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Antioxidant Activity of Typha Elephantina Inflorescence Extracts

Smita Narwal[1, 2], Supriya Agnihotri[3]*, Gurvirender Singh[4]

[1] Research scholar I.K. Gujral Punjab Technical University, Kapurthala-144603, Punjab, India

[2] Global Research Institute of Pharmacy,V.P.O. - Radaur, Distt. Yamuna Nagar-135133, Haryana, India

[3]Chandigarh College of Pharmacy, Chandigarh Group of Colleges, Landran-140307, Punjab, India

[4] Institute of Pharmaceutical Sciences Kurukshetra University Kurukshetra-136118, Haryana, India

ABSTRACT

Abnormal levels of reactive nitrogen species and reactive oxygen species lead to cell toxicity disturbing cellular functions, cell survival and alters the cellular components and responsible for oxidative stress-related disorders like cardiovascular diseases, atherosclerosis, diabetes, and cancer. Our current study's objective is to analyze the antioxidant potential of *Typha elephantina* inflorescence petroleum ether extract (TEPE), chloroform extract (TECE) and methanol extract (TEME). DPPH, ABTS, Nitric oxide and Hydrogen peroxide free radical scavenging assays were conducted for evaluating the antioxidant and free radical scavenging activities. In DPPH assay TEME exhibited 70.97±0.62 percent inhibition at 125µg/ml, whereas standard ascorbic acid exhibited 82.08±1.29% inhibition and IC50 value of standard 15.5 was comparative to the IC50 value of TEME 28.37. In contrast to Petroleum ether and chloroform extract, TEME have significant free radical scavenging tendencies evaluated by mentioned assays. It can be estimated that methanol extract of *Typha elephantina* has potential free radical scavenging capacity due to the existence of antioxidant compounds and these compounds might serve as prospective source of natural antioxidants, to treat various oxidative stress-related diseases.

Keywords: Antioxidants, DPPH, ABTS, *Typha elephantina*, IC50, Nitric oxide

Introduction

Chemical entities with an unpaired electron in the valence shell are known as free radicals. They are very unstable because of the presence of one or more unpaired electrons, and they can withdraw electrons from new molecules, hence causing harm to them in attempt to achieve stability [1]. Free radicals may be both damaging and beneficial to our health and are formed naturally in our body as a part of the normal metabolic process [2]. If too much reactive oxygen species (ROS) production takes place and/or the level of antioxidants get reduced in our body, it can cause tissue damage and a variety of illnesses by oxidizing membrane lipids, carbohydrates, cell proteins, enzymes, and DNA, affecting critical bio components in the cell. Antioxidants are efficient at slowing down the oxidation of biomolecules [3]. Antioxidants are food derived compounds that can slow or stop oxidation by inhibiting the start and spread of oxidative chain reactions [4]. They safeguard our bodies from disease by decreasing oxidative damage to cellular components produced by reactive oxygen species (ROS). Oxidative stress can lead to various degenerative diseases such as certain cancers, cardiovascular disease, neurodegenerative disorders, diabetes, atherosclerosis, inflammatory conditions, and fetal complication [5].

Increased antioxidant activity in our bodies is the most practical approach to combat degenerative illnesses, and this may be accomplished by eating more vegetables, fruits, and edible plants. Man has been using medicinal plants to treat illnesses for ages [6]. Phytochemical found in plants have a variety of bioactivities, including antioxidant, anti- inflammatory and anticancer properties. In addition, in the quest for novel antioxidants, well-known and traditionally utilized natural antioxidants from tea, wine, fruits, vegetables, spices, and a variety of other plant species have been studied [7]. Many man-made antioxidants, like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are available commercially, and they've been proven to be toxic or mutagenic in certain situations, whereas plant-based therapies have less side effects [8]. There is always a need to learn more about the antioxidant capacity of different plant species.

Antioxidant activity of *Typha elephantina Roxb.* was evaluated using four distinct techniques in this study. *Typha elephantina* is an aquatic perennial plant. It is a member of the Typha Linn. genus, which is often known as 'Cattails.' Burning syndrome, numerous blood diseases, and bacterial infections such as erysipelas, blood coagulation disorders, Cystitis, burning during micturition, calculus, swelling, oligospermia, and ulcers have all been treated with it in the past. Phytochemical investigations found the presence of sugars, proteins, tannin, flavonoid, glycoside, resin, and alkaloids. Ethanol extracts of *Typha elephantina* fruits confirm the presence of four phytoconstituents: p entacosane, 1-triacontanol, -sitosterol, and -sitosteryl-3-O-D-glucopyranoside, as well as cholesterol, quercetin, and lanosterol, isorhamnetin, pentacosane, and plant sterols.

In order to investigate the traditional capacities of this plant in the treatment of various diseases as well as the free radical scavenging effects of other plants in same genera, the goal of this study is to assess its antioxidant capacity using in vitro methods.

Material and methods:

Collection and identification of plants material

Typha elephantina was obtained from roadside marshy regions of Kurukshetra, Haryana in the months of July – September. Dr. S. S. Yadav, Department of Botany, Maharishi Dayanand University, Rohtak, India, verified the plants.

Preparation of extracts

Plant material was coarsely ground after shade drying and then the extraction was carried out using several solvents of increasing polarity in a Soxhlet device. Initially, lipids were removed from the plant material using pet-ether (60-70°C), as extracts having lipid content hampered spectroscopic assessment of the isolated chemical. After extracting the plant material using Petroleum ether, chloroform and methanol were utilized for extraction [9]. The entire extraction process took place at constant temperature. Following the collection, the extracts were concentrated into semi-solid crude mass with the help of a rotary evaporator.

In-vitro anti-oxidant activity:

1, 1- diphenyl -2- picrylhydrazyl (DPPH) radical scavenging assay

Typha elephantina extracts were tested for their efficiency to scavenge DPPH free radicals. A solution of DPPH (0.1 mm) was created. For this, 1.9 mg of DPPH was dissolved in methanol and the final volume was made-up to 100 ml with methanol. After that, this mixture was kept in at dark place for about 30 minutes to accomplish the reaction. 1 ml of above made solution was then added to 1 ml of various concentrations of extracts (i.e 8, 15, 30, 60, and 125 µg/ml) and allowed to stand at room temperature for about 30 minutes. At 517 nm, the solution was spectrophotometrically quantified. The tests were carried out three times to enhance reproducibility.Following formula was used to calculate percent inhibition,

% inhibition= $(A_0-A_1) / A_0 \times 100$

Where, A_0 control absorbance

A1 sample absorbance.

Hydrogen peroxide scavenging (H_2O_2) assay

Humans get exposure of H_2O_2 in someway from the environment at a rate of around 0.28 mg/kg/day, with the majority of their consumption coming from leafy vegetables. H_2O_2 could penetrate our body through inhaling vapor or mist, as well as contact with the eyes or skin. H_2O_2 decomposes quickly into oxygen and water, producing OH, which is ableto cause lipid peroxidation as well as damage of DNA in body cells. Method employed to trace out the capacity various extracts of *Typha elephantina* to scavenge hydrogen [10].In phosphate buffer, make a 40 mM hydrogen peroxide solution (50 mM pH 7.4). A spectrophotometer is used to detect the quantity of hydrogen peroxide by measuring absorbance

at 230 nm. After 10 minutes, the absorbance at 230 nm of the extract is measured in distilled water. Phosphate buffer alone was considered blank [11].H_2O_2 scavenging is computed by using following formula:

H_2O_2 scavenging = $(A_{con}- A_t)/A$control×100Where,

A_{con}= absorbance of H_2O_2 + methanol

A_t = absorbance of H_2O_2 + sample extract or standard.

Nitric oxide scavenging activity

Specific nitric oxide synthases produce NO in biological tissues by metabolizing arginine to citrulline and forming NO via a five- electron oxidative process [12]. At physiological pH (7.2), the chemical sodium nitroprusside decomposes in aqueous solution, generating NO. NO interacts with oxygen in aerobic circumstances to form stable compounds (nitrate and nitrite), the amounts of which may be measured using the Griess reagent [13]. 2ml sodium nitroprusside (10 mM) diluted in 0.5 ml phosphate buffer saline (pH 7.4) is combined with 0.5 ml sample at different concentrations. After that, it is incubated at 25 degrees Celsius. 150 minutes after incubation, 0.5 ml solution is taken from added to 0.5ml of Griess reagent. After 30 minutes of incubation at RT, the absorbance of the liquid flowing intoa cuvette is measured at 546 nm [14]. This equation is used to determine the quantity of nitric oxide radical inhibition:Radical scavenging activity (%)= (Acontrol-Atest)/Acontrol×100

Where,

Acontrol = absorbance of the control (without extract) Atest = absorbance in the presence of the extract/standard.

ABTS Radical Scavenging Assay The ABTS scavenging test technique was used to assess the antioxidant properties of phytochemicals from traditional plants via cation decoloriszation method [15]. To generate ABTS radical cations, 7 mM of ABTS in solution form was treated with 2.45 mM ammonium persulfate solution. At 734 nm absorbance was measured. The optical density was measured at 734 nm after 0.1 mL of varied concentrations (20–100 g/ml) of plant extracts of *Typha elephantina* and ascorbic acid were allowed to react with 2 ml of ABTS solution [16].

Results and discussion:

DPPH scavenging activity

TEME exhibited 70.97±0.62 percent inhibition in a DPPH test at 125 µg/mL, whereas TEPE and TECE showed only 19.35±1.86 and 48.39±1.86, respectively. However, standard ascorbic acid exhibited 82.08±1.29% inhibition that was found to be comparative to that of methanol extract (Table 1). IC50 value of standard 15.5 was comparative to the IC50 value of TEME that was 28.37, while TEPE and TECE exhibited 2859 and 113.9 IC50.

Table 1: Assessment of percent radical scavenging and IC50 of *Typha elephantina* extracts and ascorbic acid evaluated by DPPH assay

DPPH	8µg/ml	15µg/ml	30µg/ml	60µg/ml	125µg/ml	IC50
TEPE	8.23±0.37	10.38±0.97	11.09±1.31	16.51±1.49	19.41±1.75	2859
TECE	17.2±0.59	23.66±0.58	31.9±0.89	42.65±1.31	48.39±1.90	113.9
TEME	31.9±1.56	41.58±1.29	53.41±0.95	60.57±0.95	70.97±0.62	28.37
ASCORBIC ACID	44.09±0.62	51.25±0.36	60.22±0.62	73.12±0.62	82.08±1.29	15.5

Hydrogen Peroxide Radical Scavenging Assay Hydrogen peroxide (H_2O_2) acts as a signal transuding molecule involved in cell-cell communication and plays a defense role in innate immunity and involved in the different biosynthetic processes. During long-term chronic inflammation, the high levels of H_2O_2 in living organisms react with iron molecules generate tremendously reactive hydroxyl radicals and these OH free radicals showed a toxic effect on cellular and tissue level [17].

As given in Table 2, different plant extracts and ascorbic acid at varying concentrations exhibited a dose-dependent hydrogen peroxide, (H_2O_2) scavenging activity. TEME exhibited 72.63±0.61 percent scavenging activity which is found to be in close proximity to that of 84.91±0.35 of standard with IC50 34.38 and 12.47 respectively. However, TEPE and TECE were not able to produce remarkable percent inhibition and IC50.

Table 2: Assessment of percent radical scavenging and IC50 of *Typha elephantina* extracts and ascorbic acid evaluated by H_2O_2 assay

	8µg/ml	15µg/ml	30µg/ml	60µg/ml	125µg/ml	IC50
TEPE	6.67±0.35	8.42±1.05	9.12±1.27	11.23±0.7	11.58±0.61	0
TECE	16.49±0.93	21.05±0.61	29.47±0.61	40.7±1.27	49.47±1.61	123.4
TEME	31.23±0.35	34.04±1.86	46.32±0.61	57.89±0.61	72.63±0.61	34.38
ASCORBIC ACID	45.61±0.7	53.33±1.27	58.6±0.93	69.12±0.93	84.91±0.35	12.47

Nitric Oxide Radical Scavenging Assay Nitric oxide acts as both a chemical messenger and a protective molecule involved in the endocrine system and the immune system. A high level of NO during infection conditions reacts with oxygen to form peroxy nitrite anions showing adverse cellular damages on membrane structure and DNA cleavage [18].
As showed in Table 3, methanol extract exhibited highest dose dependent percent inhibition in contrast to petroleum ether extract and chloroform extract. TEME at 125µg/ml showed 66.63±1.85 percent inhibition while standard ascorbic acid showed 75.4±1.23% nitric oxide radical scavenging with IC50 values of 45.96 and 15.97 respectively.

Table 3: Assessment of percent radical scavenging and IC50 of *Typha elephantina* extracts and ascorbic acid evaluated by NO assay

	8µg/ml	15µg/ml	30µg/ml	60µg/ml	125µg/ml	IC50
TEPE	2.46±0.93	5.26±0.61	7.72±2.53	8.77±0.93	10.18±1.27	26651
TECE	13.33±1.4	17.89±0.61	26.67±1.95	37.54±0.93	41.05±0.61	199.5
TEME	28.42±0.61	31.93±0.93	43.16±0.61	52.28±0.35	66.63±1.85	45.96
ASCORBIC ACID	43.51±0.93	49.47±0.61	53.33±0.7	66.32±0.61	75.4±1.23	15.97

ABTS Scavenging Activity As showed in Table 4, different plant extracts and ascorbic acid at varying concentrations exhibited a dose-dependent ABTS scavenging activity. TEME exhibited 59.96±2.22 percent scavenging which is found to be in close proximity to that of 68.02±1.6 of standard with IC50 values of 79.89 and 37.00 respectively. However, TEPE and TECE produced 5.68±1.33 and 33.91±0.68% scavenging along with 2536 and 244.8 IC50 respectively.

Table 4: Assessment of percent radical scavenging and IC50 of *Typha elephantina* extracts and ascorbic acid evaluated by ABTS assay						
	8µg/ml	15µg/ml	30µg/ml	60µg/ml	125µg/ml	IC50
TEPE	-2.42±0.97	0.53±0.64	3.11±2.66	4.21±0.97	5.68±1.33	2536
TECE	2.84±1.57	7.95±0.68	17.79±2.19	29.98±1.04	33.91±0.68	244.8
TEME	14.11±0.73	18.32±1.11	31.79±0.73	42.74±0.42	59.96±2.22	79.89
ASCORBIC ACID	30.52±1.14	37.85±0.75	42.6±0.86	58.57±0.75	68.02±1.6	37

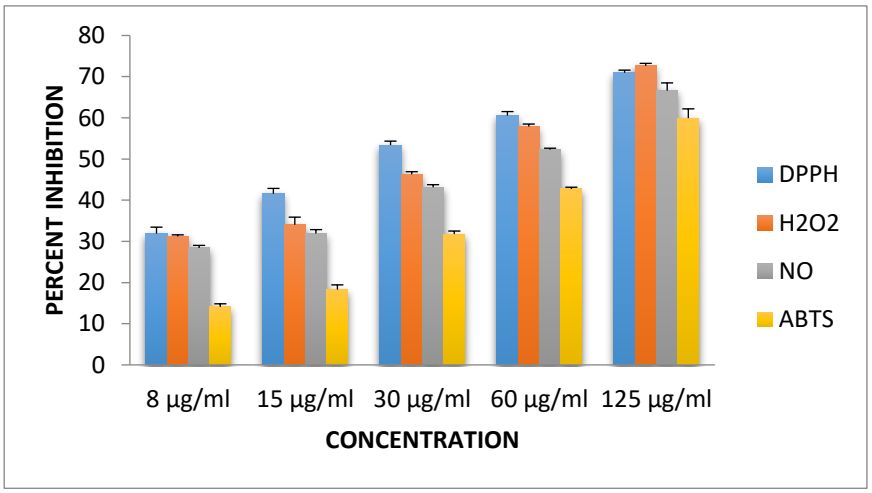

Figure 1: Results obtained by DPPH, NO and H_2O_2 ABTS scavenging assays of *Typha elephantina* methanol extract at different concentrations.

Conclusion

From current results of *in vitro* antioxidant activities against tested free radicals, it can be concluded that the methanol extract of *Typha elephantina* showed promising reducing activity and excellent free radicals scavenging activity against DPPH, hydrogen peroxide (H2O2), ABTS and NO free radical which was maybe because of secondary metabolites such as flavonoids and terpenoids, as well as a high phenolic content. These free radical scavenging activities confirm that methanol extract of *Typha elephantina* found to be having considerable antioxidant potential and promising source for antioxidants, that can actually prevent the development of oxidative stress-related diseases.

Statistical Analysis and IC50 Value All methods were repeated to obtain readings in triplicate (n=3) and the data was represented as mean standard deviation. IC50 was estimated using intercept, slope and concentration via Microsoft excel. Further analysis was obtained using graphpad prism6.0.

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