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IN-VITRO ANTIOXIDANT STUDY OF LYOPHILIZED EXTRACT OF NILAVEMBU KUDINEER CHOORANAM– A TRADITIONAL SIDDHA FORMULATION

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ABSTRACT

Nilavembu kudineer chooranam (NVK) is a traditional Siddha poly herbal formulation controls all types of fever associated with body ache. It exhibits potent antiviral activity against viruses causing Dengue and Chikungunya fever. In fevers associated with inflammatory conditions such as abscess it counteracts the inflammation and reduces body temperature. several studies have established that oxidative stress as a determinant of vascular homeostasis besides its involvement in the pathogenesis of various infectious diseases, such as chronic hepatitis C, Japanese encephalitis, leptospirosis, respiratory syncytial virus-induced acute lung inflammation, malaria, chagas cardiomyopathy, schistosomiasis, sepsis, acute herpes simplex virus type 1, measles subacute sclerosing panencephalitis, and dengue. Literature revealed that no antioxidant studies have been carried out in this traditional formulation. So in the present study the antioxidant potential of lyophilized extract of NVK was evaluated using different methods such as DPPH, nitric oxide free radical scavenging assay and FRAP reducing power assay. The results revealed that significant free radical scavenging activity was observed with NVK in DPPH and Nitric oxide free radical scavenging assay methods. The NVK exhibited potent antioxidant power in FRAP assay.

KEYWORDS: Nilavembu kudineer chooranam, DPPH assay, FRAP reducing power assay, Nitric oxide assay, Antioxidant property.

INTRODUCTION

The nilavembu kudineer chooranam (NVK, a poly herbal siddha formulation) constituted equal proportion of Nilavembu (*Andrographis paniculata*), Vilamichu ver (*Plectranthus amboinicus*), Chukku (*Zingiber officinale*), Milagu (*Piper nigrum*), Koraikizhangu (*Cyperus rotundus*), Peipudal (*Tricosanthes cucumerina*), Vettiver (*Vettiveria zizanoides*), Santhanam (*Santalum album*), and Parpadagam (*Mollugo cerviana*).[1] The NVK is a poly herbal formulation which heals all types of fever with body pain. It controls fever in a wide-ranging way through healing effects by regulating temperature, controlling inflammation and relief from body pain. Combined action of this herbal combination relieves all types of fever irrespective of the type of causative organism. It exhibits potent antiviral activity against viruses causing Dengue and Chikungunya fever.[2] It is also effective in fevers caused by infective organism such as Typhoid and Malaria. In fevers associated with inflammatory conditions such as abscess it counteracts with the inflammation and reduces body temperature. Pharmacological actions of few medicinal plants present in NVK were studied extensively. Piperine, extracted from *Piper nigrum* has anti-inflammatory, antinociceptive, and anti-arthritic property.[3,4] Neoandrographolide, one of the principal diterpene lactones, isolated from a medicinal herb *Andrographis paniculata* possesses significant antiinflammatory effects.[5] Hepatoprotective role of the whole plant of *Hedyotis corymbosa* against paracetamol overdose-induced and *Trichosanthes cucumerina* in carbon tetrachloride induced liver damage in rats was well documented.[6-8] Rhizomes of *Zingiber officinale* has antiinflammatory, cytoprotective, anti-ulcer action in non steroidal anti-inflammatory drug (NSAIDs) induced ulcer model, hepatoprotective and antioxidant property in paracetamol induced animal model.[9-11]

It is widely known that many of today's diseases are caused by oxidative stress that results from an imbalance of ROS/RNS and their pacification when endogenous antioxidant mechanisms are unable to reduce the free radicals.[12] The free radicals are scavenged by synthetic antioxidants, but due to their adverse side effects leading to cancer, search for effective and natural antioxidants has become crucial.[13] Natural antioxidants are believed to be safer and bioactive.[14] 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. With this background, the present study was aimed to evaluate the antioxidant potential of NVK using different antioxidant assay methods.

MATERIALS AND METHODS

Plant material

The plant materials were collected and authenticated by Dr. Chelladurai, Research officer (retd), Central Council for Research in Ayurvedic Sciences (CCRAS), Tamil nadu. The nilavembu kudineer (a poly herbal siddha formulation) composed of equal quantity of Nilavembu (*Andrographis paniculata*), Vilamichu ver (*Plectranthus amboinicus*), Chukku (*Zingiber officinale*), Milagu (*Piper nigrum*), Koraikizhangu (*Cyperus rotundus*), Peipudal (*Tricosanthes cucumerina*), Vettiver (*Vettiveria zizanoides*), Santhanam (*Santalum album*), and Parpadagam (*Mollugo cerviana*).

Preparation of lyophilized extract

Weighed 12.5 gm of NVK was boiled with 250 ml of water till the decoction is concentrated to 60 ml. The extract was lyophilized and used for the *in-vitro* antioxidant study.

DPPH free radical scavenging assay

Aliquots of 0.1ml of lyophilized extract (10, 20, 40, 60, 80, 100 µg/0.1ml) were mixed with 1.9ml of DPPH solution (200µM in ethanol) and incubated in dark condition for 20min at 37°C. The absorbance of the reaction mixture was recorded at 517nm. Ascorbic acid (AS) was used as standard. The radical scavenging activity was determined using the formula[15] - % scavenged (DPPH) $=[(A_o-A_1)/A_o] \times 100$, where A_0 = Absorbance of control; A_1 – Absorbance of Extract or Standard.

Nitiric oxide free radical scavenging assay

Aliquots of 2ml of sodium nitroprusside (10mM in phosphate buffered saline) were mixed with 1ml of lyophilized extract (10, 20, 40, 60, 80, 100 µg/ml) and incubated for 4 hours at 37°C. To the above solution, 0.5ml of Griess reagent was added and the absorbance was measured at 546nm. Curcumin (CUR) was used as standard. The radical scavenging activity was determined using the formula[16] - NO Scavenged (%) $= (A_0-A_1)/A_0 \times 100$, Where A_0 = Absorbance of control reaction and A_1 = Absorbance in presence of sample.

Ferric reducing antioxidant power (FRAP) assay

Weighed 2.5 ml of various concentrations of lyophilized extract was mixed with 1ml of 0.2M phosphate buffer pH-6.5. Then add 1ml of 1% potassium ferricyanide and the mixture was incubated in waterbath at 50^0c for 20mins. The reaction mixtures were rapidly cooled and 2.5ml of 10% Trichloroacetic acid was added.[17] Then, the contents was centrifuged for 10 min at 1000rpm. 2.5ml of the supernatant was added with 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride. The colour changes to green, allow standing for 10 min then the absorbance was taken at 593nm. Ascorbic acid was used as standard.[18]

RESULT AND DISCUSSION

DPPH radical scavenging activity

The DPPH assay is the most convenient method for antioxidant screening which is performed by forming a stable and reduced DPPH molecule through the donating of hydrogen (19). The results of DPPH assay are showed in table1 and graph 1. The results revealed that the percentage inhibition of NVK was increasing with the increase in concentration. The results are comparable with the standard ascorbic acid.

Table 1: DPPH Free Radical Scavenging Activity of Lyophilized Extract of NVK.

Concentration (µg/ml)	Nilavembu kudineer chooranam	Standard (Ascorbic acid)
	Percentage Inhibition	
10	80.05±1.94	94.25±6.13
20	83.99±0.17	96.83±1.25
40	85.95±0.22	95.61±2.07
60	88.98±0.33	97.26±0.65
80	91.69±0.71	97.83±0.31
100	92.61±0.07	98.29±0.06

Values are expressed as Mean±S.D of three experiments.

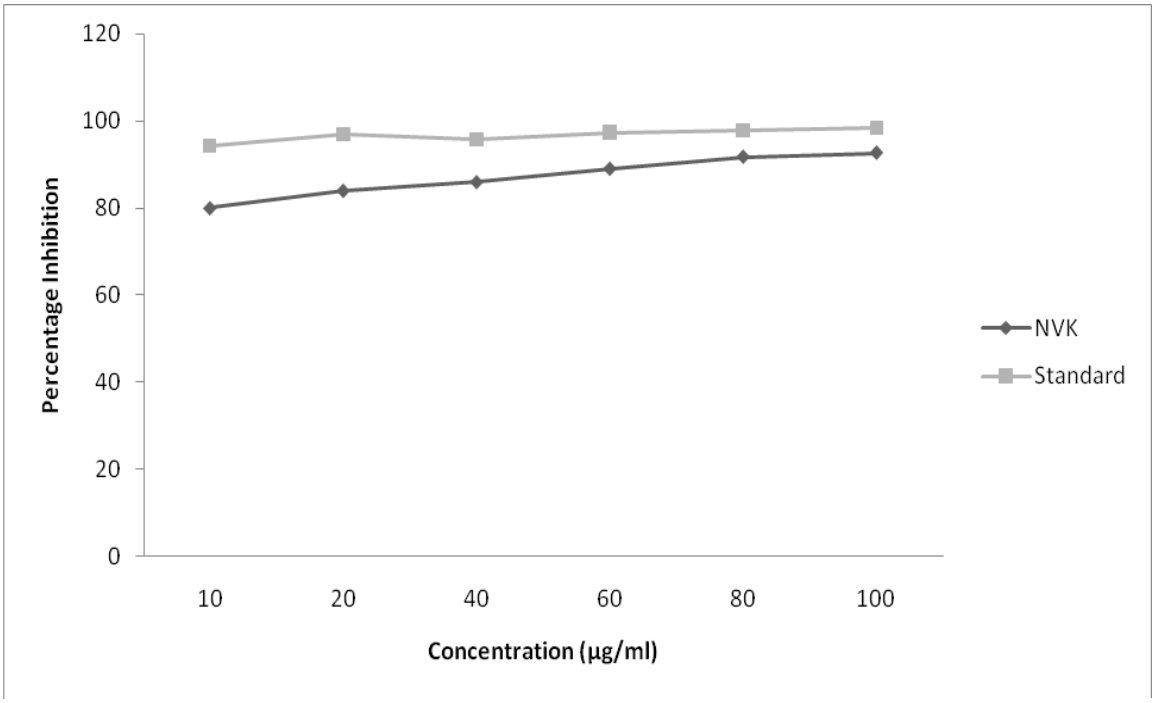

Figure 1: DPPH Radical Scavenging Activity of Lyophilized Extract of Nilavembu Kudineer Chooranam (NVK). Standard used was Ascorbic acid.

Nitric oxide radical scavenging activity

As antioxidants donate protons to the nitrite radical, the absorbance is decreased. The difference in absorbance was used to measure the extent of nitrite radical scavenging.[20] The results of nitric oxide free radical scavenging assay are showed in table 2 and graph 2. Concentration dependent percentage inhibition of NVK was observed. The percentage inhibition was high at 100 µg/ml. The results are comparable with the standard curcumin.

Table 2: Nitric Oxide Radical Scavenging Activity of Lyophilized Extract of Nilavembu Kudineer Chooranam (NVK).

Concentration (µg/ml)	Nilavembu kudineer chooranam (NVK)	Standard (Curcumin)
	Percentage Inhibition	
10	48.94±0.92	48.37±1.96
20	56.50±1.27	55.82±0.45
40	60.53±0.41	59.06±0.46
60	63.53±0.57	63.88±0.45
80	66.35±0.57	65.73±1.78
100	69.17±0.09	67.53±0.45

Values are expressed as Mean±S.D of three experiments

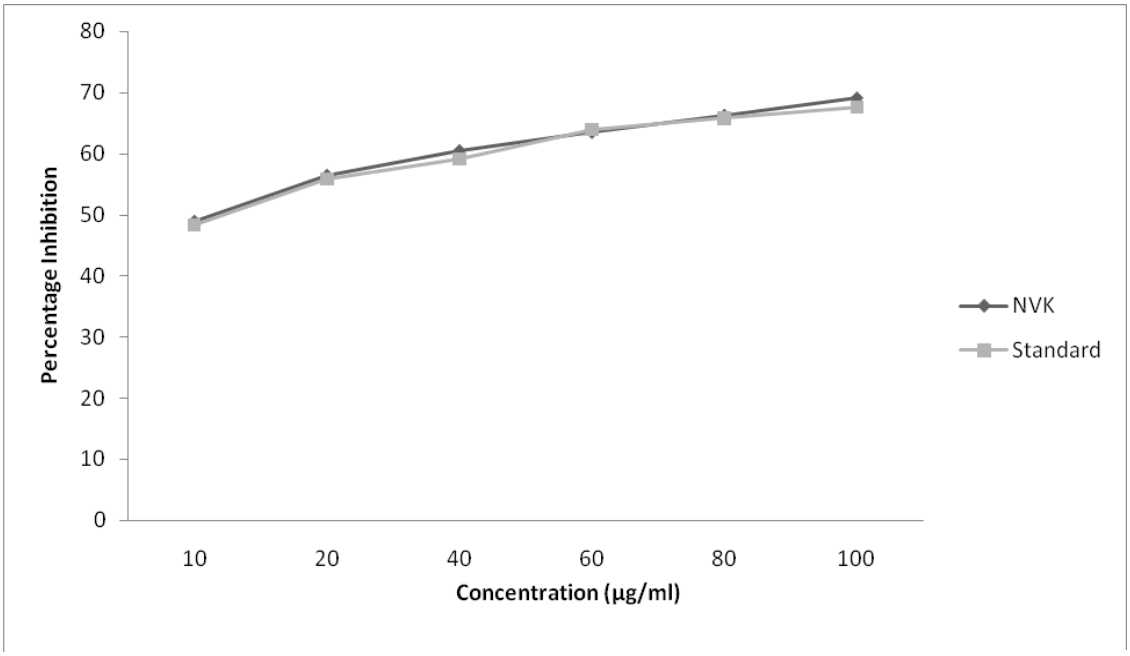

Figure 2: Nitric Oxide Radical Scavenging Activity of Lyophilized Extract of Nilavembu Kudineer Chooranam (Nvk). Standard used was Curcumin.

FRAP assay

The reduction of Fe3+ to Fe2+ by ferric reducing antioxidant (FRAP) assay is an indicator of potential antioxidant activity of natural products (21). The results of the FRAP assay are shown in table 3 and graph 3. Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. The FRAP values were obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The results revealed that the antioxidant power of NVK extract shown by increase in absorbance. The antioxidant power of sample was comparable with standard ascorbic acid.

Table 3: FRAP Assay of Lyophilized Extract of Nilavembu Kudineer Chooranam (NVK).

Concentration (µg/ml)	Nilavembu kudineer chooranam (NVK)	Standard (Ascorbic acid)
	Absorbance	
10	0.071±0.0025	0.0027±0.0002
20	0.091±0.015	0.015±0.004
40	0.114±0.024	0.073±0.003
60	0.126±0.009	0.078±0.001
80	0.137±0.008	0.086±0.002
100	0.150±0.002	0.098±0.006

Values are expressed as mean±S.D of three experiments

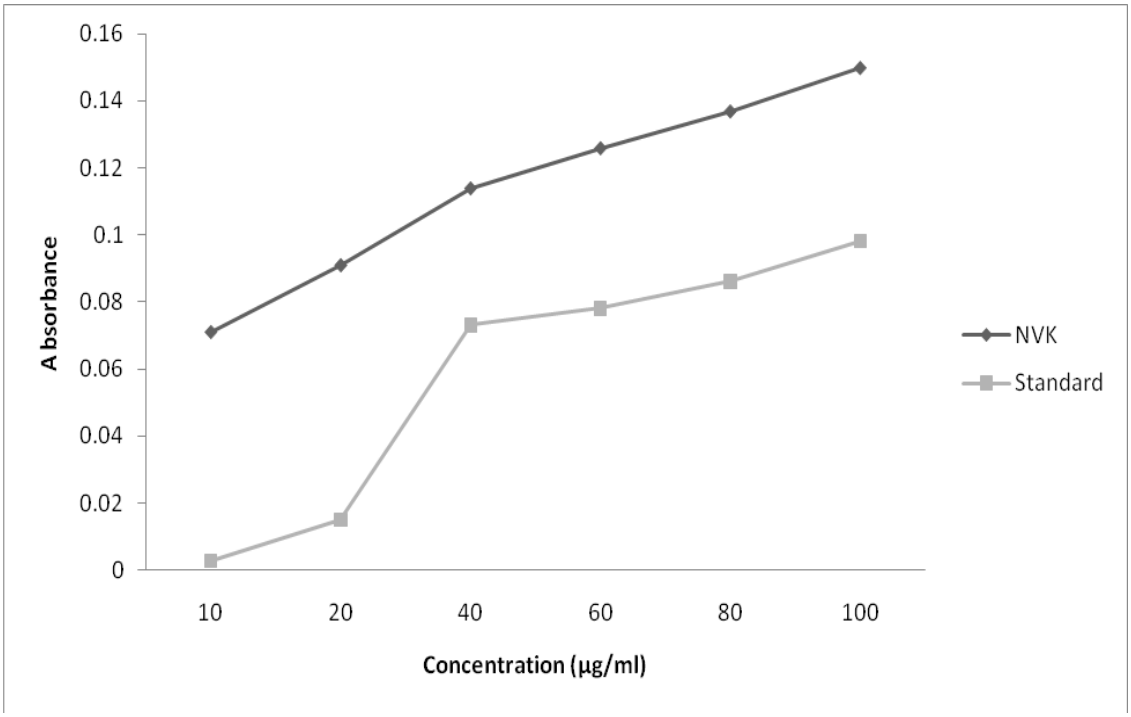

Figure 3: FRAP Assay of Lyophilized Extract of Nilavembu Kudineer Chooranam (NVK). Standard used was Ascorbic acid.

CONCLUSION

In this study, the antioxidant potency of lyophilized extract of NVK was evaluated using multiple methods like DPPH, nitric oxide free radical scavenging assay and FRAP reducing power assay. The results showed significant free radical scavenging activity with NVK in DPPH and Nitric oxide free radical scavenging assay methods which was comparable with standard ascorbic acid and curcumin respectively. The formulation also exhibited potent antioxidant power in FRAP assay which was similar to standard ascorbic acid. The result substantiates the traditional use of NVK in fever and infections. Endogenous oxidation reactions are important for the normal biochemistry of life and are especially critical for leukocyte microbial killing mechanisms in host defense to infectious diseases. The data from literature suggest that antioxidants, and therapy based on increasing antioxidant potential, have a major impact on clinical infectious diseases.

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