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In-vitro Antioxidant activity of poly herbal siddha formulation *Thanneervittan nei*

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Abstract

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms and leads to development of certain disease like cancer, atherosclerosis, cardiovascular diseases, ageing, and inflammatory diseases etc., In ancient time, the term Antioxidant has been clearly described in Siddha system of medicine as *Kaaya Karpam* (Rejuvenation Therapy). The *Kaaya Karpam* helps to prevent the risk of disease development and ageing. *Thanneervittan Nei* (TVN) is a poly herbal Siddha preparation mentioned in ancient Siddha literature. This medicine is indicated for nervous debility, urinary obstructions, leucorrhoea, gonorrhea, menorrhagia, diabetes, emaciation and tuberculosis. It is a powerful rejuvenating medicine in Siddha system. The present study is aimed to evaluate the antioxidant effect of TVN. 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 42.19 % (standard drug Ascorbic acid -83.99 %), 45.16 % (Gallic acid – 89.18 %) and 63.79 % (Gallic acid – 92.78 %) respectively and thus, findings provide evidence that TVN 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: Siddha medicine; *Thanneervittan Nei;* Antioxidant.

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 ROS in vivo. Free radicals or more generally reactive oxygen species are highly reactive species that are generated by cells during respiration and cell-mediated immune functions [1].

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[2]. Normally, there is equilibrium 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[3].

It is currently hypothesized that many diseases are due to oxidative stress that results from an imbalance between the formation and detoxification of pro-oxidants. Oxidative stress is initiated by reactive oxygen species (ROS), which are produced as a by-product of electron transport in mitochondria [4].

Prolonged oxidative stress can result in permanent damage to vital body organs, which may lead to chronic disorders such as heart diseases, diabetes, neurodegenerative diseases, cancer, and premature aging [2,5]. 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].

In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases [7, 8]. Natural antioxidants such as α-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens [9-11]. Therefore, there is a considerable interest in finding new and safe antioxidants from natural sources to replace these synthetic antioxidants [12, 13].

Recently, traditional plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids [14] which prevent free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular disease [15].

In Siddha system of medicine many 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 ageing[16].In this TVN is one of the Siddha antioxidant medicine which is widely used in practice. Therefore, the objective of this study was to assess the free radical scavenging potential of the polyherbal Siddha formulation TVN.

Materials and Methods

Thanneervittan Nei[17]*:*

The test drug *Thanneervittan Nei* has been purchased from IMPCOPS Pharmacy, Chennai, Tamilnadu. It is a poly herbal Siddha preparation prepared from twenty-nine ingredients.

Table: 1. Ingredients of *Thanneervittan Nei*

S.No	Tamil Name	Botanical Name/ English Name
1	*Nellikkai*	Amla juice
2	*Thanneervittan*	Asparagus root juice
3	*Vellari*	**Cucumis sativus**
4	*Venpooshani*	Ash Gourd fruit juice
5	*Ilaneer*	Tender Coconut
6	*Pasumpaal*	Cow's Milk
7	*Karumbu*	Sugarcane juice
8	*Nei*	Cow's ghee
9	*Chukku*	*Zingiber officinale*
10	*Milaku*	*Solanum nigrum*
11	*Thippili*	*Piper longum*
12	*Kadukkai*	*Terminalia chebula*
13	*Nellikkai*	*Phyllanthus emblica*
14	*Thanrtikkai*	*Terminalia belerica*
15	*Dhaniya*	*Coriandrum sativum*
16	*Elam*	*Elettaria cardamomum*

17	*Elavangam*	*Syzygium aromaticum*
18	*Athimadhuram*	*Glycyrrhiza glabra*
19	*Kottam*	*Costus speciosus*
20	*Seeragam*	*Cuminum cyminum*
21	*KarumSeeragam*	*Nigella Sativa*
22	*Senkazhuneerkizhangu*	Water lily rhizome
23	*AngoorThiraatchai*	Dry grapes
24	*KottiKizhangu*	Aponogeton tuber
25	*Pereechchang kai*	Dry dates
26	*Kalkandu*	Sugar candy
27	*Induppu*	Rock salt
28	*Omum*	*Trachyspermum ammi*
29	*KurasaniOmum*	*Hyoscyamus niger*

Antioxidant activity:

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay [18]

The antioxidant activity of test drug sample TVN was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample TVN extract 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 six test tubes and by serial dilution with concentration ranges from 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Ascorbic acid was 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 TVN 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 using double-beam U.V Spectrophotometer at 517 nm by using methanol as blank.

% scavenging =

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

Nitric Oxide Radical Scavenging Assay[19]

The concentrations of test sample TVN extract are made into serial dilution from 10−100µg/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 diaminedihydrochloride 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 TVN 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 done for the test samples. The absorbance was measured using a Spectra Max Plus UV-Vis microplate reader at 546 nm (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug TVN and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug TVN 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 or standards.

ABTS Assay[20]

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

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

Results and Discussion

In the present study free radical scavenging activities of TVN 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 TVN 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 Spectrophotometrically related to the number of electrons gained [21]. The DPPH radical scavenging (%) activity is shown in the Fig: 1, TVN exerted an inhibition of 42.19 % and that of Ascorbic Acid was 83.99 % at 100µg/ml.

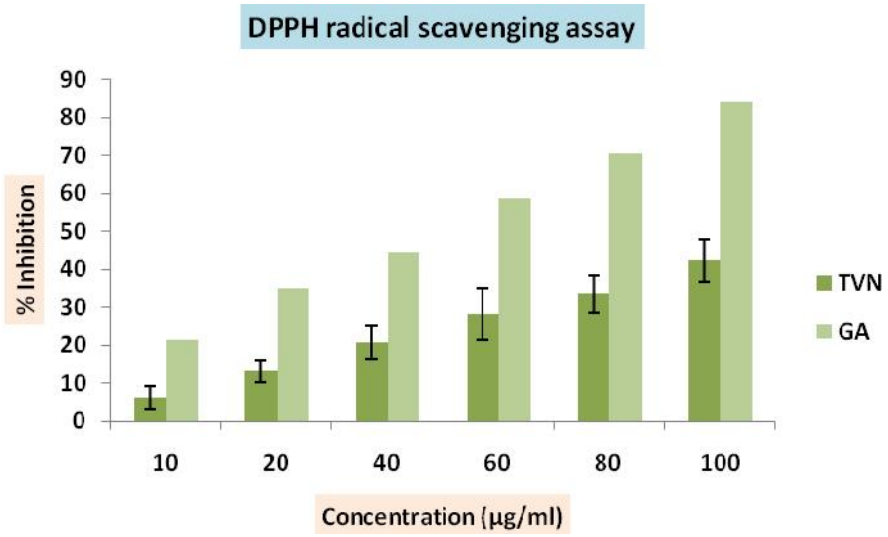

Figure 1

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 ($ONOO^-$)[2]. 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, TVN 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 Naphthylethylenediamine, forming pink colour, which was measured at 546 nm[22]. 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. [23]The Nitric Oxide radical scavenging (%) activity is shown in the Fig: 2, TVN exerted an inhibition of 45.16 % and that of Gallic Acid was 89.18 % at 100µg/ml.

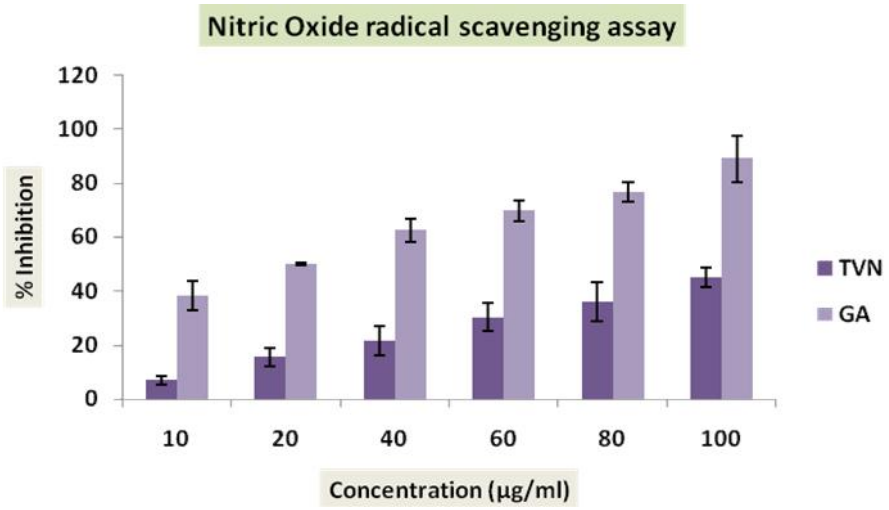

Figure 2

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 TVN. In Fig. 3, TVN extract was found to be effective in scavenging radicals and the increase was concentration-dependent. At 100µg/ml, the inhibition of the extract was 63.79 % and that of Gallic acid 92.78%. This shows that TVN presents a moderate ability to scavenge free radicals.

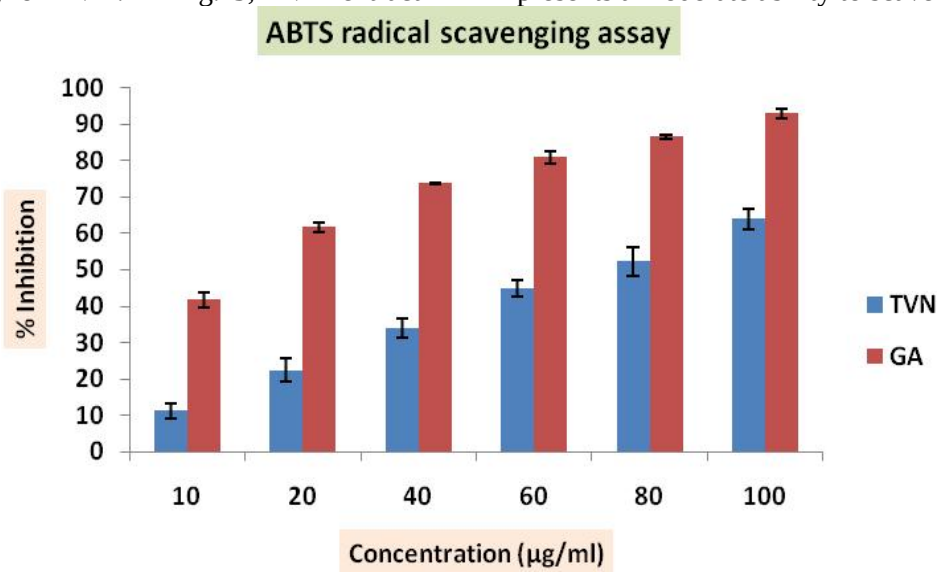

Figure 3

Conclusion

This study explores that the Siddha poly herbal preparation *Thanneervittan Nei* which possesses good antioxidant potential. Hence, further evaluation has to be carried out to isolate the specific bioactive compound.

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