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Research Article

# A STUDY ON PHYTOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACT OF SIDDHA DRUG KARISALAI KARPAM CHOORANAM IN *IN VITRO* CONDITION

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**ABSTRACT**

In Siddha system of medicine drugs consist of polyherbal formulations, containing different plants, used for the treatment of various diseases. Karisalai Karpam chooranam is a polyherbal formulation consisting seven traditionally used herbs. Since there is no information about pharmaceutical activities of antioxidant and phytochemical evaluation, the present study focused on the *in vitro* antioxidant methods where different solvent extract of Karisalai Karpam chooranam screened for 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid free radical scavenging activity, lipid peroxidation assay, superoxide scavenging activity, metal chelating assay, nitric oxide scavenging activity, and antioxidant activity in hemoglobin-induced linoleic acid. The results revealed potent scavenging activity when compared with standard. The aqueous-methanol extract of Karisalai Karpam chooranam was screened for further phytochemicals which indicated the presence of alkaloids, flavonoids, tannin, saponin, tepenoids, polyphenols and absence of glycosides, anthocyanin. These metabolites profile was recorded with thin layer chromatography. The result indicated promising antioxidant activity of crude extract and needs further exploration for their effective use in both modern and traditional system of medicines.

**Keywords:** Karisalai karpam chooranam, Siddha medicine, Phytochemical evaluation, Antioxidant.

## INTRODUCTION

There are numerous plants and traditional formulations available in siddha system of medicine for the treatment of many complication diseases. This has attracted a great deal of research interest in natural antioxidants and antioxidant based drugs/formulations for the prevention and treatment of complex diseases such as hepatic disorders, aging-related diseases. The human system creates reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical, and hydrogen peroxide by many enzymatic systems through oxygen consumption [1]. In small amounts, these ROS can be beneficial as signal transducers and growth regulators [2]. However, during oxidative stress, large amounts of these ROS may favor some human disease conditions such as cancer, hepatic diseases, cardiovascular diseases, ageing, and neurodegenerative diseases [3]. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS. Recently, many epidemiological studies have suggested that the consumption of natural antioxidants such as polyphenol-rich food, fresh fruits, vegetables, or teas have protective effects against the aforesaid diseases and this protection has been partly ascribed to the presence of several components, such as vitamins, flavonoids, and other phenolic compounds [4].

Karisalai Karpam chooranam is one of the siddha polyherbal drugs composed of seven different medicinal plants are *Eclipta alba* (Vellai Karisalai Samoolam), *Wedelia chinensis* (Manjal Karisalai Samoolam), *Indigofera tinctoria* (Neeli Samoolam), *Sphaeranthus indicus* (Kottakaranthai Samoolam), *Centella asiatica* (Vallarai Samoolam), *Acalypha indica* (Kuppaimeni), and *Coldenia procumbens* (Siru Serupadai). This drug mainly used internally for hepatic related diseases. The polyherbal medicine preparation and uses details were referred from Bogar 700. The present investigations were undertaken to study the effects of different solvent and aqueous decoction used for *in vitro* antioxidants activity of siddha medicine drug Karisalai Karpam chooranam.

## METHODS

### Collection of plant

Medicinal plants *E. alba* (Vellai Karisalai Samoolam), *W. chinensis* (Manjal Karisalai Samoolam), *I. tinctoria* (Neeli Samoolam) *S. indicus* (Kottakaranthai Samoolam), *C. asiatica* (Vallarai Samoolam) *A. indica* (Kuppaimeni), and *C. procumbens* (Siru Serupadai) composed in Karasalai Karpam chooranam were collected from Sairam Herbal garden, Department of Medicinal Botany, Sri Sairam Siddha Medical College and Research Centre, West Tambaram, Chennai-44, Tamil Nadu, India. Plants were authenticated by Dr. S. Sankaranarayanan, Head Department of Medicinal Botany.

### Preparation of extract

All the dried herbs clove, catechu, and fennel were finely powdered, and the fresh betel leaves were triturated in household mixer grinder without adding water. Then all the powdered herbs were weighed about 14.28 g and mixed evenly. The powdered material was subjected to maceration using different solvents methanol, acetone, ethyl acetate, and chloroform for 48 hrs. Aqueous decoction made into sterile distilled water in the water both 100°C for 1 hr. The extracts were filtered and evaporated to dryness and kept for further studies.

### Phytochemical analysis of Karisalai Karpam chooranam

The aqueous methanol extract of Karisalai Karpam was freshly prepared, and various chemical constituents were analyzed according to methods described by Allen [5] and Harbone [6]. The different chemical constituents tested for included tannins, saponin, glycosides, alkaloids, terpenoids, anthocynin, polyphenol, and flavonoids.

### 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay of different solvent extracts from Karisalai Karpam

ABTS radical scavenging activity of different extracts aqueous, methanol, acetone from Karisalai Karpam was determined according to Re *et al.* [7]. ABTS radical was freshly prepared by adding 5 ml of a

4.9 mM potassium persulfate solution to 5 ml of a 14 mM ABTS solution and kept for 16 hrs in the dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of vitamin C. Similarly, in the test Group 1 ml reaction mixture comprised of 950 µl of ABTS solution and 50 µl of the extract solutions. The reaction mixture was vortexed for 10 seconds and after 6 minutes absorbance was recorded at 734 nm against distilled water using an ELICO (SL150) ultraviolet-visible (UV-Vis) spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

**Superoxide radical scavenging assay of different solvent extracts from Karisalai Karpam**

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich, [8]) in the presence of the riboflavin-light-NBT system, as described earlier Tripathi and Pandey [9]; Tripathi *et al.* [10]. Each 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM ethylenediaminetetraacetic acid, NBT (75 µM) and different concentration of different solvent extract of Karisalai Karpam sample solution. It was kept in front of fluorescent light and absorbance was taken after 6 minute at 560 nm using an ELICO (SL150) UV-Vis spectrophotometer. Identical tubes with the reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample solution.

% Super oxide radical scavenging capacity=([A0-A1]/A0) × 100

Where A0 was the absorbance of control and A1 was the absorbance of organic solvent extract or standard.

**Inhibition of lipid peroxidation activity of different solvent extracts from Karisalai Karpam**

Lipid peroxidation induced by $Fe_{2+}$-ascarbate system in egg yolk by the method of Bishayee and Balasubramanian [11], was estimated as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al.* [12]. The reaction mixture contained egg yolk 0.1 ml (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and various concentrations of different solvent extracts of Karisalai Karpam in a final volume of 0.5 ml. The reaction mixture was incubated at 37°C for 1 hr. After the incubation period, 0.4 ml was removed and treated with 0.2 ml sodium dodecyl sulfate (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100°C for 1 hr. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 minutes. The butanol-pyridine layer was removed, and its absorbance at 532 nm (ELICO [SL150] UV-Vis spectrophotometer) was measured to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density of treatments with that of the control. Ascorbic acid was used as a standard.

Inhibition of lipid peroxidation (%) by the extract was calculated according to 1-(E/C) × 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample ($Abs_{532+TBA}$-$Abs_{532-TBA}$).

**Metal chelating activity of different solvent extracts from Karisalai Karpam**

Metal chelating capacity of different solvent extracts of Karisalai Karpam was measured according to the method described by Gülçin *et al.* [13]. 1 ml of different concentrations of ethanolic extract was added to a 0.05 ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine, and the mixture was shaken vigorously. After 10 minutes, the absorbance of the solution was measured at 562 nm against blank. All readings were taken in triplicate and vitamin C was used as a standard. The % inhibition of ferrozoine-$Fe^{2+}$ complex was calculated by following equation.

% Inhibition of ferrozoine-$Fe^{2+}$ complex=([A0-A1]/A0) ×100

Where A0 was the absorbance of control and A1 was the absorbance of different solvent extract.

**Nitric oxide radical scavenging activity of different solvent extracts from Karisalai Karpam**

Nitric radical scavenging capacity of different solvent extracts of Karisalai Karpam was measured according to the method described by Olabinri *et al.* [14]. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of ethanolic extract and incubated at room temperature for 150 minute. After incubation period, 0.2 ml of griess reagent (1% sulfanilamide, 2% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546 nm against blank. All readings were taken in triplicate and curcumin was used as a standard. The % inhibition was calculated by following equation.

% Nitric oxide radical scavenging capacity=([A0- A1]/A0) ×100

Where A0 was the absorbance of control and A1 was the absorbance of different solvent extract.

**RESULT**

**Phytochemical screening of Karisalai Karpam**

Phytochemical screening provides basic information about medicinal importance of a plant extract. In this study, evaluation for qualitative analysis of the chemical constituents of Karisalai Karpam extracts showed the presence of various secondary metabolites, alkaloid, saponins, flavonoid, tannins, polyphenols, and triterpenes. Anthraquinones and cardiac glycosides were not detected in aqueous-methanol extract (Table 1). Phytochemical screening indicated that the aqueous-methanol extract contained tannins and flavonoids, which are phenolic compounds. Plant phenolics are known to be antioxidants and free radical scavengers.

**Partial characterization of different solvent extract of Karisalai Karpam chooranam by thin layer chromatography (TLC)**

The methanol and acetone extract of Karisalai Karpam chooranam loaded on pre-coated TLC plates (60 $F_2$ 54 Merck) and developed with

**Table 1: Phytochemical screening of aqueous-methanol extract from Karisalai Karpam**

| S.No. | Phytochemical constituents | Result indicated | Extract of Karisalai Karpam |
|---|---|---|---|
| 1. | Alkaloids Dragendroff's reagent | Brown precipitation | + |
| | Mayer's reagent | Yellow precipitation | + |
| 2. | Flavonoids Alkaline test | Yellow coloration | + |
| | Lead acetate | Immediate precipitation | + |
| 3. | Polyphenols Ferrozine test | Blue coloration | + |
| 4. | Terpenoids Salkowski test | Brown ring | - |
| 5. | Tannins | Dark green blue | + |
| 6. | Glycosides Keller-Killani test | Reddish brown ring | - |
| | Bronbagers test | Pink color in ammonia layer | - |
| 7. | Saponins Froth test | Foam | + |
| 8. | Anthocynin ammonia test | Yellow color in ammonia layer | + |

−: Negative (absent), +: Positive (present)

a solvent system of hexane, chloroform, and methanol in the ratio of 1:0.5:0.1 was efficient to extract the antioxidant compound it was used for further studies. The developed plate was viewed under UV 240 nm and 360 nm (Table 2 and Fig. 1).

**ABTS radical activity of different solvent extract of Karisalai Karpam Chooranam**

All fractions of Karisalai Karpam Chooranam exhibited a powerful scavenging activity for ABTS radical cations in a concentration-dependent manner (Table 3), showing a direct role in catching free radicals. Maximum inhibition was observed with the aqueous decoction and methanol extract ranges from 26.38 to 72.63% at 5-20 µl/ml of Karisalai Karpam Chooranam and minimum inhibition was observed with the chloroform fraction ranges 14.41-58.89% at 5-20 µl/ml. This property may be credited to the presence of polyphenolics and flavones in the Chooranam of Karisalai Karpam. Hagerman *et al.* [15] have reported that the high molecular weight phenolics (tannins) have more abilities to quench free radicals (ABTS) and their effectiveness depends on the molecular weight, the number of aromatic rings, and nature of hydroxyl group's substitution than the specific functional groups. Free radical (ABTS) scavenging activity of Karisalai Karpam Chooranam extracts might be due to the presence of high molecular weight phenolics such as catechin and rutin derivatives.

**Inhibition of lipid peroxidation by different solvent extract of Karisalai Karpam Chooranam**

Different fractions aqueous, methanol, acetone, ethyl acetate, and chloroform of Karisalai Karpam Chooranam also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was observed with total aqueous and methanol extract with inhibition percentage 24.13-81.8 and 22.1.63-79.08 at 20 µg/ml, respectively, then other fractions (Table 4). This inhibition of lipid peroxidation possibly either due to chelation of Fe or by corner of the free radicals. Iron also is playing a major role for the formation of lipid peroxidation in the body. The process of lipid peroxidation has been suggested to proceed via a free radical chain reaction [16], which has been associated with cell membrane damage. This membranous damage has been suggested to contribute to various diseases, including diabetes. Incubation of egg yolk homogenates in the presence of $FeSO_4$ causes a significant increase in lipid peroxidation. It is possible that the high level of inhibition on lipid peroxidation displayed by the ethyl acetate fraction is related to the presence of phenolic compounds, which have been correlated with antioxidant activity [17].

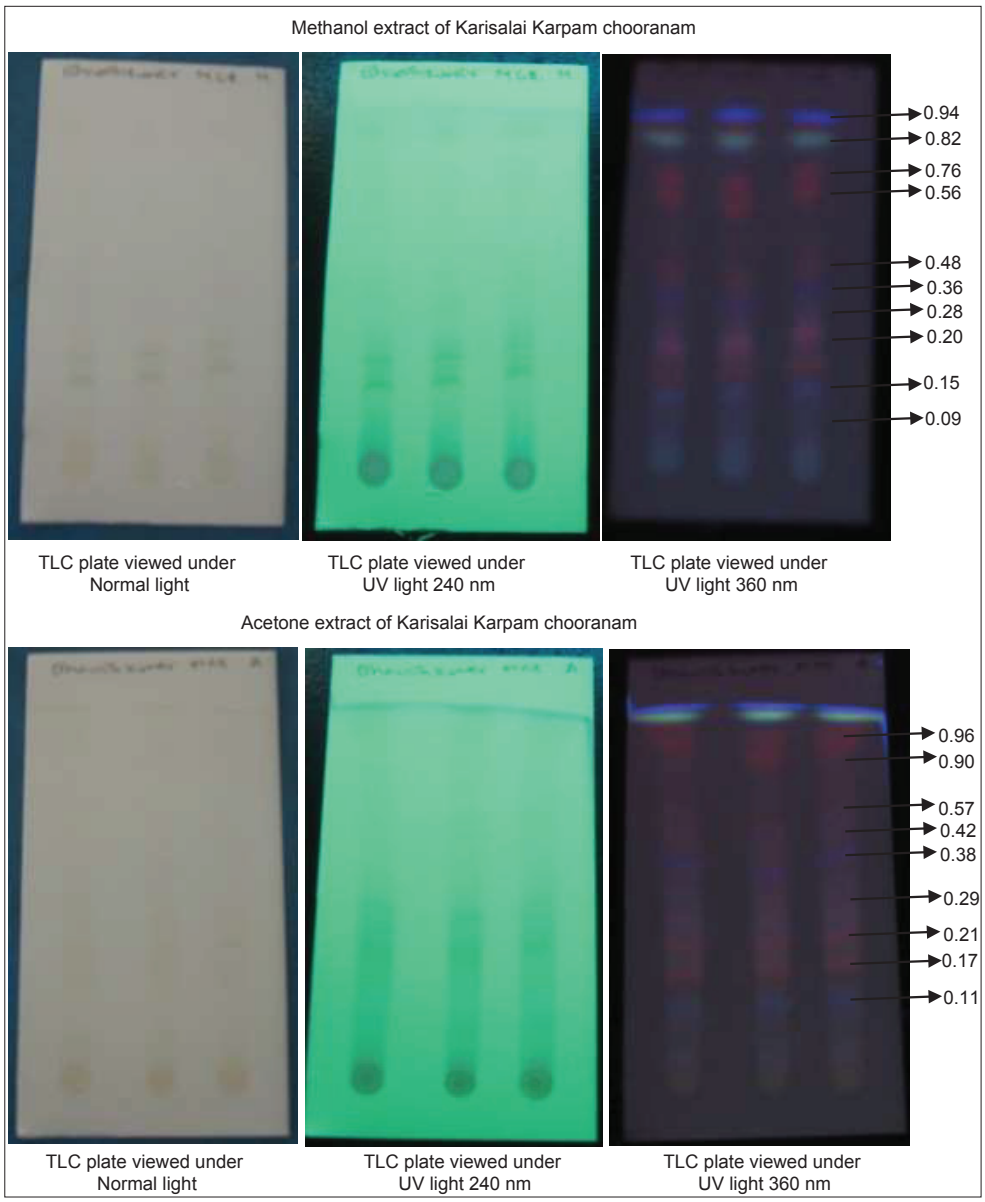


**Fig. 1: Partial characterization of different solvent extract from the Karisalai Karpam chooranam by thin layer chromatography**

**Superoxide anion scavenging activity of different solvent extract of Karisalai Karpam Chooranam**

Superoxide radicals by photochemical decrease of NBT in the occurrence of a riboflavin-light-NBT system, which is one of the standard methods. The total fractions aqueous, methanol, and acetone of Karisalai Karpam Chooranam exhibited potent scavenging activity for superoxide radicals in a concentration-dependent manner (Table 5). The aqueous decoction fraction had the highest superoxide radicals scavenging percentage 79.50±0.79 at 20 µg/ml, and the acetone fraction was least potent with 59.71±1.38 value at 20 µg/ml. Removal of superoxide in a concentration-dependent manner by any solvent fractions may be attributed to the direct reaction of its phytomolecules with inhibition of the enzymes.

**Effect of different solvent extract from Karisalai Karpam Chooranam for the Metal chelating activity**

The chelating properties of polyphenol compounds contribute to their antioxidant activity. By removing and neutralizing iron ions from iron-loaded hepatocytes, polyphenol metabolites inhibit oxidative damage. Table 6 shows the chelating effect of the Karisalai Karpam Chooranam aqueous decoction, methanol, and acetone extract on ferrous ions. Similarly, the ability of chelating ferrous ions also increased with the concentration ranges from 5 to 20 µg/ml of the Karisalai Karpam Chooranam extracts to a certain point, after that leveled off as the concentration further increased. At a dose level of 20 µg/ml, the chelating effect of the Karisalai Karpam Chooranam aqueous decoction and methanol extract could reach 84.08% and 79.24%, respectively, than other organic solvent extract. $Fe^{2+}$ has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing $Fe^{2+}$ concentration in Fenton reaction affords protection against oxidative damage. Chelating agents can inhibit radical generation by stabilizing transition metals, consequently reducing free radical damage. In addition, phenolic compounds have the potential to bind to metal ions due to their chemical structures, and have been shown to exhibit antioxidant activity through the chelation of metal ions [18].

**Nitric oxide scavenging assay of different solvent extracts of Karisalai Karpam Chooranam**

Nitric oxide injury takes 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. In the present study, five different solvent extracts of Karisalai Karpam Chooranam showed nitric oxide scavenging activity. Good result was observed at aqueous decoction and methanol extract with scavenging

**Table 2: Partial characterization of methanol and acetone extract from the Karisalai Karpam chooranam by TLC**

| S.No. | UV 240 nm Rf value | UV 360 nm Rf value | Visible Rf value |
|---|---|---|---|
| Methanol extract | | | |
| 1. | 0.28 | 0.09 | 0.28 |
| 2. | 0.30 | 0.15 | 0.20 |
| 3. | 0.34 | 0.20 | 0.15 |
| 4. | 0.36 | 0.28 | - |
| 5. | - | 0.36 | - |
| 6. | - | 0.48 | - |
| 7. | - | 0.56 | - |
| 8. | - | 0.76 | - |
| 9. | - | 0.82 | - |
| 10. | - | 0.94 | - |
| Acetone extract | | | |
| 1. | 0.35 | 0.11 | 0.28 |
| 2. | 0.40 | 0.17 | 0.20 |
| 3. | - | 0.21 | 0.15 |
| 4. | - | 0.29 | - |
| 5. | - | 0.38 | - |
| 6. | - | 0.42 | - |
| 7. | - | 0.57 | - |
| 8. | - | 0.90 | - |
| 9. | - | 0.96 | - |

UV: Ultraviolet, TLC: Thin layer chromatography

**Table 3: ABTS radical activity of different solvent extract of Karisalai Karpam Chooranam**

| Different concentration of Karisalai Karpam Chooranam | [a]Percentage of radical activity with different solvent extracts of Karisalai Karpam Chooranam on ABTS | | | |
|---|---|---|---|---|
| | Methanol extract | Aqueous extract | Acetone extract | Vitamin-C |
| 5 µl | 26.38±2.08 | 29.14±2.13 | 21.77±0.83 | 30.2±1.23 |
| 10 µl | 37.11±2.05 | 41.1±1.9 | 34.66±1.25 | 42.3±0.50 |
| 15 µl | 53.39±1.0 | 57.36±0.93 | 49.69±1.08 | 61.2±2.80 |
| 20 µl | 72.69±1.25 | 74.23±0.91 | 65.95±0.57 | 76.12±0.87 |

All the observations in different groups showed significant ($p<0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean±SD, ABTS: 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid, SD: Standard deviation

**Table 4: Inhibition of lipid peroxidation induced by $FeSO_4$ using egg yolk homogenates as lipid-rich media by different fractions of Karisalai Karpam Chooranam**

| Different concentration of Karisalai Karpam Chooranam | [a]Inhibition percentage of lipid peroxidation with different organic solvent extracts | | | |
|---|---|---|---|---|
| | Aqueous extract | Methanol extract | Acetone extract | Vitamin-C |
| 5 µl | 24±2.49 | 22±1.63 | 13±1.24 | 26±1.5 |
| 10 µl | 43±0.71* | 41±0.86* | 36±2.08* | 46±1.2 |
| 15 µl | 62±1.69 | 60±1.69 | 53±1.28 | 66±1.7 |
| 20 µl | 81±2.05 | 79±0.83 | 75±1.2 | 84±1.05 |

There was a significant (*$p<0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean±SD, SD: Standard deviation

**Table 5: Superoxide anion scavenging activity of different solvent extract of Karisalai Karpam Chooranam**

| Different concentration of Karisalai Karpam Chooranam | [a]Inhibition percentage of superoxide anion scavenging with different organic solvent extracts | | | |
|---|---|---|---|---|
| | Methanol extract | Aqueous extract | Acetone extract | Vitamin-C |
| 5 µl | 21.9±0.86 | 25.08±0.79 | 14.48±0.75 | 27.05±0.55 |
| 10 µl | 36.74±0.85 | 42.75±0.82 | 31.8±0.3 | 44.18±0.62 |
| 15 µl | 56.74±0.95 | 61.13±1.04 | 51.23±0.99 | 65.23±1.04 |
| 20 µl | 71.37±1.11 | 79.50±0.95 | 67.13±1.05 | 82.12±0.85 |

All the observations in different groups showed significant ($p<0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis), [a]Mean±SD, SD: Standard deviation

**Table 6: Effect of different solvent extract from Karisalai Karpam Chooranam for the Metal chelating activity**

| Different concentration of Karisalai Karpam Chooranam | [a]Percentage of Metal chelating with different organic solvent extracts | | | |
|---|---|---|---|---|
| | Methanol extract | Aqueous extract | Acetone extract | Vitamin-C |
| 5 µl | 23.21±0.89 | 25.81±0.81 | 21.25±1.22 | 28.52±0.65 |
| 10 µl | 35.47±1.06 | 37.31±0.77 | 30.99±0.66 | 39.21±0.55 |
| 15 µl | 52.82±0.79 | 58.78±0.9 | 47.8±1.53 | 61.45±0.1 |
| 20 µl | 79.24±1.08 | 84.08±1.51 | 72.04±1.17 | 88.23±1.82 |

All the observations in different extract showed significant ($p<0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean±SD, SD: Standard deviation

ranges 81.04±1.67 at 20 µg/ml than acetone extracts compared with 87.41±0.63 at 20 µg/ml for vitamin C which served as positive control (Table 7). The present study demonstrated three different solvent acts as nitric oxide scavenging due to extracts contain polyphenol compounds; free radicals are scavenged and, therefore, can no longer react with nitric oxide, resulting in less damage. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, and involved in the regulation of various physiological processes. At present, it is increasing evidence to suggest that nitric oxide and its derivatives produced by the activated phagocytes may have a genotoxic effect and may contribute in the multistage carcinogenesis process [19]. By antioxidant defense systems, the 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 [20].

**Antioxidant activity in hemoglobin-induced linoleic acid of different solvent extracts of Karisalai Karpam Chooranam**

The antioxidant activity of Karisalai Karpam Chooranam different solvent extracts was determined using the hemoglobin induced linoleic acid system. The bioactive compounds in the extract were electron donors and could react with free radicals to convert them into more stable products and to terminate radical chain reactions. The maximum inhibitory activity was 88% in 20 µl/ml concentration of aqueous extract of Karisalai Karpam Chooranam (Table 8). ROS promotes the free radical attack resulting in lipid peroxidation [21]. The Karisalai Karpam Chooranam aqueous extract showed maximum activity in hemoglobin-induced linoleic acid system due to the presence of high phenolic content and most of the polyphenol compound freely soluble water, and thus acting as potential antihemolytic extract.

## CONCLUSION

According to data achieved from the present study, three different solvents methanol, aqueous extract, and chloroform crude extract of Karisalai Karpam Chooranam was found to be an effective antioxidant in different *in vitro* assays, including total antioxidant activity determination by ABTS radical, superoxide anion radical scavenging, lipid peroxidation, and nitric oxide scavenging when it is compared to standard antioxidant compounds used as vitamin C. Further exact antioxidant compounds isolation and characterization and *in vivo* studies under processes.

**Table 7: Nitric oxide scavenging activity with different solvent extracts of Karisalai Karpam Chooranam**

| Different concentration of Karisalai Karpam Chooranam | [a]Percentage of nitric oxide scavenging with different solvent extracts | | | |
|---|---|---|---|---|
| | Methanol extract | Aqueous extract | Acetone extract | Vitamin-C |
| 5 µl | 27.93±0.84 | 30.28±1.07 | 25.35±0.80 | 32.08±1.5 |
| 10 µl | 43.19±0.65 | 45.07±1.08 | 40.84±0.76 | 47.05±1.80 |
| 15 µl | 62.22±0.71 | 63.84±0.19 | 60.79±0.77 | 66.25±0.56 |
| 20 µl | 79.57±1.12 | 81.45±0.97 | 78.4±0.85 | 84.69±0.48 |

All the observations in different extract showed significant ($p<0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean±SD, SD: Standard deviation

**Table 8: Antioxidant activity in hemoglobin induced linoleic acid of different solvent extracts of Karisalai Karpam Chooranam**

| Different concentration of Karisalai Karpam Chooranam | [a]Percentage of antioxidant activity in hemoglobin-induced linoleic acid with different solvent extracts | | | |
|---|---|---|---|---|
| | Methanol extract | Aqueous extract | Acetone extract | Vitamin-C |
| 5 µl | 20±0.97 | 23±2.15 | 19.2±1.10 | 25.21±1.5 |
| 10 µl | 38±1.35 | 44±3.0 | 37.5±1.46 | 48.10±2.4 |
| 15 µl | 59±1.11 | 65.33±2.3 | 58.6±1.20 | 68.23±1.9 |
| 20 µl | 75±1.55 | 79.4±1.95 | 73±1.69 | 81.23±1.7 |

All the observations in different extract showed significant ($p<0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean±SD, SD: Standard deviation

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