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Research Article

EVALUATION OF ANTIOXIDANT PROPERTIES OF POLY HERBAL SIDDHA PREPARATION *KARISALAI KARPA CHOORANAM*

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ABSTRACT

In recent years, there has been a great deal of attention toward the field of free radical chemistry. Free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, DNA and trigger a number of human diseases. Hence the researchers are searching a potent antioxidant drug from natural resource. In ancient time it has been clearly mentioned in Siddha system of medicine as *Kaaya Karpam* Therapy (Rejuvenation). *Karisalai Karpa Chooranam* (KKC) is a powerful poly herbal Siddha preparation mentioned in ancient Siddha literature. This medicine is indicated for *Paandu* (Anaemia), *Kaamalai* (Jaundice), *Kalleral veekkam* (Hepatomegaly), *Sobai* (Generalized edema), Skin diseases and helps to enhance the immune system. It is a powerful rejuvenating medicine in siddha system and used as a *Kaayakalpam*. This study is aimed to screen the antioxidant effect of KKC. In this study DPPH, Nitric Oxide and ABTS radical scavenging studies were performed. The results of this study shows that the percentages of inhibition in DPPH, Nitric Oxide and ABTS radical scavenging studies are 48.4 % (standard drug Ascorbic acid -78.64%), 50.7 % (Gallic acid – 86.2%) and 60 % (Gallic acid – 91.16%) respectively and thus, our findings provide evidence that KKC could be a potential source of natural antioxidant and it may be used as rejuvenating medicine for vast therapeutic effects, gives a powerful body, mind and soul with long-lasting life.

KEYWORDS: *Karisalai Karpa Chooranam*, Siddha Medicine, Antioxidant activity.

INTRODUCTION

Oxidation in living organisms is essential for the generation of energy during catabolism but these metabolic processes result in the continuous production of free radicals and reactive oxygen species (ROS) *in vivo.* Free radicals or more generally ROS are highly reactive species that are generated by cells during respiration and cell-mediated immune functions [1]. Free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, and pesticides [2]. The instability and reactivity of free radicals due to the lone electron in the outer shell can cause them to attack specific bio-molecules in the body such as protein and lipids[3]. Normally, there is a balance between the quantity of free radicals generated in the body and the antioxidant mechanisms which scavenge/quench these free radicals preventing them from causing deleterious effects in the body[2]. The antioxidant mechanisms include endogenous and exogenous systems such as catalase and vitamin antioxidants, respectively. When the generation of free radicals exceeds the scavenging capacity of the cell's endogenous systems, the excess free radicals seek stability through electron pairing with biological macromolecules of healthy cells such as proteins, lipids, and DNA. The pairing of the free radicals with bio-molecules can eventually result in the induction of lipid peroxidation which leads to cancer, atherosclerosis, cardiovascular diseases, ageing, and inflammatory diseases [2,4]. Prolonged oxidative stress can result in permanent damage to vital body organs, which could eventually lead to chronic disorders such as heart diseases, diabetes, cirrhosis, malaria, neurodegenerative diseases, AIDS, cancer, and premature aging [3,5]. It has been noted that about 95% of the pathologies observed in people above 35 years of age are associated with production and accumulation of free radicals [6].

Natural antioxidants are considered to be safe and bioactive.[7] The antioxidants from natural sources are the only alternative to synthetic antioxidants in counteracting the free radicals associated disease[3]. The antioxidant activities of phenolic compounds are mainly due to the redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers, in addition to their metal chelating potential. The antioxidant activity of phenolics plays an important role in the adsorption or neutralization of free radicals. [8]

In recent years, various species of plants have been used in preparation of drugs and are consumed as food due to their antioxidant activities [9]. The extracts of medicinal plants and natural products have become a great source of antioxidant and antiageing properties[10]. Recently, much attention has been directed towards the development of ethnomedicines with strong antioxidant properties but with low cytotoxicity.[11] Therefore, antioxidants with free radical scavenging activities of medicinal plants may have great relevance in the prevention of diseases and in therapeutic properties. [12] Plants, rich in their phytochemical compounds, are good sources of antioxidants and radical scavengers.[5]

In Indian traditional system of medicine like Siddha system many more herbs are used in antioxidant medicinal formulations which are called as *Kaaya Kalpa* medicines. These formulations have been used to treat the illness and help to regenerate the degenerative conditions and also help to prevent the aging. KKC is a poly herbal Siddha preparation mentioned in ancient Siddha literature.

Modern pharmaceuticals and nutraceuticals are currently out of reach of a large proportion of the human population in developing countries.[13] This necessitates the use of other sources of human knowledge to provide common health benefits. Thus, herbal medicines are now regarded as important but underutilized tool against the disease.[14] The main objective of the study was to determine the antioxidant activity of KKC by DPPH, Nitric Oxide and ABTS radical scavenging assay.

MATERIALS AND METHODS

Karisalai Karpa Chooranam [15]

The test drug *Karisalai Karpa Chooranam* has been purchased from SKM Siddha and Ayurveda Pharmacy, Erode, Tamilnadu. It is a poly herbal Siddha preparation prepared from seven herbal ingredients (Table:1) which has been used for *Paandu* (Anaemia), *Kaamalai* (Jaundice), *Kalleral veekkam* (Hepatomegaly), *Sobai* (Generalized oedema), Skin diseases and helps to enhance the immune system. It is a powerful rejuvenating medicine in siddha system and used as a *Kaayakalpam*.

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

The antioxidant activity of test drug sample KK was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . Sample KK 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 were used as standard was prepared in same concentration as that of the sample extract 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 KK 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.

$$\%\text{scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The effective concentration of test sample KGR 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 KK 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% naphthyl ethylene diamine dihydro chloride 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 KK 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 KK and standard was calculated and recorded. The

percentage nitrite radical scavenging activity of the test drug KK and gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

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

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

ABTS Assay

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug KK against2,2'-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 (10-100µg/ml) 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 KK was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample KK was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

Radical scavenging (%)

$$= \left(\frac{(A)_{control} - (A)_{sample]}}{(A)_{control}} \right) x_{100}$$

RESULTS AND DISCUSSION

Polyphenols are a large and diverse class of compounds, many of which occur naturally in a wide range of food and plants. The flavonoids are the largest and best studied group among polyphenols. A range of plant polyphenols is either being actively developed or already currently sold as dietary supplements and/or herbal, derived medicines. Although these compounds play an unknown role in nutrition (non-nutrients), many of them have properties including antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of genome12. Antioxidant quality is a measure of the effectiveness of the antioxidant(s) present as a pure compound or a mixture [16].The percentage scavenging and IC50 values were calculated for all models.

In the present study free radical scavenging activities of KKC was evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. The reactivity of KKC was analyzed with DPPH, a stable free radical. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stechiometrically related to the number of electrons gained [17].The DPPH radical scavenging (%) activity is shown in the Fig: 1, KKC exerted an inhibition of 48.4 % and that of Ascorbic Acid was 78.64 % at 100µg/ml and the IC50 value of the extract was 102.8 µg/ml, while that of Ascorbic Acid was 48.7µg/ml.

It is widely recognized that many of today's diseases are due to the oxidative stress that results from an imbalance between formation of ROS/RNS and their neutralization when endogenous anti-oxidant mechanisms are unable to quench the free radicals.[18] The free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity, search for effective and natural antioxidants has become crucial[19]. Natural antioxidants are believed to be safer and bioactive. [20]

Nitric oxide (NO) is generated from amino acid Larginine by vascular endothelial cells, phagocytes, and certain cells of the brain. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion (ONOO−)[3]. The antioxidants from natural sources could be the alternative to synthetic antioxidants in counteracting oxidative stress associated diseases. A great number of naturally occurring substances have been recognized to have antioxidant abilities and various *in vitro* methods have been used to assess their free radical scavenging and antioxidant activity. Therefore, in the present study, KKC at different concentrations were assessed for their nitrite free radical scavenging activity in an *in vitro* model. The nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The nitrite ions diazotize with sulphanilamide acid and couple with naphthyl ethylenediamine, forming pink colour, which was

measured at 546 nm[21]. As antioxidants donate protons to the nitrite radical, the absorbance is decreased. The decrease in absorbance was used to measure the extent of nitrite radical scavenging. [22]

ABTS radical scavenging activity

The ABTS.+ scavenging assay, which employs a specific absorbance (734 nm) at a wavelength remote from the visible region and requires a short reaction time, can be used as an index that reflects the antioxidant activity of KKC. In Fig. 2, KKC extract was found to be effective in scavenging radicals and the increase was concentration-dependent. At 100µg/ml, the inhibition of the extract was 60 % and that of Gallic acid 91.16 %. The IC50 of Galic acid was 23.79 µg/ml while the KKC extract was 76.33 µg/ml. This shows that KKC presents a moderate ability to scavenge the ABTS radical. The antioxidant activities against ABTS or DPPH were correlated with the concentration, chemical structures, and polymerization degrees of organ antioxidants [23].

Table: 1. Ingredients of *Karisalai Karpa Chooranam*

S.No	Tamil Name	Botanical Name
1	*Vellai Karisalai*	*Eclipta prostrata*
2	*Manjal Karisalai*	*Wedelia chinensis*
3	*Kuppaimeni*	*Acalypha indica*
4	*Seruppadai*	*Coldenia Procumbens*
5	*Vallarai*	*Centella asiatica*
6	*Neeli*	*Indigofera tinctoria*
7	*Kottai Karanthai*	*Sphaeranthus indicus*

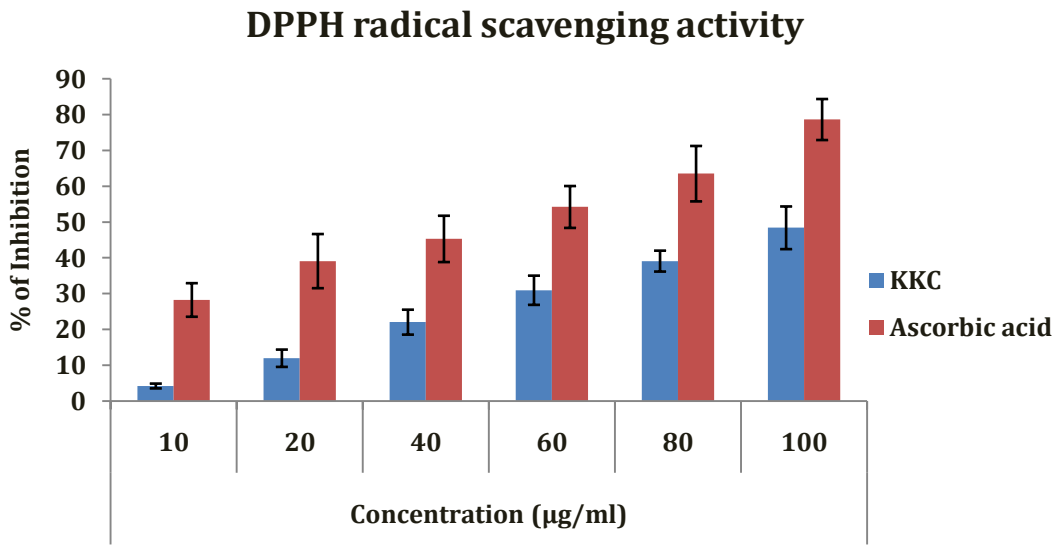

Figure: 1. shows the percentage of inhibition of KKC on DPPH radical scavenging activity

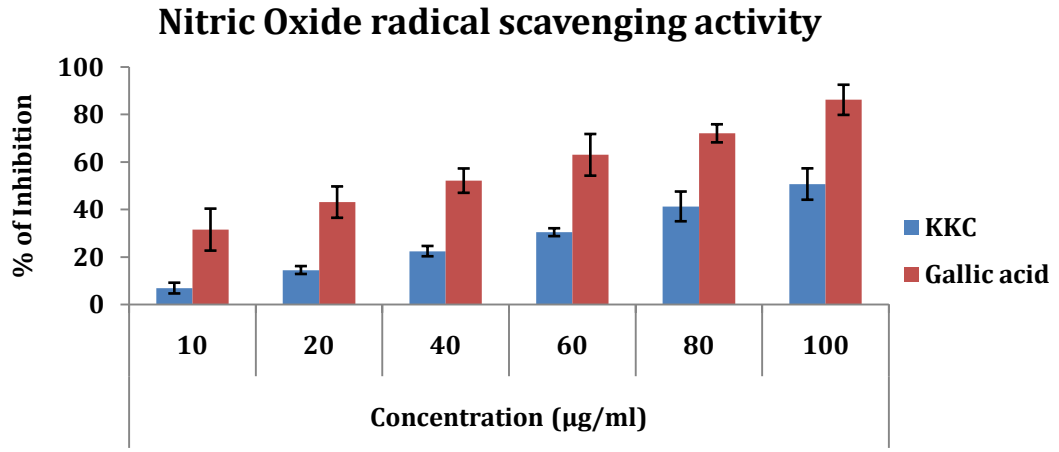

Figure: 2. Shows the percentage of inhibition of KKC on Nitric Oxide radical scavenging activity

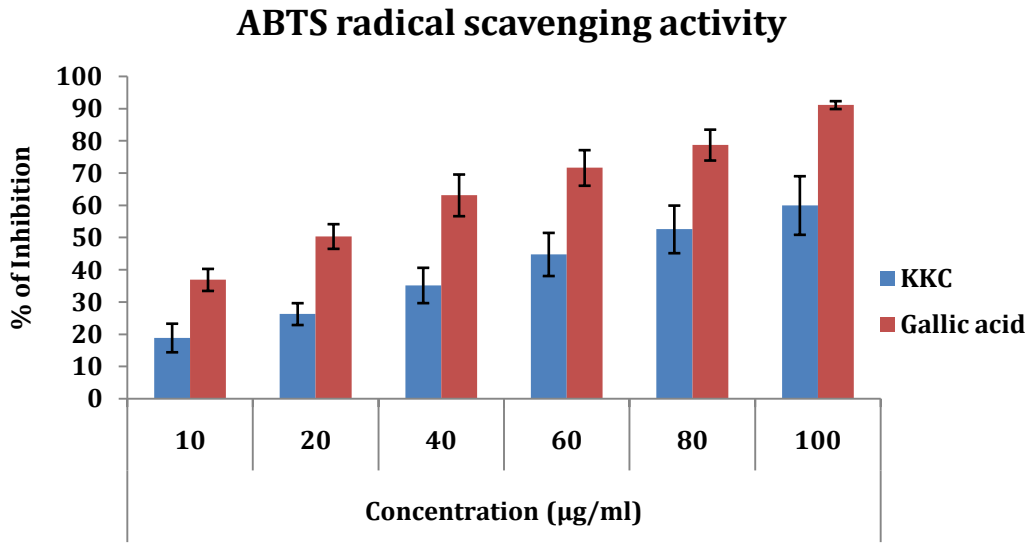

Figure: 3 Shows the percentage of inhibition of KKC on ABTS radical scavenging activity

CONCLUSION

The present investigation suggests that the Siddha poly herbal preparation *Karisalai Karpa Chooranam* which possesses good antioxidant potential is a better supplement for the diseases associated with oxidative stress.

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