxidative stress. Continuous ROS production and prolonged exposure to oxidative stress would lead to the pathophysiology of diseases such as diabetes, inflammation, and neurological disorders [6]. Natural antioxidants in food and beverage, e.g., tea, fruit, and vegetables, could react with free radicals and thus provide protection from age-related degenerative diseases [7]. Chiu et al. studied the antioxidant efficacy of protein-rich pearl powder in a randomized placebo-controlled trial with 20 participants [4]. The abundance of protein in pearl powder imparted its overall antioxidant activity. Both in vivo and in vitro studies indicated that pearl powder is an effective antioxidant and could potentially be used to treat multiple age-related degenerative diseases. Shao et al. showed that pearl powder is effective in beauty treatment and resistant to aging. However, particle size may influence antioxidant activity. Ultra-nano and ultra-micro pearl powders showed better free radical scavenging effects than water-soluble pearl powder [8]. Yang et al. reported that pearl powder bestow antihemolytic and antioxidant activities to protect human erythrocytes from 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), which induces oxidative stress and causes damage to membrane proteins/lipids with concomitantly reduced hemolysis [3]. The protection of erythrocytes afforded by pearl powder is worthy of further molecular studies in elucidating the mechanism of action involved to help to discover new and effective therapies against diseases [3]. Getting a glass skin is a goal of new facial cosmetic goods. A recent randomized, placebo-controlled experiment on the use of blue pearl pigment reported that it could generate the perception of blue light effect, contributing to the transparency and gloss on Korean women's faces [9 - 16].

Importance of the following assays

2. Materials and Methods

The study drug was prepared as per Siddha formulary of India-1 and was purchased from GMP certified Siddha pharmaceutical company-Earth India Naturals (EIN). The purchased drug was subjected to the following antioxidant assays.

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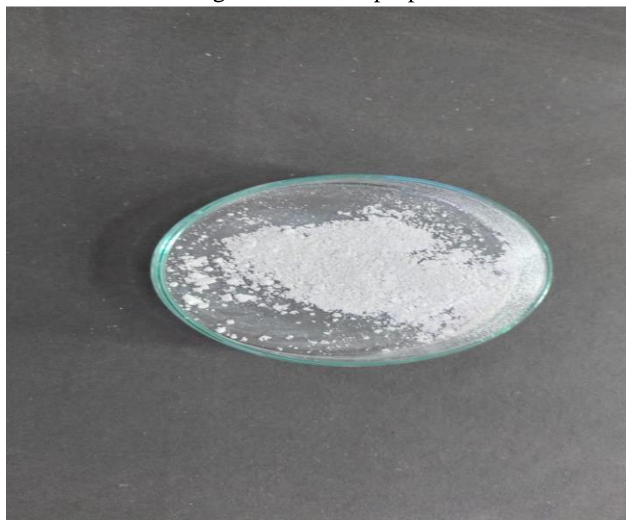
Figure-1. Muthu parpam



Antioxidant property

The antioxidant property of plants sample (WCF, CNL, CCL and CRL) was analyzed using following methods.

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of test drug sample MPM was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample MPM at the concentration of 10 -100 µg/ml along with standard ascorbic acid were utilized for detection of DPPH scavenging assay. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample MPM at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100µg/ml) was noted after 15 min incubation period at 37^{0}C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

The effective concentration of test sample MPM required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations.[17]

Nitric Oxide Radical Scavenging Assay

The concentrations of test sample MPM are made into serial dilution from 10–100 µg/mL and the standard gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the test drug (10–100 µg/mL) and incubated at 25°C for 180 mins. The test drug MPM was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the test drug but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The absorbance was measured at 546 nm using a Spectra Max Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug MPM and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug MPM and gallic acid were calculated using the following formula:[18]

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100,$$

where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

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ABTS Assay

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug MPM against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals. The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1 : 44, v/v). To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of test sample at the concentration of 10-100µg/ml in DD water and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug MPM was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample MPM was calculated using the following equation: The ABTS scavenging effect was measured using the following formula[19]:

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

Hydrogen Peroxide Radical Scavenging Assay

A hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the test sample MPM (different concentration ranging from 10-100µg/ml) were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). After adding 0.6 mL hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. BHA was used as the positive control. The percentage inhibition of the test drug MPM and standard was calculated and recorded. The percentage radical scavenging activity of the test drug MPM and BHA were calculated using the following formula [20]:

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100$$

3. Results

Percentage inhibition of test drug MPM on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of MPM	% Inhibition of Ascorbic Acid
10 µg/ml	12.48 ± 11.39	24.55 ± 10.56
20 µg/ml	16.7 ± 11.19	28.83 ± 9.211
40 µg/ml	20.43 ± 11.03	51.23 ± 9.973
60 µg/ml	24.71 ± 9.851	60.78 ± 5.466
80 µg/ml	32.23 ± 8.197	72.79 ± 4.855
100 µg/ml	38.25 ± 8.528	88.87 ± 5.318

Data are given as Mean ± SD (n=3)

IC50 Values for DPPH radical scavenging Assay by MPM and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
MPM	143.4 ± 25.97
ASCORBIC ACID	57.96 ± 2.129

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug MPM on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of MPM	% Inhibition of Gallic Acid
10 µg/ml	17.64 ± 9.523	33.31 ± 4.069
20 µg/ml	21.92 ± 7.54	38.88 ± 6.358
40 µg/ml	26.89 ± 7.291	50.84 ± 1.96
60 µg/ml	30.02 ± 7.393	60.65 ± 2.232

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80 µg/ml	32.77 ± 6.297	82.29 ± 4.314
100 µg/ml	35.32 ± 4.755	93.68 ± 0.8381

Data are given as Mean ± SD (n=3)

IC50 Values for Nitric Oxide radical scavenging assay by MPM and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
MPM	170.4 ± 13.05
GALLIC ACID	36.94 ± 4.983

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug MPM on ABTS radical scavenging assay

Concentration (µg/ml)	**% Inhibition of MPM**	**% Inhibition of Gallic Acid**
10 µg/ml	7.066 ± 4.704	23.83 ± 4.652
20 µg/ml	13.65 ± 2.903	40.16 ± 6.003
40 µg/ml	22.04 ± 2.486	53.66 ± 2.671
60 µg/ml	26.75 ± 4.167	70.57 ± 0.5831
80 µg/ml	33.72 ± 3.348	84.66 ± 6.642
100 µg/ml	36.97 ± 3.665	90.36 ± 1.275

Data are given as Mean ± SD (n=3)

IC50 Values for ABTS radical scavenging assay by MPM and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
MPM	133.4 ± 10.04
GALLIC ACID	37.34 ± 0.9758

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug MPM on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	**% Inhibition of MPM**	**% Inhibition of BHA**
10 µg/ml	6.655 ± 2.483	33.99 ± 1.176
20 µg/ml	11.68 ± 1.777	44.1 ± 1.104
40 µg/ml	15.27 ± 1.113	57.54 ± 1.007
60 µg/ml	20.79 ± 2.852	60.57 ± 0.9853
80 µg/ml	26.44 ± 2.742	76.74 ± 0.8691
100 µg/ml	30.71 ± 1.061	84.52 ± 0.8133

Data are given as Mean ± SD (n=3)

IC50 Values for Hydrogen peroxide radical scavenging assay by MPM and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
MPM	172.9 ± 9.099
BHA	33.9 ± 1.966

Data are given as Mean ± SD (n=3)

4. Discussion

In this study, the study drug Muthu parpam, has been evaluated for antioxidant activity with an approach to evaluate its therapeutic effects for eye diseases such as Cataract . Cataract is a condition that causes blindness in 17 million people and is the most significant cause of vision loss, around 47.9%. The formation of cataracts is linked to both the production of reactive oxygen species

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(ROS) such as peroxides, super-oxides, and hydroxyl radicals during mitochondrial oxidative metabolism. Progression of eye lens opacities occur due to oxidative stress as free radicals are highly reactive molecules. Their outer orbitals have one or more unpaired electrons and can be neutralized by electron-donating compounds, such as antioxidants. [21]

The trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 12.48 ± 11.39 to 38.25 ± 8.528 % when compared with standard ascorbic acid with percentage inhibition ranges from 24.55 ± 10.56to 88.87 ± 5.318%. The IC50 value of the trial drug was found to be 143.4 ± 25.97 (µg /ml) when compared with standard ascorbic acid with (IC50 value 57.96 ± 2.129 µg/ml). The NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 17.64 ± 9.523 to 35.32 ± 4.755% when compared with standard gallic acid with percentage inhibition ranges from 33.31 ± 4.069 to 93.68 ± 0.8381 %. The corresponding IC50 value of the trial drug was found to be 170.4 ± 13.05 (µg /ml) when compared with standard gallic acid with (IC50 value 36.94 ± 4.983 µg/ml)

Further the trial drug were screened for ABTS radical scavenging assay and the percentage inhibition ranges from 7.066 ± 4.704 to 36.97 ± 3.665 % when compared with standard gallic acid with percentage inhibition ranges from 23.83 ± 4.652 to 90.36 ± 1.275 % .The corresponding IC50 value of the trial drug was found to be 133.4 ± 10.04 (µg /ml) when compared with standard Gallic acid with (IC50 value 37.34 ± 0.9758 µg/ml)

The hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 6.655 ± 2.483 to 30.71 ± 1.061 % when compared with standard BHA with percentage inhibition ranges from 33.99 ± 1.176 to 84.52 ± 0.8133 %.The corresponding IC50 value of the trial drug was found to be 172.9 ± 9.099 (µg /ml) when compared with standard BHA with (IC50 value 33.9 ± 1.966 µg/ml).

where all antioxidant properties found are extremely effective in treating free-radical-induced diseases such as cardiovascular and inflammatory diseases, cancer etc., our study contributes to the fact that *Muthu parpam* has free radical scavenging activity especially DPPH among all other tested assays. The free radical DPPH, which is widely used to evaluate the ability of compounds to operate as free-radical scavengers and hydrogen suppliers, is a rapid, simple, and inexpensive method for testing antioxidant capabilities. The DPPH test relies on the elimination of DPPH, a stabilized free radical. DPPH is indeed a dark-colored crystalline compound made up of free-radical particles that are stable. In particular, it is a well-known radical and a popular antioxidant test. The DPPH test is used to estimate antioxidant activity based on the process through which antioxidants limit lipid oxidation, resulting in DPPH free-radical scavenging and therefore determining free-radical scavenging potential.[22]. Reports also indicate good solubility and bioavailability of calcium from these natural sources of calcium.[23] .Calcium derived from oysters exhibited good efficacy in suppressing the formation and proliferation of oral squamous cell carcinoma.[24].A study found that 55 brands of calcium supplements can be classified into seven categories based on the major ingredient in them and three or more categories were found to be derived from marine organisms mainly oyster/clamshells, algae, shark cartilage, and chelated calcium products . In addition, calcium from marine organisms has good bioavailability and biological function [25]. Since antioxidants play a major role in cancer as well as in degenerated diseases and biologically derived calcium and its derivatives are a major source of *Muthu parpam*, its antioxidant effect may be linked with the presence of biological calcium which is highly bioavailable

5. Conclusion

Cataracts are caused by a variety of factors, including tissue changes caused by aging in which proteins and lens fibers begin to break down, resulting in blurred or unclear vision, diabetes complications that cause high sugar levels in the aqueous humor, and oxidative stress caused by free radicals such as ROS. The way to neutralize ROS and other free radicals is with natural antioxidants. Antioxidants can donate electrons to make ROS and other free radicals less reactive. Based on the results obtained from the In-vitro anti-oxidant assay for the sample MPM it was concluded that the siddha formulation MPM has promising anti-oxidant activity in the estimated assays.

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