IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2018 Vol.:12, Issue:2

In Vitro Evaluation of Antioxidant Activity of Kusta Gaja Kesari (KGK) - A Siddha Herbo Mineral Formulation

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

A.Sureka[1*], C. Mary Sharmila [2], R. Chithra Devi [3], N. J. MuthuKumar [4], V. Banumathi [5]

[1] *Emergency Medical Officer, National Institute of Siddha, Tambaram Sanatorium.*

[2] *Resident Medical Officer, National Institute of Siddha, Tambaram Sanatorium.*

[3] *House Officer, National Institute of Siddha, Tambaram Sanatorium.*

[4] *Associate Professor, Hospital Superintendent, National Institute of Siddha, Tambaram Sanatorium.*

[5] *Director, National Institute of Siddha, Tambaram Sanatorium.*

Submission: 22 April 2018
Accepted: 28 April 2018
Published: 31 May 2018

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Kusta Gaja Kesari, Anti Oxidant Activity, Siddha, Skin Diseases.

ABSTRACT

AIM - The aim of the present study is to evaluate the In vitro free radical scavenging property of Kusta Gaja Kesari a herbo-mineral formulation mentioned in the Siddha classical texts "Siddha Vaidhya Thirattu" which is commonly and widely used to treat *kuttam, Ven Kuttam, megham* and chronic skin diseases. **METHODS** – The evaluation of the In vitro antioxidant activity was carried out using DDPH ASSAY, Nitric Oxide Radical scavenging assay, ABTS Assay, Hydrogen Peroxide Radical scavenging assay at different concentrations (10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml). **RESULTS** - The results obtained for the varied concentration levels showed a percentage inhibition of 6.17 to 58.33% in DPPH assay, 20.96 to 75.08% by NO radical scavenging assay, 30.04 to 76.42% by ABTS radical scavenging assay,11.06 to 59.95% by hydrogen peroxide radical scavenging assay. The IC 50 values of KGK for the above mentioned assays are 84.28, 64.61, 43.72, 78.47 respectively. **CONCLUSION** - It can be concluded that the Siddha herbo-mineral formulation KGK has a promising antioxidant activity in the estimated assays and hence can be effectively implemented in the treatment of skin diseases as indicated in the Siddha classical texts.

INTRODUCTION

Skin diseases rank fourth in the list of ailments causing non- fatal disease burden. According to the Global Burden Disease Study 2010, fungal skin diseases, subcutaneous diseases, and acne are the most prevalent diseases worldwide[1]. Skin diseases create a negative impact affecting the quality of life and are the most common diseases in the primary care settings in tropical areas. Reactive oxygen and reactive nitrogen species of free radicals are commonly produced in a normal healthy skin. These free radicals are responsible for a regulated cell multiplication, differentiation, senescence, and death [2]. Oxidative stress sets in when there is an imbalance between the free radicals and the endogenous antioxidants. Review of various studies point out the role of free radicals in the etiopathogenesis of various dermatological disorders, onset of cutaneous neoplasia and the therapeutic benefits of antioxidants in skin diseases[3].

In Siddha literature *"Yugi vaithiya chinthamani"* skin diseases are classified into "18 kuttam". All the skin pathologies under the 18 kuttam, in Siddha medicine are viewed as derangements of the three Doshas (vatam, pitham, kabham), the five elements (space, air, water, earth and fire) and the seven dhatus (Saaram, senneer, oon, Kozhuppu, enbu, Moolai, sukilam /sronitham). In Siddha system of medicine, numerous literature with elaborate medicinal preparations for skin diseases have been documented. One such of a Siddha medicinal preparation mentioned in "Siddha Vaidhya Thirattu" is Kusta Gaja Kesari (KGK) indicated for skin diseases like *kuttam, Ven Kuttam, megam, naatpatta thol noigal.* KGK is a herbo-mineral formulation prepared with the extracts of the fruit of savuri pazham (Trichosanthes tricuspidata). The constituents of Kusta Gaja Kesari are known to possess the following properties in siddha system of medicine. Abraga chenduram (mica) is given along with old rice water to treat kuttam (leprosy). Aya chenduram - Ayam (iron) has the general property to cure venkuttam (leucoderma).

"Paandu venkuttam parunthoola noi sobai ....."

Rasa parpam is indicated for thaemal (tinea infection) affecting head, limbs, trunk, and joints[4].

Trichosanthes tricuspidata leaf juice is used in the oil preparation for venkuttam (leucoderma). The fruit is smashed well, mixed with coconut oil, boiled and is used for ulcers in ear, nose, and ears[5].

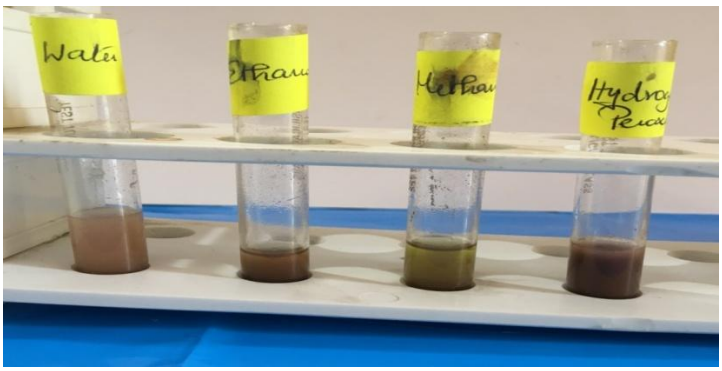
In this study, the author has aimed to study and evaluate the antioxidant activity of Kusta Gaja Kesari, a Siddha herbo-metallic drug commonly used in the treatment of various skin diseases.

MATERIALS AND METHODS

Sample Description

State	Solid
Appearance	Reddish Brown
Nature	Coarse Powder
Odor	Strong Metallic

Solubility Assay



S.No	Solvent Used	Solubility
1.	Water	Sparingly Soluble
2.	Methanol	Freely Soluble
3.	Ethanol	Freely Soluble
4.	Hydrogen Peroxide	Soluble

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

The antioxidant activity of test drug sample KGK was determined using the 2,2 -diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample KGK was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution 1ml, 2ml, 4ml, 6ml 8ml and 10ml of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10 ml whose concentration was then10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Ascorbic acid used as standard was prepared in the same concentration as that of the test drug by using methanol as solvent. 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 KGK 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.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

The effective concentration of test sample KGK 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 [6].

Nitric Oxide Radical Scavenging Assay

The concentrations of test sample KGK are made into serial dilution from 10−100 µg/mL and the standard used is gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride

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 KGK 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 drugKGK and standard was calculated and recorded.[7] The percentage nitrite radical scavenging activity of the test drug KGK 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 KGK 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 the test sample (10-100µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured at 734 nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug KGK was measured following the same procedures described above and was used as positive controls[8]. The antioxidant activity of the test sample KGK 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 KGK (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 drugKGK and standard was calculated and recorded[9]. The percentage of radical scavenging activity of the test drug KGK and BHA were calculated using the following formula:

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

RESULTS

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

Concentration (µg/ml)	% Inhibition of KGK	% Inhibition of Ascorbic Acid
10 µg/ml	6.176 ± 2.353	39.63 ± 2.796
20 µg/ml	19.51 ± 2.449	53.33 ± 2.222
40 µg/ml	30.1 ± 3.594	61.85 ± 2.796
60 µg/ml	35.98 ± 5.56	71.11 ± 2.94
80 µg/ml	47.75 ± 6.792	79.22 ± 8.569
100 µg/ml	58.33 ± 4.454	89.63 ± 0.2313

Data are given as Mean ± SD (n=3)

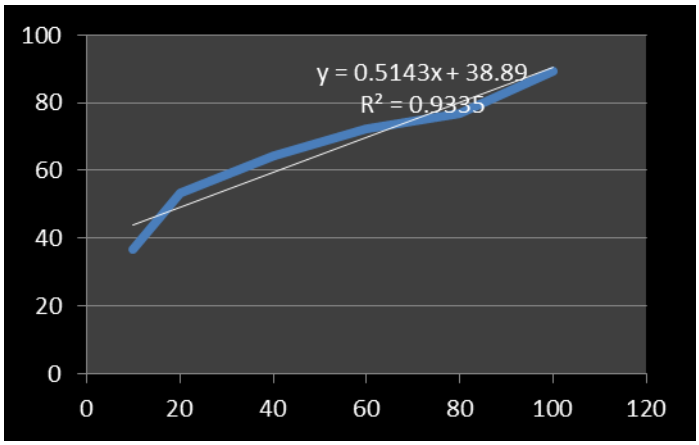
Table 1a: IC50 Values for DPPH radical scavenging Assay by KGK and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	20.45 ± 1.37
KGK	84.28 ± 9.896

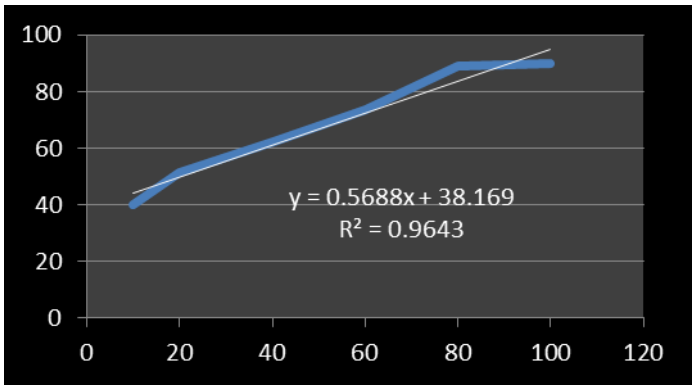
Data are given as Mean ± SD (n=3)

Figure 1: Percentage inhibition of STD on DPPH radical scavenging assay

Triplicate 1



Triplicate 2



Triplicate 3

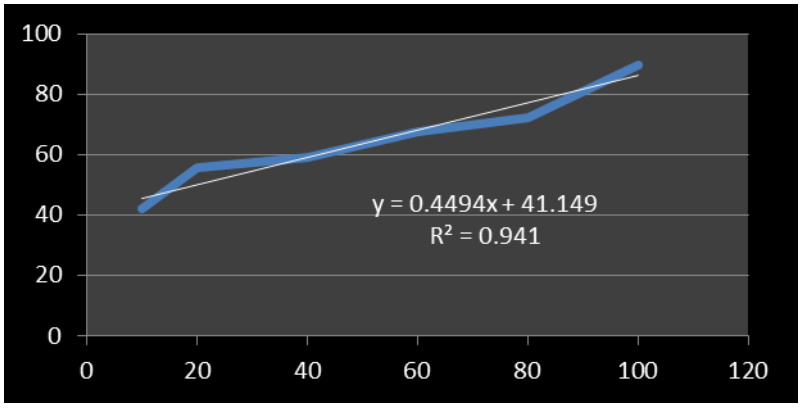
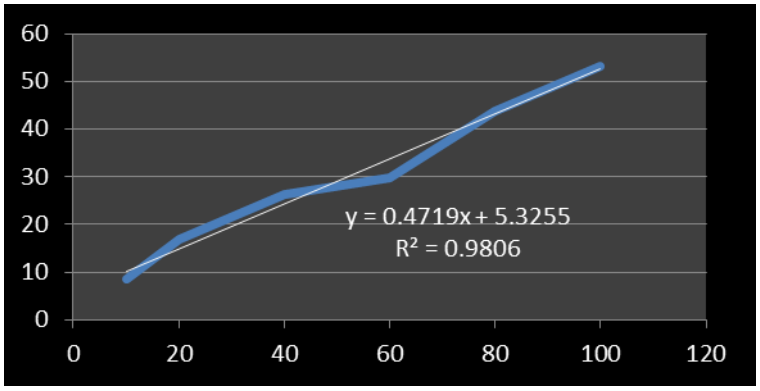
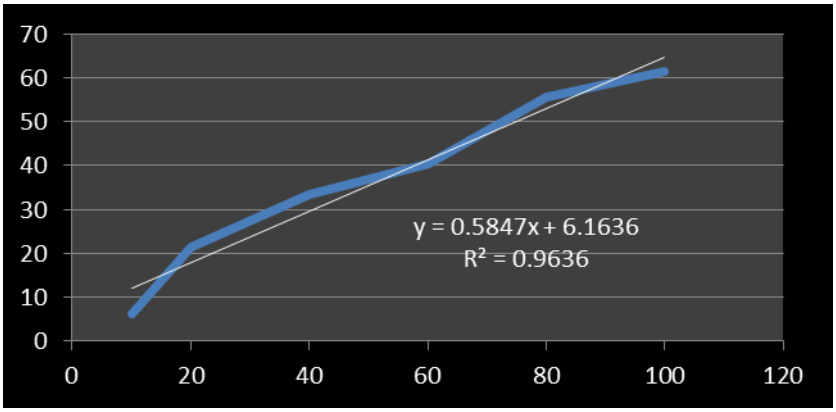


Figure 1a: Percentage inhibition of KGK on DPPH radical scavenging assay

Triplicate 1



Triplicate 2



Triplicate 3

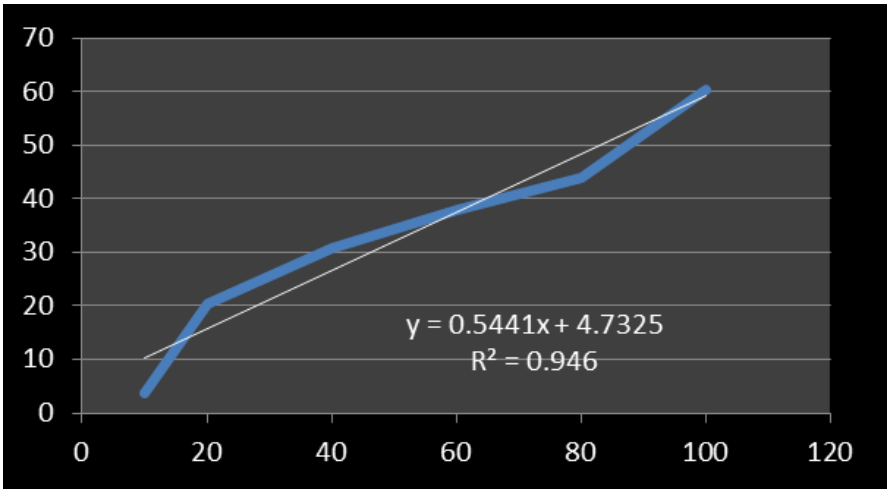


Table 2: Percentage inhibition of test drug KGK on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of KGK	% Inhibition of Gallic Acid
10 µg/ml	20.96 ± 2.151	31.49 ± 8.853
20 µg/ml	28.84 ± 2.706	43.09 ± 6.612
40 µg/ml	36.01 ± 1.075	52.14 ± 5.137
60 µg/ml	43.54 ± 2.151	63.01 ± 8.786
80 µg/ml	53.57 ± 4.346	72.07 ± 3.817
100 µg/ml	75.08 ± 5.922	86.2 ± 6.369

Data are given as Mean ± SD (n=3)

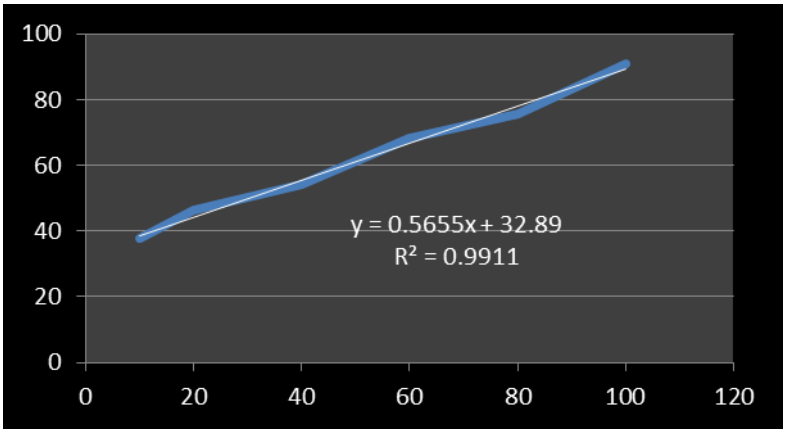
Table 2a: IC50 Values for Nitric Oxide radical scavenging assay By KGK and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
KGK	64.61 ± 2.365
GALLIC ACID	37.21 ± 11.79

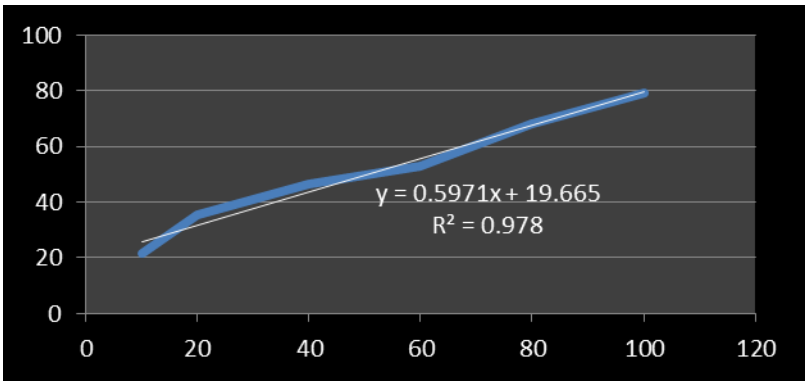
Data are given as Mean ± SD (n=3)

Figure 2: Percentage inhibition of STD on NO radical scavenging assay

Triplicate 1



Triplicate 2



Triplicate 3

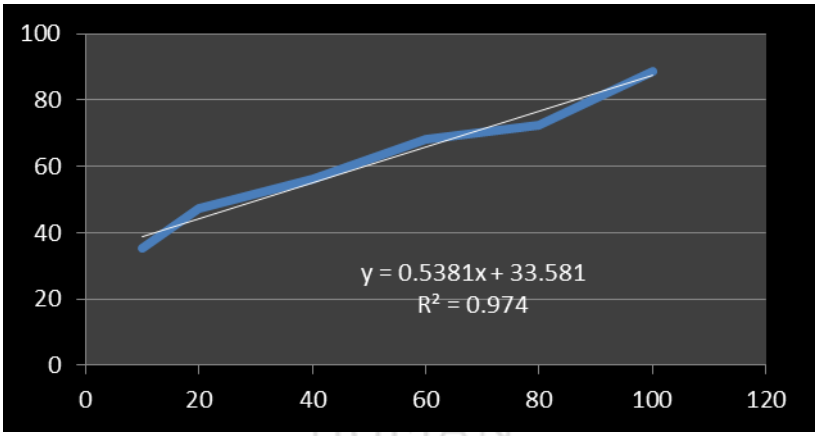
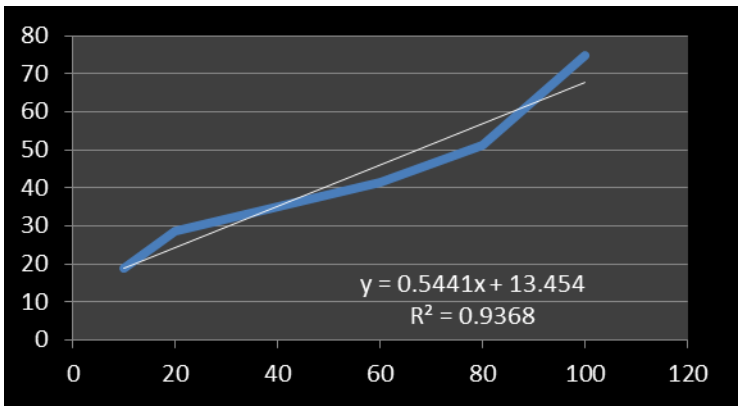
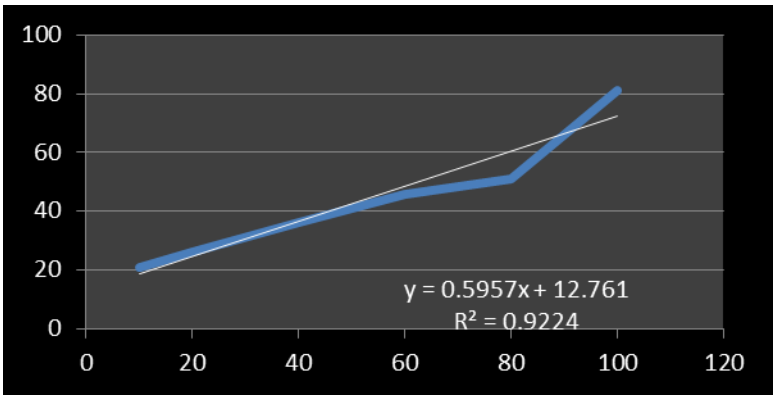


Figure 2a: Percentage inhibition of KGK on NO radical scavenging assay

Triplicate 1



Triplicate 2



Triplicate 3

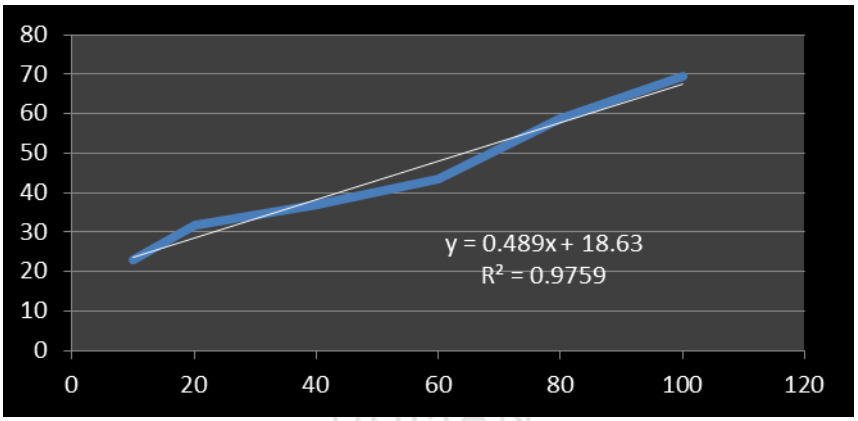


Table 3: Percentage inhibition of test drug KGK on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of KGK	% Inhibition of Gallic Acid
10 µg/ml	30.04 ± 1.883	48.74 ± 3.994
20 µg/ml	42 ± 4.981	57.88 ± 2.791
40 µg/ml	48.88 ± 3.817	66.67 ± 1.611
60 µg/ml	57.58 ± 8.158	74.05 ± 2.436
80 µg/ml	68.81 ± 4.901	79.33 ± 3.705
100 µg/ml	76.42 ± 3.817	94.45 ± 0.2196

Data are given as Mean ± SD (n=3)

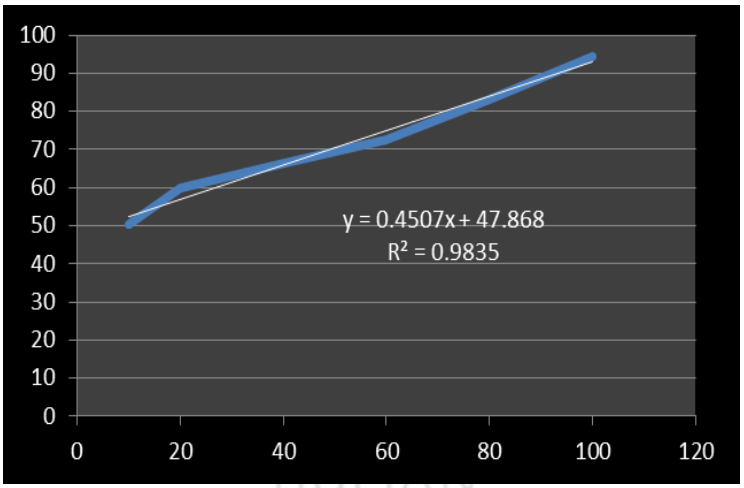
Table 3a: IC50 Values for ABTS radical scavenging assay By KGK and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
KGK	43.72 ± 6.82
GALLIC ACID	7.346 ± 4.854

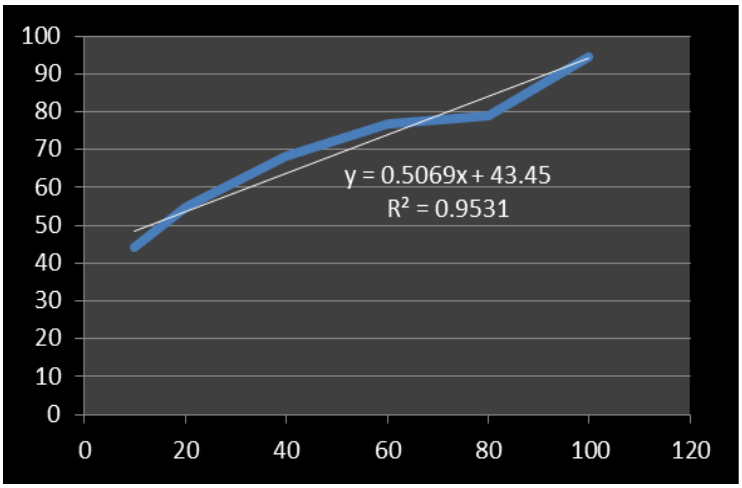
Data are given as Mean ± SD (n=3)

Figure 3: Percentage inhibition of STD on ABTS radical scavenging assay

Triplicate 1



Triplicate 2



Triplicate 3

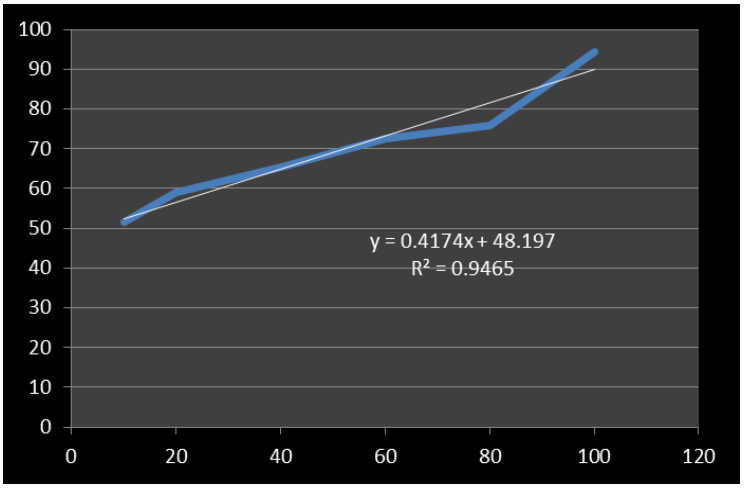
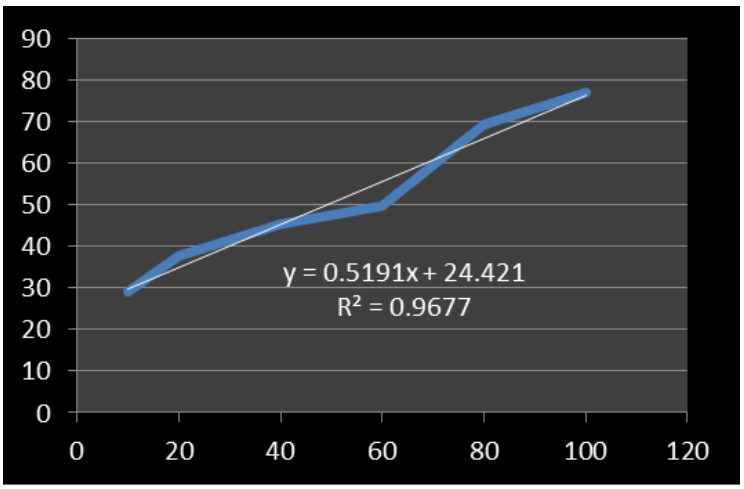
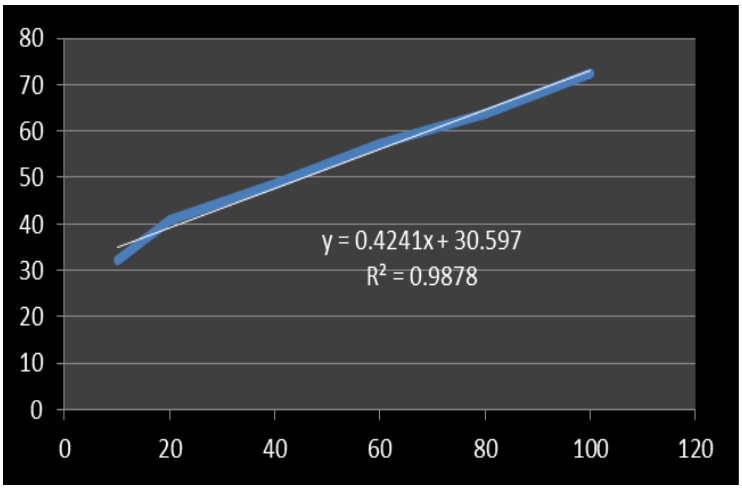


Figure 3a: Percentage inhibition of test drug KGK on ABTS radical scavenging assay

Triplicate 1



Triplicate 2



Triplicate 3

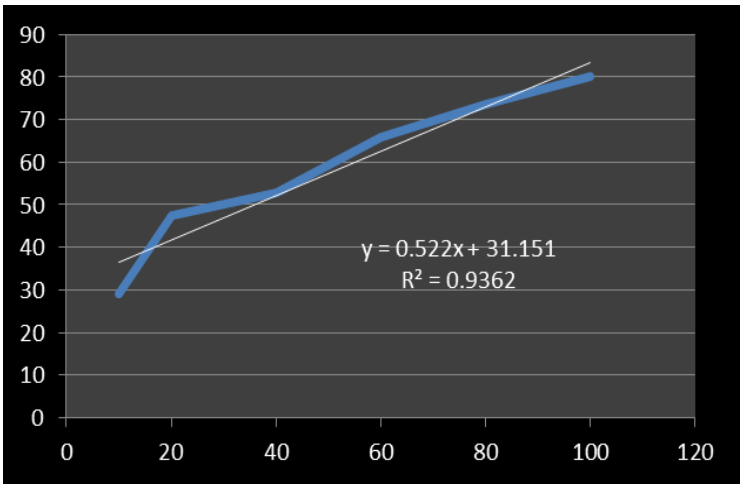


Table 4: Percentage inhibition of test drug KGK on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of KGK	% Inhibition of BHA
10 µg/ml	11.06 ± 2.066	43.53 ± 3.342
20 µg/ml	18.63 ± 1.193	57.05 ± 4.687
40 µg/ml	30.68 ± 1.033	66.05 ± 8.119
60 µg/ml	38.95 ± 1.033	76.8 ± 4.801
80 µg/ml	53.07 ± 1.578	84.59 ± 3.538
100 µg/ml	59.95 ± 3.321	92.49 ± 1.68

Data are given as Mean ± SD (n=3)

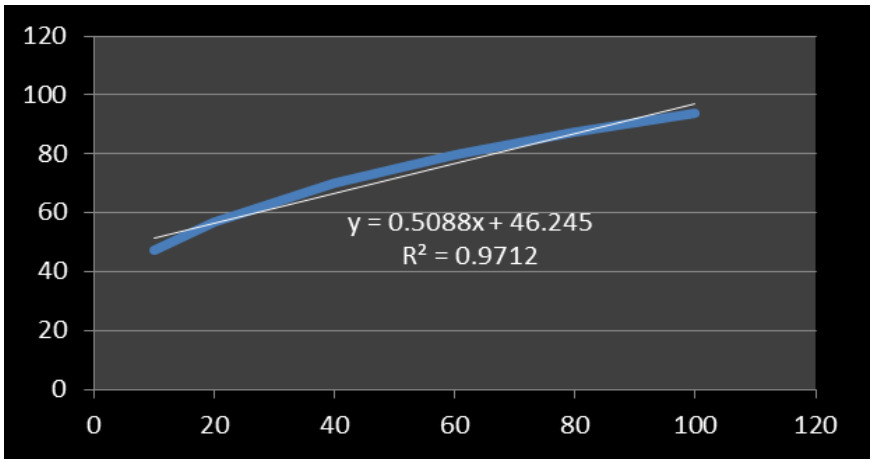
Table 4a: IC50 Values for Hydrogen peroxide radical scavenging assay by KGK and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
KGK	78.47 ± 1.137
BHA	12.1 ± 9.196

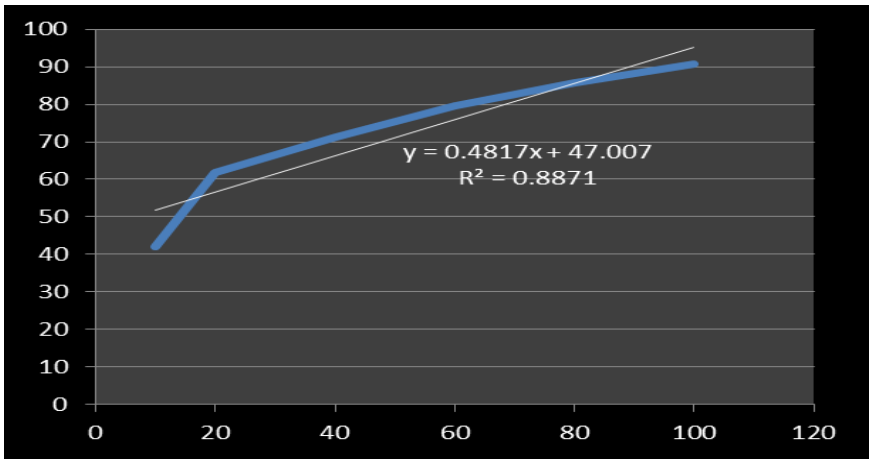
Data are given as Mean ± SD (n=3)

Figure 4: Percentage inhibition of STD on Hydrogen Peroxide radical scavenging assay

Triplicate 1



Triplicate 2



Triplicate 3

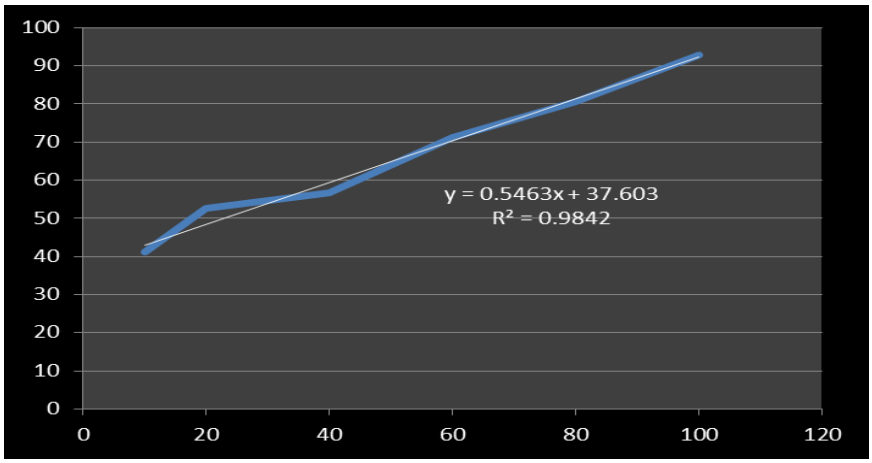
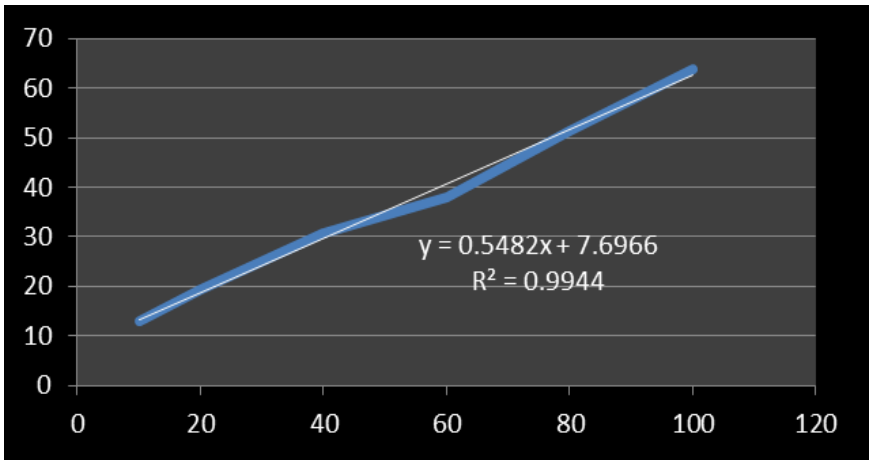
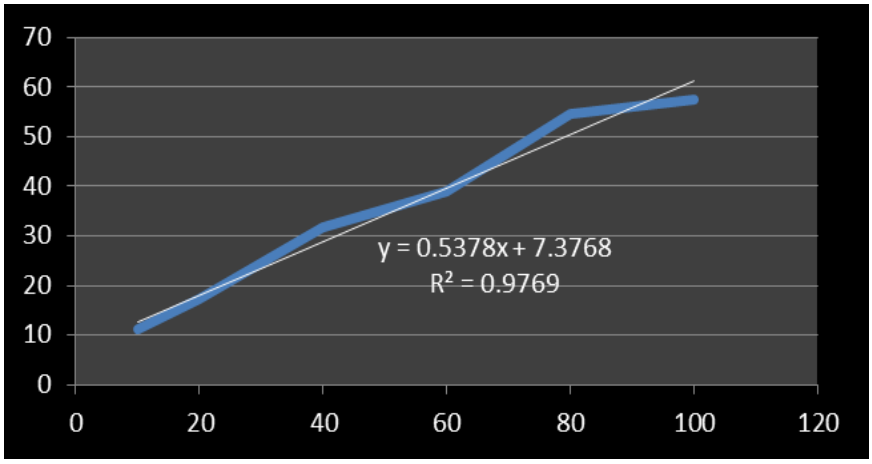


Figure 4a: Percentage inhibition of KGK on Hydrogen Peroxide radical scavenging assay

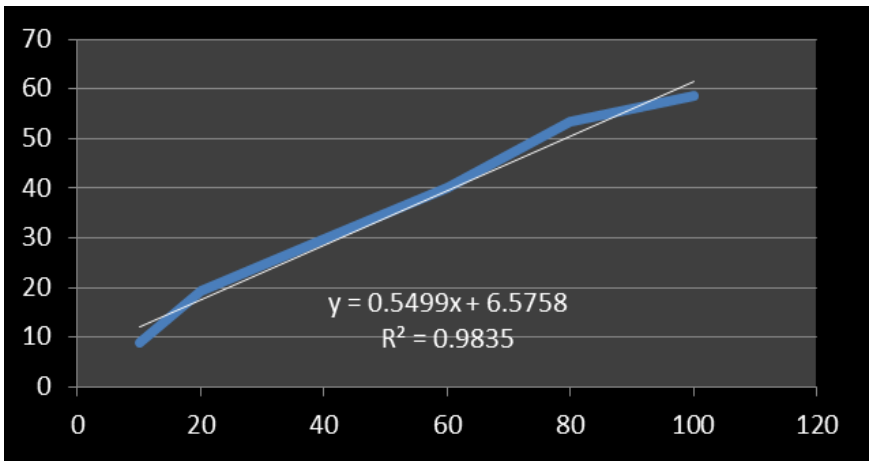
Triplicate 1



Triplicate 2



Triplicate 3



DISCUSSION

DPPH radical scavenging activity: Trial drug KGK were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 6.17 to 58.33 % when compared with standard ascorbic acid with percentage inhibition ranges from 39.63 to 89.6 %. The IC50 value of the trial drug was found to be 84.28 (µg /ml) when compared with standard ascorbic acid with (IC_{50}value 20.45µg/ml). The results are given in tables 1,1a and graphically represented in figure 1,1a.

NO radical scavenging activity: NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 20.96 to 75.08 % when compared with standard gallic acid with percentage inhibition ranges from 31.49 to 86.2 %.The corresponding IC50 value of the trial drug was found to be 64.61(µg /ml) when compared with standard gallic acid with (IC_{50}value 37.21µg/ml). The results are given in tables 2,2a and graphically represented in figure 2,2a.

ABTS radical scavenging activity: Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 30.04 to 76.42 % when compared with standard gallic acid with percentage inhibition ranges from 48.74 to 94.45%. The corresponding IC50 value of the trial drug was found to be 43.72 (µg /ml) when compared with standard gallic acid with (IC_{50}value 7.34µg/ml). The results are given in tables 3,3a and graphically represented in figure 3,3a.

Hydrogen peroxide radical scavenging activity: Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 11.06 to 59.95 % when compared with standard BHA with percentage inhibition ranges from 43.53 to 92.49 %. The corresponding IC50 value of the trial drug was found to be 78.47 (µg /ml) when compared with standard BHA with (IC_{50}value 12.1µg/ml). The results are given in tables 4,4a and graphically represented in figure 4,4a.

CONCLUSION

Based on the results obtained from the In vitro antioxidant assay for the sample KGK, it was concluded that the Siddha formulation KGK has promising antioxidant activity in the estimated assays. Hence KGK can be beneficial utilized in the management of skin diseases as mentioned in Siddha literature.

REFERENCES

1. Roderick J.Hay, Mohsen Naghavi. "The Global Burden of Skin Disease in 2010: An Analysis of the prevalence and impact of skin conditions". Journal of Investigative Dermatology. June 2014: volume 134(6): 1527-1534.
2. Pastore S, Korkina L." Redox imbalance in T cell-mediated skin diseases". Mediators of inflammation 2010. 2010 861949.August 4.
3.Varadraj V.Pai, Pankaj Shukla, Naveen Narayanshetty Kikkeri. "Antioxidants in dermatology".Indian Dermatology Online Journal. April- June 2014: volume 5(2): 210-214.
4.Murukesa Muthaliyar K.S, Gunapadam Porutpanbu Nool Part – 1: Siddha Materia Medica: Medicinal Plants Division.7th edition. Chennai. Department of Indian Medicine and Homeopathy: 2003.
5.Thyagarajan. R, Gunapadam Thathu – Jeeva vaguppu Part II and III: Siddha Materia Medica.4th Edition. Chennai. Department of Indian Medicine and Homeopathy: 2004.
6.Badami, Omprakash, Dongr SH, Suresh B. "In-vitro Antioxidant property of Solanum pseudocapsicum leaf extract". Indian Journal of Pharmacology. 2005: volume 37: 251-252.
7. Panda B. N, Raj A. B, Shrivastava N. R, and Prathani A. R. "The evaluation of nitric oxide scavenging activity of Acalypha indica Linn Root". Asian Journal of Research in Chemistry. 2009: volume 2(2): 148–150.
8. Pellegrini N, Ying M, and Rice-Evans C. "Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2,2′-azobis (3-ethylbenzothine-6-surfonic acid) radical cation decolorization assay". Methods in Enzymology. 1999: volume 299:384–389.
9. Ruch R. J, Cheng S.-J, Klaunig J. E. "Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea". Carcinogenesis. 1989:10(6):1003–1008

.