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Anti-oxidant activity of *Nyctanthes arbor-tristis* Linn.

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Abstract:

Studies on natural compounds are of interest in now a day, due to their satisfactory health benefits with low toxicity. Herbs and spices have been added to different types of food to impart flavour as well as to improve storage stability due to their anti-oxidant effects. Anti-oxidants play a major role in curing degenerative diseases. In current scenario many synthetic antioxidants are commonly used but due to their toxic and carcinogenic effects, their use has been restricted by legislative rules. So the search for natural anti-oxidants is essential. In the present study, the flower of Nyctanthes arbor-tristis Linn was extracted with n-butanol. Total phenolic content of the extracts was determined by Prussian blue method was found to be 1220 mg catechin is equivalent to 100 g of flower powder. The antioxidant activity of the extracts was evaluated through in vitro model systems such as DPPH Radical scavenging, total anti-oxidant activity and reducing power. The % Inhibition by extract (86%) as well as BHT is comparable by considering I% of BHT is 100%. Total antioxidant activity was found to be 248mg/100 g. Reducing power in terms of EC_{50} value of the extract is excellent (12.10), which is comparable with that of BHT (12.26). The results of the present study indicate that Nyctanthes arbor-tristis Linn. flower is a good source of antioxidant and phenolics.

Key words: *Nyctanthes arbor-tristis Linn., Phenolics, DPPH, BHT, Catechin*

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1. Introduction

Herbs and spices have been added to different types of food to impart flavour as well as to improve storage stability due to their anti-oxidant effects. The active principle responsible for this is phenolics [1]. Chemopreventive potential of herbs and spices due to phenolic substances contribute their use as anti-inflammatory, antimutagenic, anticarcinogenic and anti-tumour agents [2]. In current scenario many synthetic antioxidants are commonly used but due to their toxic and carcinogenic effects, their use has been restricted by legislative rules [3]. So there is urgency of natural anti-oxidants for food and pharmaceutical industry. In this connection search for natural anti-oxidant is essential. Chhattisgarh is known as herbal state rich with forest flora is the area of interest to search for natural anti-oxidant. Nyctanthes arbor-tristis 'a night flowering sad tree' of family Oleaceae (Nyctaginaceae) is well

known in Chhattisgarh as most versatile medicinal plant having a wide spectrum of biological activities [4]. In traditional medicine it is used against various human diseases from antiquity [5, 6]. Literature review suggests that this plant is rich with phytosterols, phenolics, tannins, flavonoids, glycosides and saponins [7, 8]. So in this connection to explore this plant scientifically suitable plan has been designed for in-vitro anti-oxidant activity as a preliminary Pharmacological Screening.

2. Materials and Methods

2.1. Chemicals and reagents:

All chemicals used were of analytical reagent grade and were procured from E. Merck India Ltd. (Mumbai, India).

2.2. Collection of Plant materials:

Nyctanthes arbor-tristis Linn. Flowers were collected from tribal region near to Bilaspur, Chhattisgarh. Taxonomic identification of the plant material was confirmed by the taxonomists. The voucher specimen has been deposited at the Herbarium of the Department of pharmacy GGV, Bilaspur, India.

2.3. Preparation of Phenolic Extract:

The flowers are dried under shade and grinded into fine powders. Phenolic compounds were extracted according to the method described by Variyar et al., 1988 [9]. 100g of powder was extracted with 500 ml of methanol: water (80:20) by maceration for 7 days. The extract was concentrated to remove methanol and the remaining aqueous solution was extracted with n-butanol. The n-butanol fraction containing phenolic compunds were stidied for antioxidant activity.

2.4. Determination of Total Phenolics:

Total polyphenol content was determined in the n-butanol fraction of extract by Prussian blue method [10]. Aqueous solution of catechin 100 µM was prepared. For preparation of calibration curve 0.6, 1.2, 1.8, 2.4 and 3.0 ml of standard catechin solution were taken. To this 0.2 ml aqueous solution (0.008 M) of K3[Fe(CN)6] and 0.2 ml solution of FeCl3 (0.1 M) in 0.1 M HCl added to make volume 3 ml. The absorption of the blue colored solution was read after 5 min at 700 nm and the calibration curve was drawn. Three milliliters of the sample was under study were mixed with same reagents and the absorption was measured as described above. All determinations were performed in triplicate and expressed as milligram of catechin equivalent per 100 g of fresh sample.

2.5. DPPH Radical scavenging Assay:

The DPPH radical scavenging activity of standard and samples were determined by the method described by Brand-Williams and co-workers [11]. 50, 75, 100, 250, 500 µg of the sample and standard solutions were prepared in methanol. To 1 ml solution of DPPH (0.04M), 4 ml of various concentrations of the extracts was added. Then the mixture was shaken vigorously and allowed to stand for 30 min in room temperature the absorbance of the resulting

solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1800) running with UV-Probe software . Inhibition of free radical DPPH in percent (I %) was calculated in following way $I(\%) = 100\times (A_0-A_1) / A_0$ Where A_0 is the absorbance of the control and A_1 is the absorbance of the test compound. BHT was used as standard.

2.6. Total antioxidant capacity (Phosphomolybdenum reduction Assay)

Spectrophotometric method was adopted for the determination of total antioxidant capacity. To the 0.1ml of the extract (15mg/ml) in water 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added in eppendorf tube. The tubes were incubated at 95^0C for 90 min. After cooling to room temperature; the absorbance of the aqueous solution of each was measured at 695nm against blank. BHT was used as standard. Total antioxidant capacity in terms of BHT equivalent was determined [12].

2.7. Reducing power

Extracts of 5mg/ml-20mg/ml were prepared in methanol. To 1 ml of the extract 2.5 ml of 200 m M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide was added. Then the mixture was incubated at 50^0C for 20 min. After incubation 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged for 10 min. 2.5 ml of the upper layer was taken and mixed with 2.5 ml of de-ionized water again to it 0.5 ml of 0.1% ferric chloride was added. Finally the absorbance was measured at 700 nm in a UV-visible spectrophotometer. BHT was used as standard [13].

3. Result and Discussion

Total phenolic content of the phenolic fraction of *Nyctanthes arbor-tristis Linn.*in the present study reveals that 1220 mg catechin is equivalent to 100 g of flower powder of *Nyctanthes arbor-tristis Linn.* Studies by various researchers' states that flower of *Nyctanthes arbor-tristis Linn.* is rich with Essential oil, nyctanthin, d-mannitol, tannin and glucose, carotenoid, glycosides, β-monogentiobioside ester of α-crocetin, β-monogentiobioside-β-D monoglucoside ester of α-crocetin, β-digentiobioside ester of α-crocetin [14-18]. So these are may be responsible for high catechin equivalent. DPPH is long-lived nitrogen radical. Antioxidants react quickly with DPPH and tend to decrease its oxidation ability. In the present study Inhibition % of extract as well as BHT is comparable by considering I% of BHT is 100%. The natural antioxidants might directly react with or quench the stable cation radical, which is reflected as their antioxidant activity. *Nyctanthes arbor-tristis Linn.* flower with all the ingredients show total antioxidant activity of 248mg/100 g. Reducing power in terms of EC_{50} value of the extract is excellent (12.10), which is comparable with that of BHT (12.26). The excellent reducing power of the sample may be due to the hydrogen donating abilities of the active constituents. More antioxidant capacity in herbs comes from the ingredients other than antioxidant vitamins, indicating the presence of other potentially important antioxidants. The

phenolic compounds are dominant antioxidants distributed widely in the plant kingdom that exhibit scavenging efficiency on free radicals and reactive oxygen species.

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