



## Assessment Of *Invitro* Nitric Oxide Scavenging Activity Of Ethanolic And Aqueous Extracts Of *Trianthema Decandra* (Aizoaceae)

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*Plants have provided mankind with herbal remedies for many diseases for many centuries and eventody. In India herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced such as Ayurveda, Unani and Sidha. Trianthema decndra commonly known as Vellasharani (Tamil), Gadabani (Hindi), having promising theraputic values with its various phytoconstituents. Preliminary Phytochemical investigation was carried out on the ethanolic and aqueous extracts of Trianthema decandra. It indices presence of Alkaloids, Amino acids, phenols, Tannins, Carbohydrates, Flavonoids, Saponins, glycosides and Volatile oils. We are also quantitatively estimated total phenolic content, tannins and favanioids by using spectrophotometer. The total phenolic content was  $680.23 \pm 3.03$  mg/g, tannin content was  $524 \pm 4.03$  mg/ g while the flavonoid content was  $215.42 \pm 4.52$  mg/g. The ethanolic and aqueous extracts were screened for antioxidant activity using Nitric oxide scavenging activity method, which showed significant persentage of inhibition in dose dependent manner. As antioxidant therapy is found to be useful in complicated disease status related with free radical activity. This is the first research report regarding *Invitro* evaluation of Nitric oxide scavenging activity of ethanolic and aqueous extracts of Trianthema decandra. The present study might be extended for the formulation and evaluation of different antioxidant herbal dosage forms.*

**Keywords:** Alkaloids, Antioxidants, Azoaceae, Nitric Oxide, Tannins, *Trianthema decandra*, Total Phenolic Content.

### 1. Introduction:

Free radicals have been shown to be harmful as they react with important cellular components such as proteins, DNA and cell membrane<sup>1</sup>. The body on the other hand, requires free radicals for immuneresponses. However, an overload of these molecules had been linked to certain chronic diseases of heart, liver and some form of cancers<sup>2</sup>. Atoms of oxygen or Nitrogen having central unpaired electron are called as reactive oxygen or Nitrogen species<sup>3, 4</sup>. The role of Nitric oxide (NO) in numerous disease state have generated a considerable discussion over the past several years since the Journal Science named it the molecule of the year in 1992. NO is important bioregulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial activity. Low concentration of NO are sufficient in most cases to effect these beneficial functions. However, during infections and inflammations, formation of NO is elevated and may bring about some undesired delitrious effects<sup>5</sup>. Experiments have demonstrated that NO plays a catabolic role in the development of osteoarthritis and mediates the inflammatory response, it is also involved in the degradation of matrix metalloproteins, inhibits the synthesis of both collagen and proteoglycons<sup>6</sup>. The NO molecule is very unstable and react with oxygen produce intermediates such as NO<sub>2</sub>, N<sub>2</sub>O<sub>4</sub>, N<sub>3</sub>O<sub>4</sub>. The stable products nitrate, Nitrite and peroxynitrite when reated with superoxide<sup>7</sup>.

The molecular mechanism as to how NO enhances cancer development has become an active area of research. NO mediates S-nitrosylation of key enzymes and regulatory proteins plays critical role. Key proteins S-nitrosylated which enhance tumor development include several caspases involved in apoptosis, PTEN the tumor suppressor protein, Bcl-2 the mitochondrial protein which protects from apoptosis, OGG1 the DNA repair protein and methionine adenosyl transferase the liver protein which synthesizes adenosylmethionine. The S-adenosylation of these proteins enhance DNA mutations, prevents cancer cell apoptosis and enhances oncogenic cell growth<sup>8</sup>. Problems with memory and social functions in patients with Schizophrenia may result from an imbalance in the brain's Nitric oxide system. An experimental result shows that rats with characteristics of schizophrenia regain normal functions if they receive drugs that reduce the production of nitric oxide in the brain<sup>9</sup>. Herbal drugs have been used by mankind since time immemorial to treat various disorders and as an alternative to the synthetic compounds, as they have been considered either non-toxic or less toxic. The traditional Indian system of Medicine, Ayurveda is based on the principle of balance and counter balance. Ayurveda (Ayu= life, Veda=Knowledge) extensively uses the plant derived compound formulations for the treatment of various ailments after a careful study into the type of the disease<sup>10</sup>.

According to ayurveda, the *Trianthema decandra* is acrid, sweet, digestive and astringent to bowels and good for sour throat, blood impurities and to cure ulcers<sup>11</sup>. The plant possesses hypoglycaemic, antibacterial effects. *Trianthema decandra* Linn. belongs to the family Azoaceae. It is a fast growing weed, prostrate, glabrous succulent and annual weed. All the parts of plants can be used medicinally. In India, extract is used for anaemia, diabetes which reduces blood sugar level quickly<sup>12</sup>.

It is commonly known as Gadabani (Hindi) and Vellaisharani (Tamil). It has been used in various parts of Asia, Australia and South America for curing various diseases. All parts of this plant is used for treating diseases. Leaves and roots are having significant hepatoprotective activity<sup>13</sup> anti inflammatory<sup>14</sup>, used for treating jaundice, ophthalmic<sup>15</sup>. The whole plant powder is astringent, sweet, sour, diuretic, digestive and anthelmintic. It is astringent, refrigerant, carminative, digestive, febrifuge, constipating and stomachic and antibacterial<sup>16,17</sup>. In addition, pharmacological evaluation of this plant concerning their antidiabetic, hypolipidemic, anti bacterial, antipyretic<sup>18,19</sup>.

The roots applied to the eye curing bacterial infections and it is also given in combination with ginger as a cathartic. It is also used for corneal ulcers, itching, dimness of sight and night blindness. The leaves containing huge amounts of vitamin C, and used to treat edema. The juice of leaves is used to treat the black quarter. The decoction of the herb is used as a vermifuge and is useful in rheumatitis. It is also used as an antidote to alcoholic poison<sup>20</sup>.

Eventhough more research works has been carried out on these plant, there is no sufficient scientific data available regarding Nitric oxide scavenging activity of *Trianthema decandra* (whole plant). Therefore our aim in this study was to evaluate the antioxidant activity of ethanolic and aqueous extracts of *Trianthema decandra* by Nitric oxide scavenging activity method.

## 2. Materials And Methods:

### Plant Material

The fully mature, fresh plant of *Trianthema decandra* was collected from Midhulanagaram, Mellacheruvu village, Chittoor district, Andhra Pradesh. The plant was identified and authenticated by Dr.S.B.Narasimha Reddy, Professor, Department of Botany, S.G.Govt.Degree college, Piler and voucher specimen (No.JCP/2012/143) was deposited in the Herbarium of the same department. The whole plant was air dried at room temperature (25°C) for 30 days and converted into fine powder with an automix blender, the powder was kept in a deep freezer until the time of use.

### Preparation of Extracts

500 gm of dry fine powder was suspended in 1.5 liters of methanol and double distilled water separately then

stirred magnetically for 24 hours at room temperature. The extract were double filtered by using musline cloth and whatmann No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40°C using rotary vacuum evaporator (Buchi labortechnik AG, Switzerland) to obtain crude extract. The dried EETD and AETD (Ethanolic & Aqueous extracts of *Trianthema decandra*) was stored in vacuum desiccators under controlled conditions till it used for experimental purpose.

#### Drugs and Chemicals used:

The Sodium Nitroprusside (SNP), Griess reagent, Ascorbic acid and Methanol (95% V/V) were obtained from S.D fine chemicals, Mumbai. All the chemicals and solvents were of analytical grade.

#### Preliminary Phytochemical Screening:

1 gm of the ethanolic and Aqueous extracts of *Trianthema decandra* were dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (w/v). The standard methodology of Harborne (1998)<sup>21</sup> and Kokate (2001)<sup>22</sup> were adopted for the phytochemical screening.

#### Determination of Total Phenolic content, Tannins and flavonoids

The total phenolic content in the extracts were determined using Folin-ciocalteau reagent according to the Malic and Singh (1980)<sup>23</sup>. Tannin content was determined by Folin-Denis reagent according to the method of Schandrel (1970) using tannic acid as standard<sup>24</sup>. The flavonoids were estimated by earlier reported method (Ivan *et al.*, 2004)<sup>25</sup>.

#### Nitric Oxide generation and assay of Nitric Oxide scavenging method.

Nitric Oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO<sup>26,27</sup> which interact with molecular oxygen to produce Nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO<sup>28</sup>. Sodium Nitroprusside (10 mM) in phosphate buffer saline (PBS) was mixed with different concentrations of extract (100-1000 µg/ml) of the drug dissolved in ethanol and water and incubated at 25°C for 180 minutes. The samples from above were reacted with Griess reagent (1% Sulphanilamide, 0.1% Naphthylenediamine dichloride and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with Naphthylenediamine dichloride was read at 546 nm and referred to the absorbance of ascorbic acid, used as a positive control treated in the same way with Griess reagent<sup>29</sup>.

Nitric Oxide scavenged (%) =  $A_{\text{Control}} - A_{\text{Test}} / A_{\text{Control}} \times 100$

Where,  $A_{\text{Control}}$  = Absorbance of control reaction

$A_{\text{Test}}$  = Absorbance in the presence of the samples of extracts.

### 3. Result And Discussion:

Preliminary phytochemical analysis showed the presence of Phenols, Tannins, Saponins, Volatile oils, Glycosides, Flavonoids, Alkaloids, Aminoacids like phytoconstituents (Table-1) may be responsible to show a potent antioxidant activity. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl group<sup>30</sup>. The phenolic content may contribute directly to the antioxidant activity<sup>31</sup>. It has been suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans<sup>32</sup>. Consequently, the antioxidant activity of methanolic extract are often explained by their total phenolic content, tannins and flavonoid contents with good correlation. The total phenolic content in the ethanolic extract of *Trianthema decandra* was 680.23 ± 3.03 mg/g, tannin content was 524 ± 4.03 mg/g while the flavonoid content was 215.42 ± 4.52 mg/g. These results demonstrate that tannins represents the main group of phenolic compounds in *Trianthema decandra*<sup>33</sup>.

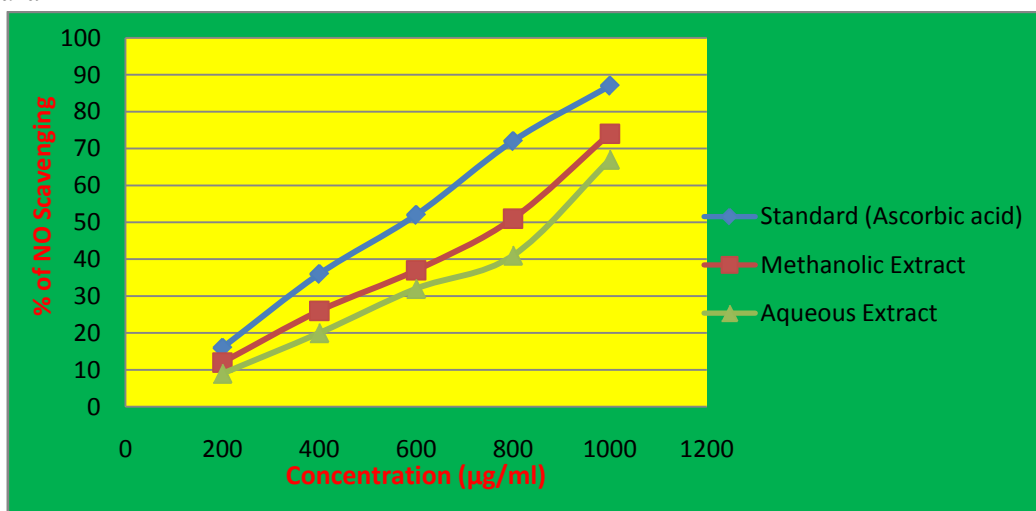
**Table-1: Priliminary phytochemical screening of ethanolic and aqueous extracts of *Trianthemadecandra***

<i>PHYTOCHEMICALS</i>	<i>ETHANOLIC</i>	<i>AQUEOUS</i>
Alkaloids	+	+
Amino acids	+	+
Anthraquinones	-	
Flavonoids	+	+
Glycosides	+	+
Phytosterols	-	-
Saponins	+	+
Steroids	-	-
Tannins	+	+
Phenols	+	-
Volatile oils	+	-

+ Presence, - Absence

**Evaluation Of Nitric Oxide Scavenging Acivity**

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radicals that plays many role as an effector molecules diverse biological system including neuronal messenger, vasodilatation, antimicrobial and anti tumour activities<sup>34</sup>. suppression of released NO may be partially attributed direct NO scavenging, as the extracts of *Trianthea decandra* decreased the amount of nirite generated from the decomposition of SNP invitro.

**Figure 1: Nitric oxide (no) scavenging activity of ethanolic and aqueous extracts of *Trianthea decandra***

The scavenging of NO by the extracts was increased in dose dependant manner. Figure -1 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The

ethanol and Aqueous extracts showed maximum activity of 76% and 68% respectively at 1000 µg/ml where as ascorbic acid was 89.06% at the same concentration. The Ic 50 values were found to be 800 µg/ml, 900 µg/ml for ethanolic and Aqueous extracts and 600 µg/ml for ascorbic acid respectively. The given results are diverted to ethanolic and aqueous extracts of *Trianthema decandra* is having NO scavenging activity but less than ascorbic acid.

#### 4. Conclusion:

The ethanolic and aqueous extracts of *Trianthema decandra* exhibited significant antioxidant activity. The results are compared with ascorbic acid and the activity may be related to the phenolic contents and flavanoids in this plant extract. Since reactive oxygen species are important contributors to several serious ailments. In the present study, the observed NO scavenging activity of the ethanolic and aqueous extracts of *Trianthema decandra* might be useful for the development of newer and more potent natural antioxidants. Furthermore, detailed studies on isolation, characterization of phytochemicals, pharmacological and biochemical investigation is needed to elucidate the mechanism of action and will helpful in projecting this *Trianthema decandra* as a therapeutic target in antioxidant research.

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