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In vitro antioxidant activity in ethanolic leaf extract of *Tabernaemontana divaricata* (L.).

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Abstract: Present study is focussed to analyze *In vitro* antioxidant activity in leaf of *Tabernaemontana divaricata* (L.). In this study different concentrations of ethanolic leaf extract (200, 400, 600, 800 and 1000µg/ml) are used to perform various assays (DPPH radical scavenging, Superoxide anion radical scavenging, Nitric oxide scavenging and hydrogen peroxide scavenging) for the determination of antioxidant potential in leaves. Concentration based scavenging activity was observed in all assays. From these results, this study expose that the leaf have the ability to act against free radicals.

Key words: Antioxidant activity, Free radical scavenging, *Tabernaemontana divaricata* (L.).

Introduction

Free radicals are rickety molecule. One type of free radicals is referred as reactive oxygen species. It contains more than one unpaired electron molecules in outer orbital. Peroxyl radical (ROO·), superoxide anion ($O2^{-}$), ozone (O_3), hydroxyl ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) are some precedent of reactive oxygen species (ROS) (1,2,3). Because of its unbalanced state, they react with vicinity molecules to reach constancy and instigating free radical producing chain reactions.

Extreme generation of these molecules leads to antioxidant diminution inside the body and begets disproportion among free radicals and antioxidant defense in human cause the state known as oxidative stress (4). Uncontrolled level of ROS play a part in the cause of changes in cellular operations as well as several oxidative stress mediated syndromes such as cancer, diabetes, neurodegenerative problems, nephrological troubles and cardiovascular complaints (5,6,7).

Antioxidants can act as free radical scavengers, chelating agent and have the ability to limit lipid peroxidation process. Antioxidant defense system precludes the cell damage by terminating sequential reactions of ROS production because of the donation of electron to the free radicals (8,9). Antioxidant activity can be assisted by means dietary supplements. Intake of these compounds can decrease the threat of diseases caused by free radicals. In developing countries, plants are employed as a conventional medicine owing to its enhanced understanding attribute, harmless nature and efficiency. Plants have medicinal properties because of the presence of natural antioxidants (10,11,12).

Tabernaemontana divaricata (L.) is a ornamental plant with medicinal properties belonging to Apocynaceae family. It can be called as Crepe jasmine (English) and Nandiyavattam (Tamil) (13, 14). Leaves of this plant have been used to cure ulcers and rheumatism (15).

Materials and Methods

Preparation of extract:

Tabernaemontana divaricata (L.) leaves (Double flower type) were collected and washed. It was gloom dried and milled. 30g leaf powder had been mixed with ethanol (200ml) and extraction was done for 24 hours. After extraction, extracts were concentrated by evaporation and further it was dispersed under vaccum. The dehydrated extract was stocked in sealed vials for future use.

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DPPH radical scavenging assay (16):
Different concentrations of ethanol extracts of leaf (200, 400, 600, 800 and 1000µg/ml) was used. 1ml ethanolic solution of DPPH (0.3mM) was mixed with 2.5ml of different concentrations of extract and kept in room temperature. After 30min, the absorbance was deliberated at 518 nm.
Scavenging capacity (%) = 100 – [(A_S - A_B) × 100/A_C]
Ethanol (1ml) and extract solution (2.5ml) was used as a blank, while DPPH solution with ethanol was used as a negative control. The positive control (standard) was DPPH with 1mM Morin.

Superoxide anion radical scavenging assay (17):
In this assay, each 3ml reaction mixture contained 0.05M of PBS (pH 7.8), Methionine (13mM), Riboflavin (2µM), 100µM of EDTA, NBT (75µM) and 1ml of ethanolic extract of leaf with different concentrations from 200 - 1000µg/ml and 1mM rutin was used as the positive control. All tubes were placed facing towards a fluorescent light (725 lumens, 34 W). After 20min of reaction, optical density values were taken at 560 nm. This whole compilation was covered in a box **lined** coated with aluminium foil. Superoxide anion radical inhibition was estimated through the expression given below

Inhibition (%) = [(A_0 – A_S)/A_0] x 100

Where, A_0 - Control absorbance and A_S – Sample absorbance.

Nitric oxide radical scavenging assay (18):
The leaf extract was taken with various concentrations from 200 − 1000µg/ml and positive control was 1mM gallic acid. 10mM sodium nitroprusside in phosphate buffered saline (0.5ml) was added to 1ml of extract then kept for 180min in 25˚C. The extract was mixed with an equal volume of freshly prepared Griess reagent (1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride). Control samples were prepared without the extracts. Absorbance was calculated at the wavelength of 546 nm. The percentage of nitrite radical scavenging was assessed using given formula,
Nitrite radical scavenging (%) = [(A_C – A_S)/A_C] x 100
Whereas, A_C - Control absorbance and A_S – Sample absorbance.

Hydrogen peroxide scavenging assay (19):
Hydrogen peroxide (40mM) was made in phosphate buffer (pH 7.4). In distilled water, different volume of leaf samples ranging from 200 -1000µg/ml were prepared then combined with 0.6ml of H_2O_2. Later than 10 minutes, Optical density values of H_2O_2 scavenged was identified at 230nm. Phosphate buffer without hydrogen peroxide was used as blank. Positive control was ascorbic acid.

The hydrogen peroxide scavenging was deliberated by the given formula

Scavenged H_2O_2 (%) = [(A_C – A_S)/A_C] x 100,

where A_C is the absorbance of the control and A_S is the absorbance of the sample

Statistical analysis:
Assays were performed in triplicates and the values are expressed as mean ± standard deviation. Graphs were plotted for the same and discussed in results section.

Results and Discussion

In plant secondary metabolites, phenolic compounds are important one. It contains various biological actions like anti-allergic, antibacterial, anti-inflammatory and anticarcinogenic nature. Flavonoids have the capacity to decrease chain reactions of free radicals by neutralize it (20, 21, 22).

Figure (1) depicts DPPH Radical Scavenging Activity in ethanolic leaf extract of *T. divaricata* (L.). Deactivation of free radicals can occur because electron or hydrogen molecules move from phytochemical compounds (23). The percentage of DPPH radical inhibition was directly proportional to extract concentration. DPPH radicals was neutralized well (9.9%) at higher concentration of extract as well as standard (Morin) exhibited 16.5% of inhibited radicals.

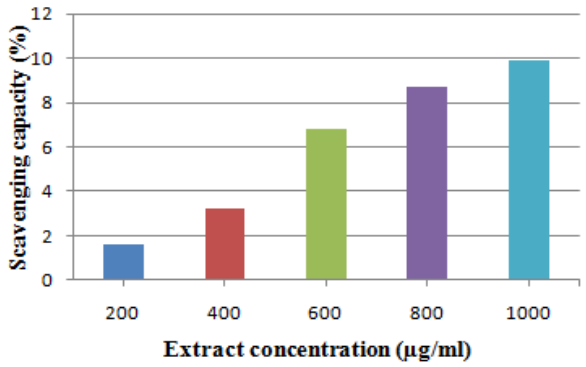

Figure 1: DPPH radical scavenging effect of ethanolic leaf extract of *T. divaricata* (L.)

One type of powerful reactive oxygen species is Superoxide anion radical. During energy

transduction in mitochondria, electrons leak to oxygen then convert into this radical form. It can also transform into injurious forms for example hydroxyl radical and hydrogen peroxide. These molecules have deleterious effect on proteins, carbohydrates and DNA (24, 25). From figure (2) the maximum scavenging of Superoxide anion radicals (26.6% inhibition) was observed at 1000µg/ml of extract concentration. It is observed that the standard Rutin also inhibits 38.3% of radicals at greater concentration of 1000µg/ml.

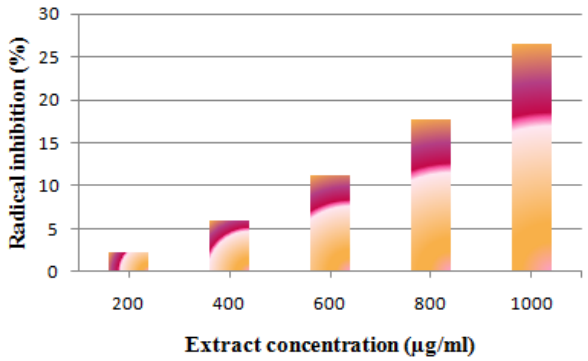

Figure 2: Activity of ethanolic leaf extract of *T. divaricata* (L.) on superoxide anion radicals

Endothelial cells, macrophages and neurons can secrete nitric oxide (NO). Several body processes are controlled by nitric oxide. When surplus of NO react with oxygen produce the molecule such as nitrite and peroxynitrite (26, 27, 28, 29). It can act as free radicals and play a part in the pathogenesis of various diseases. Figure (3) shows the inhibition percentage of radicals was 16.9% at maximum extract concentration. In case of standard gallic acid, 27.6% of radical inhibition was observed.

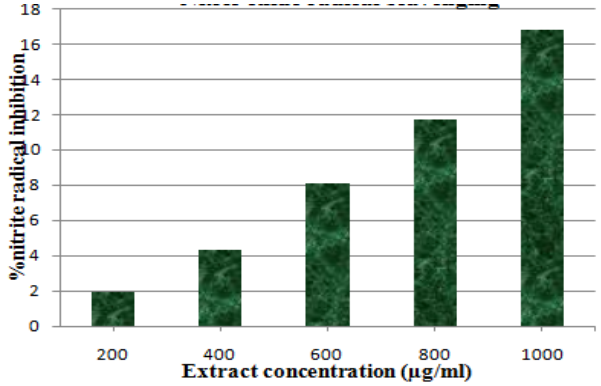

Figure 3: Effect of ethanolic leaf extract of *T. divaricata* (L.) against Nitrite radical action

In reactive oxygen species (ROS), hydrogen peroxide is non radical type; sometimes change into hydroxyl radicals in cells. Any one of the compound can donate electron to H_2O_2 leads to the reduction of H_2O_2 to water. This reaction prevent cell by retarding the production of hydroxyl radicals (30). Figure (4) reveals that the better percentage inhibition of H_2O_2 (25.3%) identified at greater concentration of extract. In case of ascorbic acid exposed 29.2% of impeded H_2O_2 at higher concentration.

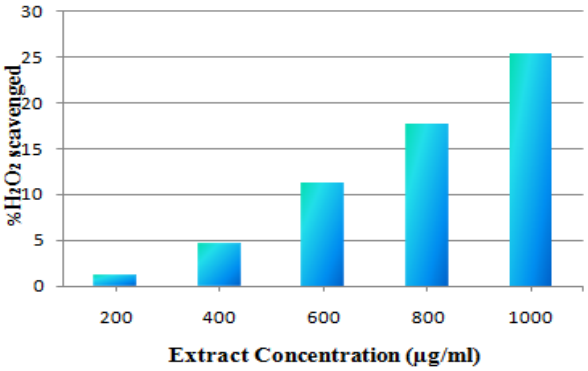

Figure 4: Action of ethanolic leaf extract of *T. divaricata* (L.) counter to H_2O_2 radicals

Due to the occurrence of antioxidant compounds (phenolic compounds, flavonoids, carotenoids and vitamins) in plant, it has the capacity to block series reactions of free radicals (31). When increase in the concentration of leaf extracts, percentage of radicals inhibition also elevated in all assays.

Conclusion

Increased number of ROS leads to detrimental outcome on macromolecules under pathological circumstances. Now a day there is a need for antioxidant containing food supplements. Present study reveals that the leaves of T. *divaricata* (L.) contain variety of phytocompounds and it can protect the body through neutralization of free radicals. Future work will be accomplished to find about the DNA damage and its protection by the mechanism of antioxidant compounds present in ethanolic extract of T. *divaricata* (L.) leaves.

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