

Research Article



Antioxidant and Acetylcholine esterase Inhibitory Activities of *Nalla Marunthu A* Poly Herbal Formulation.

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ABSTRACT

The importance of traditional herbal medicinal system has now gained vital importance. India is blessed with rich and diverse heritage of cultural traditions. Plants are prospective source of various medicinal agents in different countries. About 60 to 90% of populations in the developing countries use plant-derived medicine. Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases. Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found *in vitro* to have antimicrobial properties. Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses. Therefore, it is of interest to investigate the analysis of Antioxidants and, Acetyl Choline esterase inhibitory activities of *Nalla marunthu* were screened.

Keywords: Neuro pharmacology, Secondary metabolites, Phytochemicals, Antioxidants, Acetyl choline esterase.

INTRODUCTION

Medicinal plants play a pivotal role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various human ailments because they contain the components of therapeutic value¹. In addition, plant based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine². *Croton bonplandianus* belongs to the family of Euphorbiaceae or Caster family. A much branched woody herb and 20-50 cm tall, branches stellate hairy to glabrous. Leaves alternate or subopposite, shortly petiolate; lamina 1.2-3.2 cm long, narrowly ovatelanceolate, apex acute, cuneate at base. Inflorescence terminal, 5-7 cm long, flowers laxly distributed. Male flowers small, white occupies the upper portion of the inflorescence. Female flowers few at the base of the inflorescence. Capsule 0.45 x 0.4 cm, oblong-ellipsoid, shallowly 3-lobed. Actions and uses: Juice of 3-4 leaves is given for 3-4 days to cure cough. Seed paste is applied locally on eczema and ringworm to cure. Latex is used to heal cuts and wounds. EtOH (50%) extract of plant is hypotensive and spasmolytic. The leaf extract shows antiviral activity against tomato spotted with virus coepia. The plant extract is also effective against green gram leaf curl disease³.

Trianthema portulacastrum Linn. is a herb used in Ayurvedic medicine. *Trianthema portulacastrum* Linn, belonging to the family Aizoaceae, is one of the common weed, which has enormous traditional uses against diseases and some bioactive compounds have been isolated from this weed. It is an exotic weed and a native of tropical America. It is growing throughout most tropical

countries, such as Baluchistan, Ceylon, and India⁴. It is now naturalized throughout India in cultivated fields, river beds, waste ground, etc⁵. Its infestation is very common in various agricultural and vegetable crops, such as mustard, maize, pigeon pea, mung bean, potato, onion, cotton, soybean, pearl millet, and sugarcane, especially during the rainy seasons. This is not cultivated commercially, but it is found throughout India as a tropical problematic terrestrial weed by virtue of its infestation in plains, river beds, and in wastelands. It also grows automatically in cultivated fields with agriculture and vegetable crops, especially in the rainy seasons⁶. *Amaranthus polygonoides* is a herb the pharmacological properties of Amaranth products are considered of vital importance. For reducing tissue swelling the leaves are well thought-out to be constructive, and they have a cleansing effect too. The plant has also been used curatively for diarrhea, dysentery, excessive menstrual flow, ulcers and intestinal hemorrhaging. For the treatment of intestinal bleeding, excessive menstruation, diarrhea and other related problems, a tea made from its leaves are used⁷.

The plant *Indigofera tinctoria* belongs to the family of Fabaceae, its commonly known as "True Indigo" and called as Neeli or Avuri in Tamil. *I. tinctoria* is a shrub, distributed throughout the India. The different parts of the plants are used for variety of diseases. The seeds of *I. tinctoria* containing galactomannon composed of galactose and mannose. Roots and leaves are used in epilepsy and hydrophobia. The aerial parts of *I. tinctoria* used in treatment of antiproliferative activity in human lung cancer⁸. Different solvent extracts of *I. tinctoria* showed antibacterial activity⁹. Dry powder of *I. tinctoria* used to treatment of asthma¹⁰. The leaves of *I.*



tinctoria used as anti-inflammatory traditionally. Indirubin is the active compounds isolated from *I. tinctoria* leaves active as effective anticancer drug Indigotin is the active compounds isolated from this leaves possess hepatoprotective activity^{11,12}.

MATERIALS AND METHODS

Collection of samples

The poly herbal formulation (*Nalla marunthu*) is used for this experiment. The herbal formulation was prepared as available by the available literature.

Preparation of Herbal medicine

The herbal formulation *Nalla marunthu* was prepared in the department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Bharath University, Chennai, India. The equal volume of shade dried leaves of *I. tinctoria*, *A. polygonoides*, *T. portulacastrum* and *C. bonplandianus* were taken in to marter and pistle, the plant material was coarsely powdered, then filtered by muslin cloth and the filtrate was used for further extraction.

Preparation of extracts

1000 grams of *Nalla marunthu* was packed in separate round bottom flask for sample extraction using solvent namely Chloroform. The extraction was conducted by 250 ml of the solvent mixture for a period of 24 hours. At the end of the extraction the respective solvent were concentrated under reduced pressure and keep it in water bath (at 50°C). Now the extracted experimental solutions were stored in refrigerator.

Determination of Antioxidant activity (DPPH free radical scavenging activity).

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity¹³. Ethanolic solution of DPPH (0.05 mM) (300 l) was added to 40 l of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation. Percent (%) inhibition of DPPH activity = $[(AB - AA) / AB] \times 100$ Where AA and AB are the absorbance values of the test and of the blank sample, respectively¹⁴.

Acetyl cholinesterase (Acetyl cholinesterase) activity

Acetyl cholinesterase activity The enzymatic activity was measured using an adaptation of the method described¹⁵

500l of DTNB 3 mM, 100l of AChI 15 mM, 275l of Tris-HCl buffer 50 mM, pH 8 and 100l of each ethanolic extract, decoction or essential oil fraction were dissolved in ethanol, water or DMSO, respectively, and were added to a 1 ml cuvette. This cuvette was used as blank. In the reaction cuvette, 25 l of buffer were replaced by the same volume of an enzyme solution containing 0.28 U ml⁻¹. The reaction was monitored for 5 min at 405 nm. Velocities of reaction were calculated. Enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer instead of inhibitor. Inhibitory activity was calculated from 100 subtracted by the percentage of enzyme activity. Data presented here are the average of three replicates.

RESULTS AND DISCUSSION

Antioxidant properties of *Nalla marunthu*

Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species. Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma and parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources¹⁶. Some of the in vivo free radicals play a positive role in phagocytosis, energy production and regulation of cell growth etc. However, free radicals may also be damaging. Free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources¹⁷. Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals¹⁸. They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers¹⁹. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases²⁰. There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants. Hence, the present study was aimed at measuring the relative content of phenolics and antioxidant capacities of chloroformic extract of *Nalla marunthu*. Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress²¹. DPPH is a protonated radical having the characteristic absorption maxima at 517 nm which decreases with the scavenging of the proton radical by natural plant extracts. Hence, DPPH finds applications in the determination of the radical scavenging activity of plant materials²².



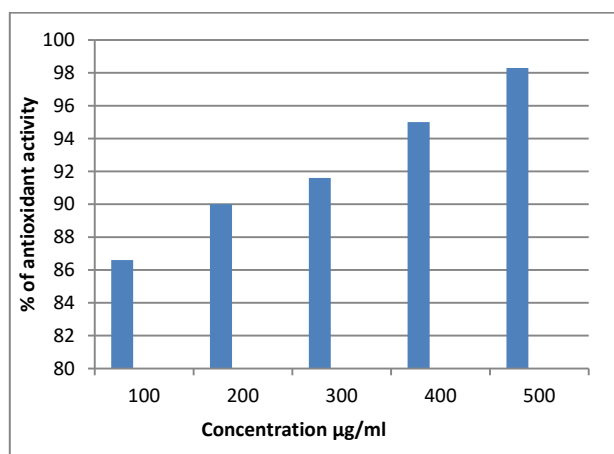


Figure 1: The percentage antioxidant activities of Chloroformic extract of *Nalla marunthu*

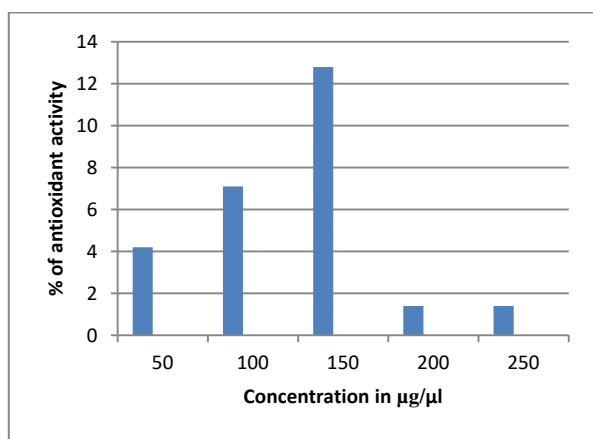


Figure 2: Percentage Ascorbic acid activity of Chloroformic extract of *Nalla marunthu*.

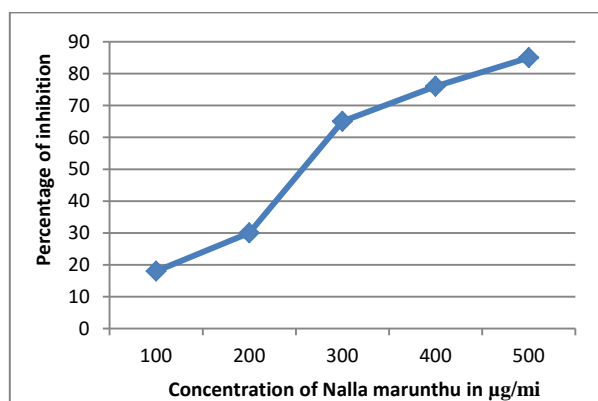


Figure 3: Acetylcholinesterase (Acetyl cholinesterase) inhibitory effects of *Nalla marunthu*.

Use of plants as a source of medicine has been inherited and is an important component of the health care system. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Neurodegenerative disease, is characterized by low levels in the brain of the neurotransmitter, acetylcholine. Clinical treatment of this disease is palliative and relies mostly on enhancing cholinergic function by stimulation of cholinergic

receptors or prolonging the availability of acetylcholine released into the neuronal synaptic cleft by use of agents which restore or improve the levels of acetylcholine. Inhibition of acetyl cholinesterase enzymes which breakdown acetylcholine, are considered as a promising strategy for the treatment of AD. A potential source of Acetyl cholinesterase inhibitors is provided by the abundance of plants in nature, and natural products continue to provide useful drugs and templates for the development of other compounds. Present efforts aimed at the acetyl choline esterases inhibitory activity and antioxidant effects of chloroform extract of a poly herbal formulation²³.

CONCLUSION

A search for more efficient agents with fewer side effects has resulted in the screening of several medicinal plants for possible activity. Furthermore, in vivo activity of the active compounds needs to be determined in animal models and human subjects, so as to determine their efficacy in a metabolic environment. Such future studies will be necessary to expand the existing, limited therapeutic arsenal for the majority of neurodegenerative diseases, especially for those therapies with side effects that limit their effectiveness.

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