IN-VITRO ANTI-OXIDANT ACTIVITY AND QUALITATIVE BIOCHEMICAL ANALYSIS OF PANDUKU KUDINEER –A SIDDHA POLYHERBAL FORMULATION

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***Abstract*-**
Background: Siddha system of medicine is one of the oldest system of medicine in India.Pandu Noi is one of the disease out of 4448 diseases mentioned in Siddha literatures.Yugi Muni has described about Pandu Noi in "Yugi Vaithiya Chinthamani'. Paandu can be compared with Anemia. Panduku Kudineer is a polyherbal drug indicated for Pandu Noi which is mentioned in the Siddha Literature "Anubava Siddha Maruthuva Muraigal ".Aim: The aim of the study is to analyze the in-vitro anti oxidant activity and biochemical analysis of Panduku Kudineer. Methodology: The present study was undertaken to evaluate anti oxidant activity of Panduku Kudineer by using DPPH(2,2-Diphenyol 1-2 picrylhydrazyl) Assay , Nitric oxide radical scavenging assay ,ABTS(2,2'azino-bis assay), Hydrogen peroxide radical scavenging assay.The drug PK has antioxidant property which may act as potential therapeutic agent for management of paandu noi.Result: The study result concludes that Panduku Kudineer has significant Anti-oxidant activity.

Keywords: **Pandu Noi, Anemia,Panduku kudineer,Anti-oxidant activity.**

INTRODUCTION:

Anemia is a major nutritional global health problem.Anemia is defined as a reduction in the oxygen carrying capacity of the blood as observed by reduced levels of hemoglobin concentration and red cell mass leading to tissue hypoxia.It affects the persons of all ages,sex and economic group.In India anemia is the most common nutritional problem affecting more than half of the population, particularly in children and pregnant women where the incidence is 50 to 97%.Some herbs act directly to induce the resolution of anemia and others act pleiotropically through their anti-oxidant activity by increasing oxidative stress resistance or by triggering cellular mechanisms. Hence this study is carried out to study the Anti-oxidant activity of Panduku Kudineer by in-vitro assays and Biochemical analysis.

MATERIALS AND METHODS:

DRUG SELECTION:

The Siddha formulation Panduku kudineer is mentioned in Siddha literature Anubava siddha maruthuva muraigal written by Arutthandhai.S.Injaasimuthu, P.Paandikumar and S.Mutheeswaran on page no 18

INGREDIENTS OF PANDUKU KUDINEER:

1.Mukkirattai vaer (Boerhavia diffusa)
2.Kizhanelli samulam (Phyllanthus Amarus)
3.Arugu vaer (Cynodon dactylon)
4.Milagu(Piper Nigrum)

AUTHENTICATION OF RAW MATERIALS:

The raw drug was identified and authenticated by Medicinal Botanist and faculties of Department of Gunapadam at Govt.Siddha Medical College,Palayamkottai,Tamilnadu.

PROCESS OF DRUG PREPARATION: The ingredients of the trial drug were purified according to the proper methods described in Siddha Classical literature. All the purified drugs were powdered separately and mixed together and stored in a tight container. 5gm of PK was weighted accurately and 120 ml of water added boiled,filtered into 15 ml of decoction and took concentrated sample weight per ml.

ANTI-OXIDANT ACTIVITY EVALUATION OF PANDUKU KUDINEER:

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

The antioxidant activity of test drug sample PK was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample PK 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 PK

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 37^0C. 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 PK 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.

Nitric Oxide Radical Scavenging Assay

The concentrations of test sample PK 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 PK 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 PK and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug PK and gallic acid were calculated using the following formula:

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.

ABTS Assay

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug PK 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 PK was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample PK was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

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

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 PK (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 PK and standard was calculated and recorded. The percentage radical scavenging activity of the test drug PK and BHA were calculated using the following formula:

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

BIOCHEMICAL ANALYSIS OF PANDUKU KUDINEER:

Preparation of the extract: 5gms of the drug was weighed accurately and placed in a 250ml clean beaker then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made to 100ml with distilled water. This fluid is taken for analysis.

RESULTS

Table 1
Percentage inhibition of test drug PK on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of PK	% Inhibition of Ascorbic Acid
10 µg/ml	4.475 ± 1.069	17.9 ± 8.51
20 µg/ml	8.868 ± 0.5255	31.06 ± 10.47
40 µg/ml	13.44 ± 2.472	53.18 ± 10.14
60 µg/ml	17.24 ± 1.904	62.61 ± 5.171
80 µg/ml	22.52 ± 1.885	74.35 ± 6.919
100 µg/ml	25.22 ± 1.061	90.24 ± 5.925

Data are given as Mean ± SD (n=3)

Table 2: IC50 Values for DPPH radical scavenging Assay by PK and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
PK	147.5 ± 87.66
ASCORBIC ACID	40.97 ± 10.72

Data are given as Mean ± SD (n=3)

Table 3: Percentage inhibition of test drug PK on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of PK	% Inhibition of Gallic Acid
10 µg/ml	21.64 ± 16.98	16.75 ± 6.633
20 µg/ml	25.92 ± 15.46	31.52 ± 4.987
40 µg/ml	31.81 ± 14.3	44.02 ± 3.892
60 µg/ml	36.39 ± 13	54.28 ± 5.649
80 µg/ml	41.44 ± 10.95	77.07 ± 4.731
100 µg/ml	48.68 ± 8.879	89.03 ± 2.463

Data are given as Mean ± SD (n=3)

Table 4: IC50 Values for Nitric Oxide radical scavenging assay by PK and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
PK	100.4 ± 32.42
GALLIC ACID	48.83 ± 5.709

Data are given as Mean ± SD (n=3)

Table 5: Percentage inhibition of test drug PK on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of PK	% Inhibition of Gallic Acid
10 µg/ml	4.548 ± 1.453	12.95 ± 2.766
20 µg/ml	10.28 ± 4.861	33.22 ± 9.787
40 µg/ml	19.18 ± 7.63	50.42 ± 5.953
60 µg/ml	26.03 ± 12.56	74.25 ± 3.158
80 µg/ml	32.55 ± 11.5	78.38 ± 1.287
100 µg/ml	40.65 ± 12.84	89.86 ± 0.046

Data are given as Mean ± SD (n=3)

Table 6: IC50 Values for ABTS radical scavenging assay by PK and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
PK	136.4 ± 60.25
GALLIC ACID	43.48 ± 4.022

Data are given as Mean ± SD (n=3)

Table 7: Percentage inhibition of test drug PK on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	**% Inhibition of PK**	**% Inhibition of BHA**
10 µg/ml	7.495 ± 6.934	19.79 ± 2.619
20 µg/ml	14.38 ± 7.855	30.82 ± 2.441
40 µg/ml	17.57 ± 5.95	45.49 ± 2.204
60 µg/ml	23.92 ± 7.004	48.8 ± 2.15
80 µg/ml	28.31 ± 8.542	66.44 ± 1.865
100 µg/ml	35.75 ± 7.345	86.3 ± 4.219

Data are given as Mean ± SD (n=3)

Table 8: IC50 Values for Hydrogen peroxide radical scavenging assay by PK and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
PK	152.2 ± 29.92
BHA	52.28 ± 3.668

Data are given as Mean ± SD (n=3)

S.NO	EXPREMENTS	OBSERVATION	INFERANCE
1	**Test for calcium:** 2ml of the above prepared extract taken in a clean test tube.to this add 2ml of 4% ammonium oxalate solution.	No white precipitate is formed	Absence of calcium
2	**Test for sulphate:** 2ml of the extract is added to 5%barrium chloride solution.	A white precipitate is formed	**Indicates the presence of sulphate**
3	**Test for chloride:** The extract is treated with silver nitrate solution.	A white precipitate is formed	**Indicates the presence of chloride**
4	**Test for carbonate:** The substance is treated with concentrated HCL.	No brisk effervescence is formed	Absence of carbonate
5	**Test for starch:** The extract is added with weak iodine solution.	Blue colour is formed	**Indicates the presence of starch**
6	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium Ferro cyanide.	Blue colour is not formed	Absence of ferric iron
7	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	Blood red colour is formed	**Indicates the presence of ferrous iron**
8	**Test for phosphate:** The extract is treated with ammonium molybdate and concentrated nitric acid**.**	Yellow precipitate is not formed	Absence of phosphate
9	**Test for albumin:** The extract is treated with esbach's reagent**.**	Yellow precipitate is not formed	Absence of albumin
10	**Test for tannic acid:** The extract is treated with ferric chloride.	Blue black precipitate is formed	**Indicates the presence of tannic acid**
11	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolorised	**Indicates the presence of unsaturated compounds**
12	**Test for the reducing sugar:**5ml of benedict'qualidative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minute	No colour change occurs	Absence of reducing sugar
13	**Test for amino acid:**One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.	Violet colour is formed	**Indicates the presence of amino acid**
14	**Test for zinc:** The extract is treated with potassium Ferro cyanide.	White precipitate is not formed	Absence of zinc

Table 9 : Biochemical Analysis

DISCUSSION:

I. DPPH radical scavenging activity

Trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 4.475 ± 1.069 to 25.22 ± 1.061% when compared with standard ascorbic acid with percentage inhibition ranges from 17.9 ± 8.51 to 90.24 ± 5.925 %. The IC50 value of the trial drug was found to be 147.5 ± 87.66 (µg /ml) when compared with standard ascorbic acid with (IC50 value 40.97 ± 10.72 µg/ml).

II. NO radical scavenging activity

NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 21.64 ± 16.98 to 48.68 ± 8.879 % when compared with standard gallic acid with percentage inhibition ranges from 16.75 ± 6.633 to 89.03 ± 2.463 %. The corresponding IC50 value of the trial drug was found to be 100.4 ± 32.42 (µg /ml) when compared with standard gallic acid with (IC50 value 48.83 ± 5.709 µg/ml)

III. ABTS radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 4.548 ± 1.453 to 40.65 ± 12.84 % when compared with standard gallic acid with percentage inhibition ranges from 12.95 ± 2.766 to 89.86 ± 0.046 % .The corresponding IC50 value of the trial drug was found to be 136.4 ± 60.25 (µg /ml) when compared with standard Gallic acid with (IC50 value 43.48 ± 4.022 µg/ml)

IV. Hydrogen peroxide radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 7.495 ± 6.934 to 35.75 ± 7.345 % when compared with standard BHA with percentage inhibition ranges from 19.79 ± 2.619 to 86.3 ± 4.219 %.The corresponding IC50 value of the trial drug was found to be 152.2 ± 29.92 (µg /ml) when compared with standard BHA with (IC50 value 52.28 ± 3.668 µg/ml)

V. Biochemical anlaysis reveal the presence of Sulphate, Ferrous iron, Chloride, Starch, Tannic acid, Unsaturated compounds, Amino acid.

CONCLUSION:
Based on the results obtained from the In-vitro anti-oxidant assay for the sample Panduku Kudineer it was concluded that the siddha formulation Panduku Kudineer has promising anti-oxidant activity in the estimated assays.This result can be further analyzed in-vivo and more promising results may be expected.

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