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In vitro anti-oxidant activity and acute toxicity, of ethanol extract of root tubers of *Asparagus gonoclados*

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Abstract
The primary concern of this study was to assess the phytoconstituents of ethanol extract of *Asparagus gonoclados* root tubers and to evaluate the *In vitro* antioxidant activity and any possible toxicity of *Asparagus gonoclados*. Phytochemical analysis of Ethanol extract revealed the presence of phytosterols, flavonoids, phenols, tannins, steroids, triterpenes, and proteins. *In vitro* antioxidant activity of the ethanol extract of *Asparagus gonoclados* was analyzed by DPPH, NO and H_2O_2 methods, and the ethanol extract at the concentration of 100 µg/ml exhibited the highest activity. Acute oral toxicity tests were also performed in 4 groups of rats with ethanol extract administered orally at the doses of 2000, 3000, and 4000 mg/kg. bwt. No momentous changes were observed after 30 min and up to 24 h, which imply the nontoxic nature of the *A.gonoclados* root tubers.
From the above outcome of the results, it is concluded that the ethanol extract of root tubers of *Asparagus gonoclados* possess impressive antioxidant and nontoxic properties.

Keywords: phytochemicals, anti-oxidants, *Asparagus gonoclados*, ethanol extract, acute toxicity

1. Introduction
Antioxidants are chemical constituents used for regimen various human diseases related to Cardiac, Pulmonary, Renal, muscular, brain and help to control aging process. The nature of antioxidants inhibiting or delaying the formation of free radicals and lipid peroxidation effectively works in human body that is mainly responsible for many human diseases (Karimi *et al*., 2011; Ostrowska *et al*., 2001) [14, 19]. Natural compounds present in plants have been considered for a wide range of biological properties such as antioxidant, anti-inflammatory and antimicrobial activities (Mohanty *et al*., 2014; Canadanovic-Brunet *et al*., 2009; Ao *et al*., 2008) [18, 6, 1]. Different phytochemicals such as ascorbic acid, tocopherols, carotenoids, and polyphenolic compounds and their collective activities result in the total antioxidant activity of a plant. However polyphenols exerts immence antioxidant potential and could be the beneficial antioxidants (Kim *et al.*, 2003) [16]. These antioxidant compounds are found in fruits and vegetables, leaves and all parts of the plants. So far, about 10,000 phytochemicals have been identified, and still a large percentage remains unknown. These identified phytochemicals include tannins, flavones, triterpenoids, steroids, saponins, and alkaloids (Barbosa *et al*., 2013) [3].
Natural plant compounds and their products are believed to be safer than chemical products. Therefore, toxicity studies of natural substances do not usually draw much attention as studies of chemical products. However, some natural substances are substantially toxic and may be inimical to human health (Haller *et al*., 2000; Palmer *et al*., 2003; Pittler *et al*., 2003) [11, 20, 21]. In this connection, issues regarding the actual safety of natural substances are steadily discussed (Haller *et al*., 2000; Palmer *et al*., 2003; Pittler *et al*., 2003) [11, 20, 21]. Therefore, standardized infallibility studies are essential for natural compounds from medicinal plants.
Asparagus gonoclados reported as a substitute for *Asparagus racemosus* wild, which has main source of Shatavari, ayurvedic drug in about 60 types of ayurvedic and herbal formulations and has been reported in the Indian and British pharmacopoeias and in traditional systems of medicine such as ayurveda, unani, and siddha for its medicinal usage (Bopana and Saxena *et al*., 2007) [4]. Earlier phytochemical analysis was done in *A. gonoclados* root tubers in different solvent (Chloroform, alcohol, aqueous and etc.) extracts (Madhavan *et al*., 2010; Tijare *et al*., 2012) [17, 25]. In our study we have analyzed qualitatively the phytochemicals in Ethanol extract, of *Asparagus gonoclados* and *In vitro* antioxidant activity has been evaluated by DPPH, NO and H_2O_2 methods, and also acute toxicity studies were carried out with the ethanol extract of the root tubers of *Asparagus gonoclados* to appraise the medicinal values of this plant.

2. Materials and Methods
2.1 Collection of *Asparagus gonoclados* root tubers
Root tubers of *Asparagus gonoclados* Baker L. were collected from in and around Tirumala hills and identified by taxonomist. A voucher specimen no (1307) has been deposited in the Herbarium of Department of Botany, Sri Venkateswara University, Tirupati.

2.2 Preparation of ethanol extract of the root tubers of *A. gonoclados* (AGEE)
Ethanol extract was prepared by successive solvent extraction of the root tuber powder in soxhlet apparatus at 65^0C-70^0C. The filtrate obtained was distilled and concentrated under

reduced pressure at low temperature (40^0C to 45^0C) in Buchi Rotavapor R-200 and finally freeze dried. The extract was stored at 0^0C in airtight containers until needed for further studies.

2.3 Qualitative phytochemical analysis of ethanol extract
The ethanol extract was analyzed to verify the presence of phenols, flavonoids, steroids, triterpenoids, saponins, alkaloids and glycosides by qualitative methods (Harborne *et al*., 2005).

In-vitro antioxidant activity
2.4 DPPH radical scavenging activity
The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1, 1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (25, 50, 75, and 100 µg/mL) in methanol was added to 4 ml of 0.004% (w/v) methanol solution of DPPH. Ascorbic was used as the standard, after a 30 min incubation period at room temperature; the absorbance was read against blank at 517 nm. This assay was done according to a slightly modified method of Burits *et al*., 2000 [5].

2.5 Hydrogen peroxide (H_2O_2) scavenging activity
H_2O_2 free radicals scavenging activity of the different solvent extracts of *A. gonoclados* was determined according to the method of Ruch *et al*., 1989 [24]. The H_2O_2 (0.6 ml, 40 mM) solution prepared in phosphate buffer (pH 7.4), was added to the different extracts having the concentrations 25, 50, 75 and 100 µg/mL in 3.4 ml phosphate buffer. In this method also ascorbic acid was used as the standard. The absorbance of the reaction mixture was recorded at 230 nm.

The percent of free radical scavenging of DPPH, NO and H_2O_2 were calculated using the following equation.

$$\%\ \text{of scavenging} = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried in triplicate.

2.6 Nitric oxide (NO) scavenging activity
Nitric oxide scavenging activity was measured by a slightly modified method of Green *et al*., 1982 [10]. Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 ml of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 µg/mL) of the different extracts of the *A. gonoclados* and incubated for 150 min at 25ºC and 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride) was added to the 1 ml of the reaction mixture. The standard antioxidant ascorbic acid was used as the positive control. The absorbance of the resulting reaction mixture was measured at 546 nm.

2.7 Acute toxicity studies
Acute toxicity study was done according to the method of Kifayatullah *et al*., 2015 [15]. For evaluating the acute toxicity of Ethanol extract of *Asparagus gonoclados* root tubers, the experimental animals (Male albino Wistar rats) were divided into 4 groups, each group consisting of 6 rats. 1st group of rats were given only saline water, 2nd, 3rd, and 4th groups of the animals were orally administered the extract of *Asparagus gonoclados* root tubers at the doses of 2000, 3000, and 4000 mg/kg.b.wt. (Dissolved in normal saline) respectively. Before the extract administration the animals were kept in overnight fasting and body weights were measured. After the administration of extract the animals were examined for any toxic effect for the first 4 h. Animals were observed for 3 days for any behavioral changes, toxic effects and other parameters like bodyweights, food intake, water intake, convulsions, urination, respiration, tremor, constipation, temperature, changes in eye and skin colors, etc.

3. Results and Discussion
3.1 Phytochemical analysis
The phytochemical analysis of, the ethanol extract of *A. gonoclados* roots revealed the presence of phenols, flavonoids, steroids, saponins, tannins, glycosides, and triterpenes. The saponins, phenols, and flavonoids were present in high levels in ethanol extract of *A. gonoclados*.

3.2 DPPH radical scavenging activity
In the present study, the ethanol extract of *A. gonoclados* possesses DPPH free radical scavenging activity of 73.41% at a concentration of 100 µg/ml. But this is less than that of the ascorbic acid which has shown 81.9% free radical scavenging activity at the same concentration. The IC50 values of the ethanol extract and ascorbic acid are 26.53 µg/ml and 43.02 µg/ml respectively. The free radical scavenging activity of AGEE or ascorbic acid was increased with increasing concentrations (25-100 µg/ml) of the extract or ascorbic acid (Figure 1).

3.3 H_2O_2 radical scavenging activity
At the concentration of 100 µg/ml AGEE exhibited a higher percentage of (52.5%) hydrogen peroxide radicals scavenging activity, whereas standard antioxidant ascorbic acid showed 41.4% of activity at the same concentration. The IC50 values of ethanol extract and ascorbic acid are 76.37 µg/ml and 116.00 µg/ml respectively. The hydrogen peroxide radicals scavenging activity of the AGEE extract and ascorbic acid were increased with the increasing concentrations of the extract and ascorbic acid (Figure 2).

3.4 NO radical scavenging activity
The AGEE exhibits maximum percentage (59.1%) of NO free radical scavenging activity at the concentration of 100 µg/ml, and it is higher than the standard antioxidant free radical scavenger Vit. C which has shown 51.6% of inhibition of NO radicals at the same concentration. The IC50 values of ethanol extract and ascorbic acid were 71.15 µg/ml and 76.06 µg/ml respectively. The nitric oxide radicals scavenging activity of

the AGEE extract or ascorbic acid were increased with the increasing concentrations of the extract or ascorbic acid (Figure 3).

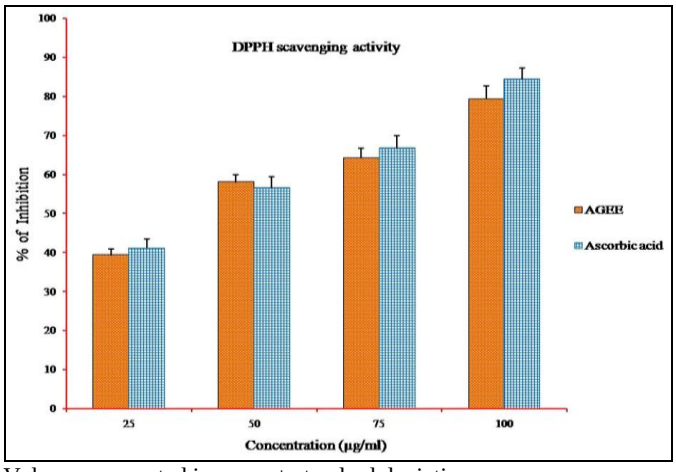
Phytochemicals have free radical scavenging activity and the ability to inhibit the free radical production. In this study the DPPH, H_2O_2, and NO scavenging activity of the ethanol extract of the root tubers of *Asparagus gonoclados* could be due to the presence of phenolic compounds (Vinayagam *et al*., 2016), Saponins (Weng *et al*., 2014) [27,28] and flavonoids (Banjarnahor and Artanti, 2014) [2] which are the major antioxidants. Many flavonoids are found to be strong antioxidants effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups (Chung, *et al*., 2006) [7]. Our study revealed the presence of phenols, flavonoids, steroids, saponins, tannins, glycosides, in the AGEE which might play an important role in improving oxidative stress (Hsu *et al*., 2006) [13]. Maximum activity of scavenging DPPH, H_2O_2, and NO radicals by the AGEE was observed at the concentration of 100 µg/ml. The percentage of inhibition of free radicals was increased with the increasing concentration of the ethanol extract or ascorbic acid. The phenolics and steroidal saponin content was higher in AGEE extract and that could have attributed to its high scavenging activity. It was determined that the hydrogen donating or the reducing nature of the plant extracts has been responsible for their free radical scavenging activity considering them as the primary antioxidants (Chung *et al*., 2006, Hsu *et al*., 2006) [7, 13]. A positive correlation was observed between the phenolic contents of the extracts and their antioxidant activity i.e., the extracts with high levels of phenols exhibit higher scavenging of DPPH free radicals. Our results are in agreement with earlier reports which correlated the total phenolic content of plants with their antioxidant activity (Tilak *et al*., 2004; Coruh *et al*., 2007; Rekha *et al*., 2012; Dileep *et al*., 2012; Prasad *et al*., 2012) [26, 8, 23, 9, 22].

The above results clearly showed that the root tubes of *Asparagus gonoclados* has potent antioxidant activity, with its phenolics, flavonoids and saponins that play major role in the free radical scavenging activity.

3.5 Acute toxicity study

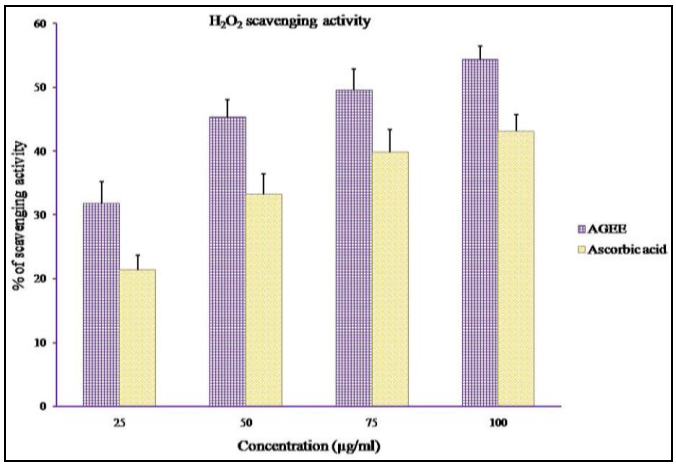
The acute toxicity effect of ethanol extract was evaluated as per the OECD guideline 423, where the limit test dose of 4000 mg//kg was used. The observations have proved that no toxic manifestations or mortality were observed after administering the doses 2000, 3000, and 4000 mg/kg orally to the animals. The monitoring of animals was done first for 4 hrs followed by 72 hrs. They did not display any changes in behavior, food intake, water intake, urination, eye color, body weights. And no death or coma was observed. So the ethanol extract of *A. gonoclados* up to the dose of 4000 mg/kg was considered as safe with no concomitant effect. The different parameters observed in the AGEE administrated rats were compared with the normal control group of rats which were administered only normal saline (Table 1).

There were no differences between the AGEE treated rats and normal control rats in all the observed parameters. The extract at all the doses did not show any side effects or mortality and seems to be safe. This suggests that the ethanolic extract of *A. gonoclados* up to the dose of 4000 mg/kg can be considered as nontoxic.



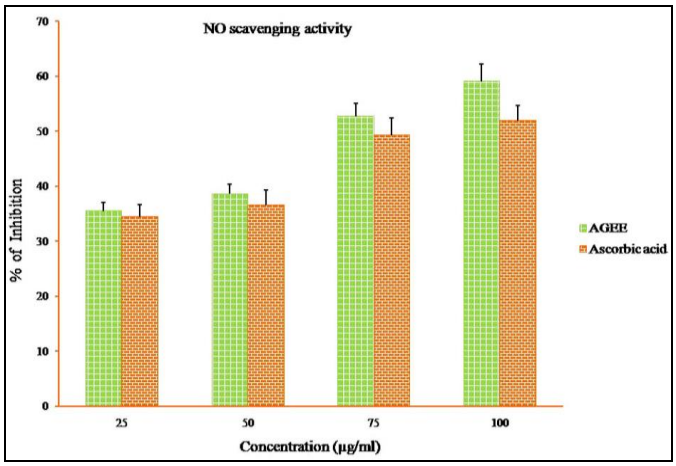
Values represented in mean ± standard deviation

Fig 1: Effect of ethanol extract of *A. gonoclados* root tubers on DPPH free radicals scavenging activity.



Values represented in mean ± standard deviation

Fig 2: Effect ethanol extract of *A. gonoclados* root tubers on H_2O_2 radicals scavenging activity.



Values represented in mean ± standard deviation

Fig 3: Effect of ethanol extract of *A. gonoclados* root tubers on NO radicals scavenging activity.

Table 1: Testing of Behavioral changes and general appearance of acute toxicity study

Parameter Observed	1st Control group	2nd group 2000 mg/kg	3rd group 3000 mg/kg	4th group 4000 mg/kg
Food intake	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal
Temperature	Normal	Normal	Normal	Normal
Bodyweight	Normal	Not Changed	Not Changed	Not Changed
Digestion	Normal	Normal	Normal	Normal
Respiration	Normal	No effect	No effect	No effect
Urination	Normal	No effect	No effect	No effect
Drowsiness	Not present	Not present	Not present	Not present
Sedation	Normal	No effect	No effect	No effect
Changes in skin	No effect	No effect	No effect	No effect
Diarrhea	Not present	Not present	Not present	Not present
Eye color	Normal	No change	No change	No change
Coma	Not identified	Not identified	Not identified	Not identified
Alive/Death	Alive	Alive	Alive	Alive

4. Conclusion

From the above results, it is concluded that the root tubers of *A. gonoclados* contain different phytoconstituents which are responsible for the *In vitro* antioxidant activity. And the ethanol extract of *A. gonoclados* was proved to be nontoxic and so it is considered safe to use for further research studies to evaluate their other beneficial effects in humans.

5. Acknowledgement

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