ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Pharmacognostical, Quantitative Phytochemical and *In-Vitro* Antioxidant Studies of the Root Extracts of *Typha Angustata Bory & Chaub.*

Mamillapalli Vani[1]*, Khantamneni Padma Latha[2], Badri Nagini[1], Saikam Naga Mounika[1], Rajulapati Jyothi[1] and Varikutti Bhavani[1].

[1]Department of Pharmacognosy and Phyto Chemistry, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Viayawada, Pin: 521108, Krishna District, Andhra Pradesh, India.
[2]Department of Pharmacology, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Viayawada, Pin: 521108, Krishna District, Andhra Pradesh, India.

ABSTRACT

The aim of the study was to carry out the pharmacognostical, quantitative phytochemical determination of flavonoids, phenolics, tannins, saponins and *in vitro* antioxidant studies by reducing power assay, scavenging of hydrogen peroxide radical assay and nitric oxide radical scavenging assay on the root extracts of *Typha angustata*. Quantitative microscopy, fluorescence analysis, physico chemical analysis has been carried out to produce quality control parameters for the *Typha angustata* roots and extracts. Quantitative determinations indicated the high amount of phenolics 107.94 ±0.70 mg/g and saponins 108.5 mg/g ±0.7 mg/g in the aqueous extract of roots of *Typha angustata*. The *in vitro* anti oxidant activity was studied at 30-180µg/ml by using quercetin as standard. The studies showed that significant activity was present in both aqueous and alcoholic extracts when compared with standard drug.Therefore the investigation on the root extracts of *Typha angustata* can be further explored inorder to study out more therapeutic benefits.

Keywords: Quality control; *Typha angustata root*; phenols; saponins; *in vitro* anti oxidant

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Phytopharmaceuticals have been used for therapy since ages. Still man is dependent on nature in search to reveal secrets of therapy. The search for new medicines goes on as the new diseases emerge. There are still no proper rigid quality control parameters to judge the authenticity of herbal products. Therefore attempts should be made to findout quality control parameters to include in official monographs. Pharmacognostic studies help in identification of herbal raw material as well as herbal extracts [1].

As a key to find route to treat many chronic disorders it is necessary to study the antioxidant potential of medicinal substances as herbs contain tannins and flavonoids as potential phyto chemical antioxidants [2].Human body produces reactive oxygen species that are oxidants during oxygen consumption. They are beneficial in small amounts but if their number is more these free radicals damage the cells and tissues. Therefore to combat this human body produces antioxidants. But over production of free radicals leads to severe damage of the system initiating several disease processes [17, 14]. There should be balance between reactive oxygen species and availability of antioxidants in the environment of cell [11]. Alternatively human being is forced to be supplemented with antioxidants. In this route herbal antioxidants take their demand being safe and efficient [8].

In order to find out new potential sources of natural antioxidants the plant selected was *Typha angustata* belonging to family *Typhaceae* as there were no scientific reports published on the roots of this plant. *Typha* plants are common aquatic weeds widely grown in waterlogged areas commonly called as cattail grown in temperate regions of the world [5]. The ethnomedicinal uses of the plant were descrbed in Indian, Chinese and Turkish medicinal systems. The leaves are used as diuretic [6].pollen in haematemesis, haematuria [19]. The shoots and flowering heads of the plant contain naringenin, isorhamnetin, quercetin, kaempferol, vanilllic acid, proto catechuic acid and typhic acid, sterols like cholesterol, β - sitosterol, typhaenoside. The roots contain linoleic and linolenic acids [9, 12].

This study focusses on morphological evaluation which includes determination of sensory characteristics , microscopic evaluation which includes study of histological characters by examining the powder characteristics, quantitative microscopical determinations, powder analysis involving fluorescence analysis, physico chemical evaluation of various parameters which include determination of ash values, extractive values, loss on drying, phytochemical evaluation which includes extraction by using alcohol and water as solvents, quantitative phytochemical evaluation by UV Spectroscopy to determine total flavonoids, tannins, phenols and *in vitro* free radical scavenging properties by reducing power assay, hydrogen peroxide radical scavenging assay and nitric oxide radical scavenging assay for the aqueous and alcoholic root extracts of *Typa angustata.*

EXPERIMENTAL

The methods used to carry out pharmacognostic, phytochemical and *in vitro* antioxidant studies on the root extracts of *Typha angustata* are described as below.

Pharmacognostic study [1, 2]

Collection & authentication of the medicinal plant

The Plant material *Typha angustata* was collected in the month of January during morning from the grounds of Vijaya institute of Pharmaceutical Sciences for women, Enikepadu, Vijayawada. Herbarium was prepared and the sample was authenticated by Dr. D.T. P. Satyanarayana Raju, plant taxonomist, Acharya Nagarjuna University, Guntur. The photographs of the plant and roots on stem, roots on rhizome were depicted in figure 1, figure 2 (a), and figure 2 (b) along with authentication letter.

ISSN: 0975-8585

Figure 1. *Typha angustata* plant

Figure 2 (a). *Typha angustata* aerial stem with roots

Figure 2 (b). *Typha angustata* roots on rhizome

ISSN: 0975-8585

Drying and pulverisation of the roots

The roots were cleaned of debris and other vegetative parts, washed properly with water and then dried in sun for a month. Some of the dried roots were used to carry out the macroscopic study. They were powdered coarsely using an electric mixer, sieved and stored in an air tight container. The photographs of dried roots and root powder of Typha angustata were depicted in figure 2(c) and figure 3.

Macroscopy of root

Materials: Dried roots of *Typha angustata*, driedroot powder of *Typha angustata*, measuring Scale, Vernier caliperse, Simple microscope (Olympus OIC).

Method: The dried roots were observed under Simple microscope. The colour, odour, taste, and shape were studied by sensory characteristics. The size *i.e.* length of the roots was measured with the help of measuring scale and width by using Vernier caliperse. The photograph was depicted in figure 4 and the results for the determination of length and width of roots of *Typha angustata* were given in table 1.

Figure 4. Measurement of length and width of roots of *Typha angustata*

Macroscopy of root powder

Method: The colour, odour, taste were studied by sensory characteristics. The details of root powder were produced in figure 3.

Powder microscopy

Observation of powder characteristics

Materials: Dried root powder of *Typha angustata,* compound microscope with camera (Olympus OLC), eyepiece micrometer (colxmpus), stage micrometer (ERMA INC), spirit lamp**,** lactophenol, phloroglucinol (olxyms), conc. Hcl (Finar-chemical limited), glycerine water.

Method: The powder was finely spread over the glass slide, heated with lactophenol for few minutes, stained with phloroglucinol and Conc.Hcl, examined under microscope. The details are depicted in figure 5 (a), 5 (b), 5 (c), 5 (d), 5 (e) and 5 (f).

Determination of length and width of phloem fibres

Materials: Dried root powder of *Typha angustata,* compound microscope with camera, eyepiece micrometer, stage micrometer, spirit lamp, lactophenol, phloroglucinol, Conc. Hcl (Merck), glycerine water, distilled water.

Method: The length and width of the phloem fibres was measured using standard procedure. The length and width for 50 fibres was calculated as intermediate, minimum and maximum values in microns and

produced as average. The results for determination of length and width of phloem fibres of roots of *Typha angustata* were given in table 2.

Powder analysis

Powder analysis using chemical reagents with naked eye

Material : Dried root powder of *Typha angustata,* Conc. Hcl (Finar), Conc. HNO_3, Conc. H_2SO_4, 5% NaOH (Finar),5% KOH (Finar), 5% $FeCl_3$, picric acid (Merck), ammonia solution (Merck).

Method: The root powder was studied with nacked eye by using the chemicals and the results were noted. The results for Powder analysis of roots of *Typha angustata* using chemical reagents with nacked eye were given in table 3.

Fluorescence analysis of the root powder

Materials : Dried root powder of *Typha angustata,* UV lamp, 1N NaOH in Methanol (Finar),1N NaOH in water, 50% Hcl, (Merck),50% H_2SO4 (Finar), 50% HNO_3, Petroleum ether (Finar), Chloroform (Merck), Picric acid (Finar), Ferric chloride (Finar), 5% Iodine solution (Merk), Methanol (Finar), Nitric acid (Merck), Ammonia solution (Finar chemical limited).

Method : The root powder was studied for fluorescence analysis under UV light (Lab India) and the results were noted after treating with different chemical reagents. The results for fluorescence analysis of the root powder of *Typha angustata* were depicted in table 4.

Physico chemical analysis of the root powder

Determination of ash value

Determination of total ash value

Materials : Dried root powder of *Typha angustata,* silica crucible, desiccators, Muffle furnace.

Method: Two grams of the powdered leaf sample was weighed and taken in a flat, thin, tarred silica crucible heated in a Muffle furnace until all the carbon is burnt off. Cool the crucible in a desiccators and the final weight of the crucible was recorded. The percentage of total ash corresponding to the air dried sample was calculated and the results were given in table 5.

Determination of Acid insoluble ash value

Materials: Dried root powder of *Typha angustata,* dilute hydrochloric acid (Finar), desiccator.

Method: The total ash was devided in to two parts. One part was taken, washed with dilute hydrochloric acid and collected in a 100 ml beaker. The whole content was boiled for five minutes and filtered using ash less filter paper and the residue was washed twice with hot water. The residue was placed in crucible. Ignite the crucible and heat gently until vapors cease to be evolved. Cool the crucible in a desiccators and the weight of residue recorded corresponding of the acid insoluble ash and the results were givenin table 5.

Determination of water soluble ash value

Materials: Dried root powder of *Typha angustata,* distilled Water, desiccators.

Method: This was determined in a similar way to acid insoluble ash using 25 ml of water in place of dilute hydrochloric acid for another half part and the results were given in table 5.

Determination of extractive value

Determination of alcohol soluble extractive

Materials: Dried root powder of *Typha angustata,* alcohol, porcelaindish, desiccator.

Method: Five grams of the dried powdered root sample was taken in 250 ml conical flask. To it 100 ml of 90% alcohol was added and left undisturbed for 24 hours. Filter it and transfer 25 ml of the filtrate to a weighed thin porcelain dish as used for the ash value determination. Cool in desiccators and weigh. Calculate the percentage (w/w) of extractive with reference to the air dried drug and the results were given in table 5.

Determination of Water soluble extractive

Materials: Dried root powder of *Typha angustata,* Porcelain dish, desiccator

Method: The steps were similar to those mentioned for alcohol soluble extractive determination. Use distilled water instead of alcohol and the results were expressed in table 5.

Determination of Petroleum ether soluble extractive

Materials: Dried root powder of *Typha angustata,* ether, porcelain dish, desiccator.

Method: The steps were similar to those mentioned, use petroleum ether instead of Alcohol and the results were givenin table 5.

Determination of moisture content (loss on drying)

Materials: Dried root powder of *Typha angustata,* desiccator, thermometer (JSGW).

Method: About 1.5 g of the powdered root sample was weighed and placed in hot air oven at 105^0C for 3 hrs. The powder was cooled in a desiccator and weighed. The loss in weight was recorded as moisture content. The results were given in table 5.

PHYTOCHEMICAL STUDY

Extraction

Materials: Dried root powder of Typha angustata, Soxhlet apparatus (JSGW), distilled Water, Methanol (Merck), Vaccum pump (Bio-tech).

Method: The roots were dried and powdered. The powder was subjected to Soxhlet extraction by using water and alcohol as solvents. The crude extract was dried using vaccum pump and weighed.

Quantitative determination

The aqueous and methanolic extracts of the roots of *Typha angustata* were screened for the total content of following phytochemical constituents according to the standard methods as follows.

Flavonoids: [7]

Materials: **Aqueous extract** of the roots of *Typha angustata***, methanolic extract** of the roots of *Typha angustata***,** 2% methanolic $AlCl_3$, quercetin (Research - labfine), Centrifuge, incubator (Bio-tech).

Method: This was assayed using the procedure described. Briefly, the extract (1.5 ml) was added to 1.5 ml of 2% methanolic $AlCl_3$ solution. The mixture was vigorously shaken on Centrifuge for 5 minutes at 200 rpm and the absorbance was read at 367 nm after 10 minutes of incubation. Quercetin was used as a standard for the calibration curve. The assay was carried out in triplicate and calculated using the equation (1).

Equation (1): **C = c.V/m**

C – Total phenolic compounds mg/gm of plant extract
c – The concentration of standard established from the calibration curve mg/ml
V – The volume of extract in ml
m -The weight of pure plant extract

Phenolics : [13]

Materials: **Aqueous extract** of the roots of *Typha angustata***, methanolic extract** of the roots of *Typha angustata***,** 0.2 N Folin Ciocalteu phenol reagent (Merck), 2% Sodium carbonate (Finar), Quercetin (Research lab fine).

Method: This was determined using Folin-Ciocalteu method. The extract (0.5 mL) was added to 10 ml deionized distilled water and 2.5 ml of 0.2 N Folin-Ciocalteu phenol reagent. The mixture was left undisturbed at room temperature for 5 minutes and then 2 ml of 2% sodium carbonate was added. The absorbance of the resulting solution was read at 780 nm and repeated three times. Quercetin was used as a standard for calibration curve. This was done in triplicate and calculated using the equation (1).

Tannins: [10]

Materials: **Aqueous extract** of the roots of *Typha angustata***, methanolic extract** of the roots of *Typha angustata* **,** 10% Na_2CO_3, incubator (Bio-tech).

Method: This was determined by Folin-Denis colorimetric method with slight modification. 0.5 was dispersed in 50 ml of distilled water and shaken. The mixture was left undisturbed for 30 minutes at 28°C and filtered through Whatman No. 1 filter paper. The filtrate (2 ml) was dispersed into a 50 ml volumetric flask and 2.5 ml of 10% $Na_2 CO_3$ solution was added. The content of each flask was made up to 50 ml with distilled water and incubated at 28°C for 90 minutes. Absorbance was read at 260 nm using the reagent blank. Tannic acid was used for the calibration curve. The procedure was repeated three times and calculated using the equation (1).

Saponins: [4]

Materials: **Aqueous extract** of the roots of *Typha angustata* **, methanolic extract** of the roots of *Typha angustata***,** isobutyl alcohol (Finar), 40% Magnesium carbonate solution (Finar), 5%$FeCl_3$ (Merck) solution.

Method: A known mass (1 g) of finely ground sample was weighed into a 250 mL beaker and 100 ml of isobutyl alcohol was added. The mixture was shaken for 5 hours to ensure uniform mixing and filtered through Whatman No. 1 filter paper into a 100 mL beaker, after which 20 ml of 40% magnesium carbonate solution was added. The resulting mixture was again filtered through Whatman No. 1 filter paper to obtain a clear colorless solution. A known volume (1 ml) of the colorless solution was pipetted into a 50-mL volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to the marked level with distilled water. It was left undisturbed for 30 minutes for blood red color to develop. The absorbance was read after color development at a wavelength of 380 nm. Standard Saponin was used for calibration curve and calculated using the equation (1).The results of quantitative screening for the root extracts of *Typha angustata* were given in table 6.

ANTIOXIDANT ACTIVITY

Reducing power assay method [18]

Materials : **Aqueous extract** of the roots of *Typha angustata***, methanolic extract** of the roots of *Typha angustata***,** U.V. Spectrophotometer (Labindia), incubator (Bio-tech), Centrifuge, Phosphate buffer (p^H 6.8), Potassium ferricyanide (1%) (Finar) , Trichloro acetic acid (10%) (Merck), $FeCl_3$ (0.5 ml, 1%).

Method: Reducing power of **aqueous and methanolic** root extracts of *Typha angustata* was carried out by preparing different concs. of the extracts (30 – 180 µg/ ml). 1 ml of each solution was mixed with

phosphate buffer (2.5ml, 0.2M, p^H 6.8) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50^0 C for 70 min. To this mixture, 2.5 ml of 10 % trichloro acetic acid (TCA) was added and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water 2.5 ml and $FeCl_3$ (0.5ml, 0.1%) was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated reducing power. The results for the antioxidant effect of different concentrations of aqueous and methanolic extracts of *Typha angustata* roots by reducing power assay were given in table 7 and were represented in graphical abstract 1.

The % scavenging was calculated by using the equation (2).

Equation (2): **Scavenging activity (%) = ($A_{Control}$ - A_{Sample}) / Control × 100**

$A_{control}$: Absorbance of solution without extract
$A_{standard}$: Absorbance of Quercetin solution
A_{sample} : Absorbance of solution with different dilutions of drug extract (30 – 180 µg/ ml)

Scavenging of H_2O_2 assay method [15]

Materials: Aqueous extract of the roots of *Typha angustata*, methanolic extract of the roots of *Typha angustata,* UV visible spectrophotometer (Lab India), H_2O_2 40 mM, Phosphate buffer pH 7.4.

Method: The ability of aqueous and methanolic root extracts of *Typha angustata*t to scavenge H_2O_2 was determined according to the method of Ruch. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of H_2O_2 was determined by absorption at 230 nm using a UV visible spectrophotometer. Then H_2O_2 solution (0.6 ml, 40 mm) was mixed to different concentrations (30 – 180 µg/ ml) of the extract dissolved in water. The absorbance of H_2O_2 at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without H_2O_2. The results for the antioxidant effect of different concentrations of aqueous and methanolic extracts of *Typha angustata* roots by scavenging of H_2O_2 assay were given in table 8 and were represented in graphical abstract 2.The percentage of H_2O_2 scavenging by the extracts and standard compound was calculated using the equation(2).

Nitric oxide radical scavenging activity [16]

Materials : Aqueous extract of the roots of *Typha angustata*, methanolic extract of the roots of *Typha angustata,* UV visible spectrophotometer (Lab India), incubator (Bio-tech), Griess reagent (Research lab fine chem.), Sodium nitroprusside (Merck), Phosphate Buffered saline (PBS).

Method: Nitric oxide was generated from sodium nitro prusside and measured by the Griess reaction. Sodium nitro prusside in aqueous solution at physiological p^H spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Griess reagent. Scavengers of nitric oxide. Sodium nitro prusside (5mm) in phosphate buffered saline (PBS) was mixed with 3.0 ml of different concentrations (10 – 320 mg/ml) of the drug dissolved in the suitable solvent system and incubated at 25^0 for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride).

The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Griess reagent. The results for the antioxidant effect of different concentrations of aqueous and methanolic extracts of *Typha angustata* roots by nitric oxide radical scavenging assay were given in table 9 and were represented in graphical abstract 3.The percentage of NO scavenged by the extracts and standard compound was calculated using the equation (2).

ISSN: 0975-8585

RESULTS AND DISCUSSION

Macroscopy of roots of Typha angustata

The colour of the outer surface of dried roots was brown and inner surface was pale yellow. The roots exhibited none odour. They possess characteristic taste. The measurements of roots for length and width were given in figure 2(c) and table 1.The shape of roots is straight and little wavy. The fracture is even. The roots are with fine root hairs attached to them on the surface.

Figure 2(c). Dried roots of *Typha angustata*

Table 1. Determination of length and width of roots of *Typha angustata*

S no.	Length	Width
1.	10 – 14.5 cm	0.01 mm – 0.2 mm

Macroscopy of root powder

The root powder is reddish brown in colour. It possess none odour and characteristic taste. The details are produced in figure 3.

Figure 3. Dried root powder of *Typha angustata*

ISSN: 0975-8585

Observation of powder characteristics

The Powder characteristics of *Typha angustata* root showed epiblema, acicular crystals of Calcium Oxalate, lignified phloem fibres, lignified xylem vessels, lignified phloem fibres containing Crystal sheath, cortex, sclerenchymatous cells of hypodermis and cortex cells. The details are depicted in figure 5 (a), 5 (b), 5 (c), 5 (d), 5 (e) and 5 (f).

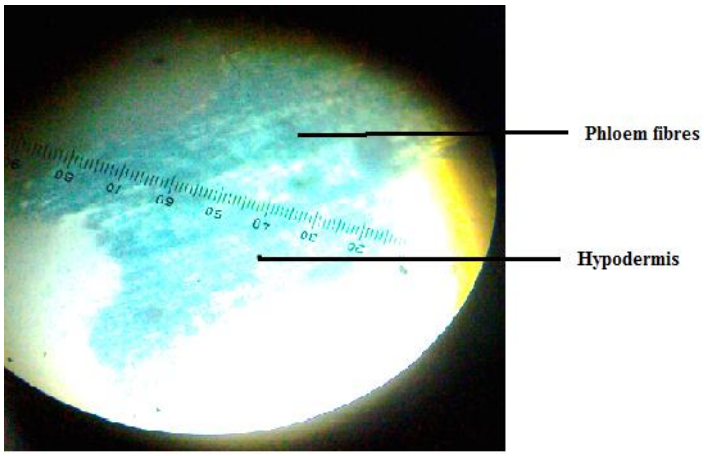


Figure 5 (a). Powder microscopy of roots of *Typha angustata*

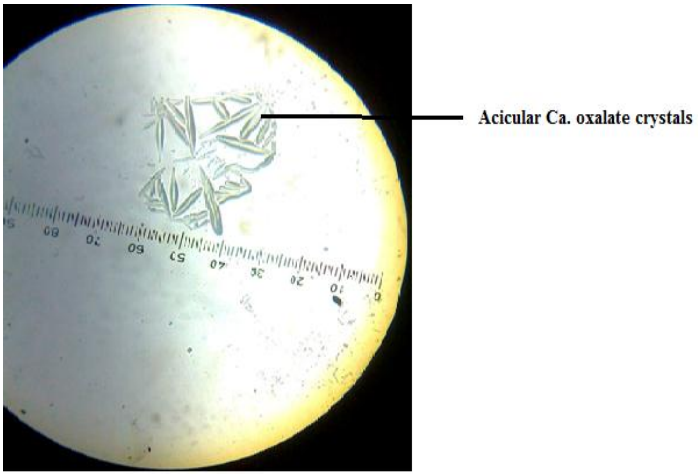


Figure 5 (b). Powder microscopy of roots of *Typha angustata*

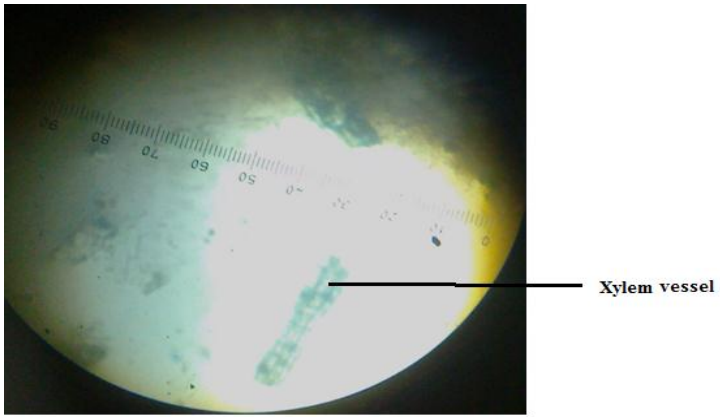


Figure 5 (c). Powder microscopy of roots of *Typha angustata*

ISSN: 0975-8585

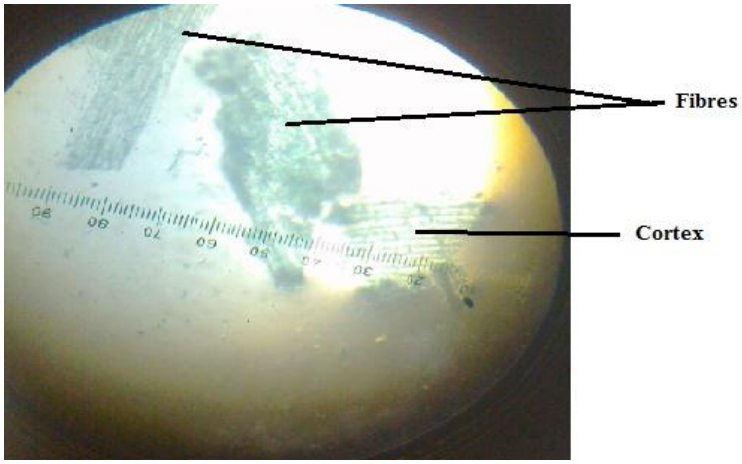


Figure 5 (d). Powder microscopy of roots of *Typha angustata*

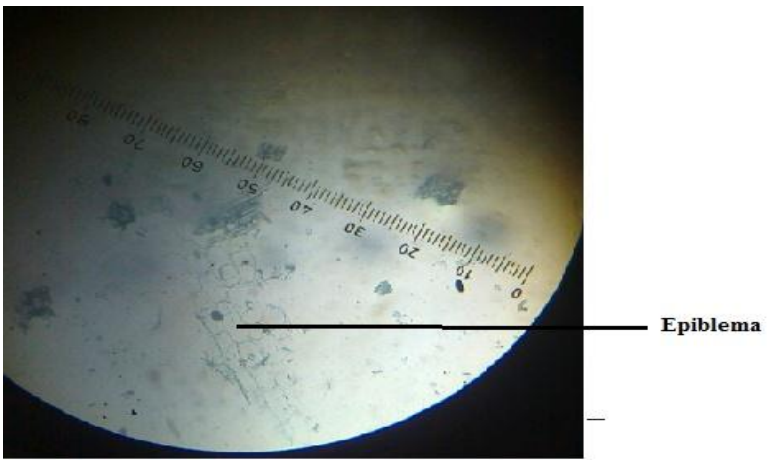


Figure 5 (e). Powder microscopy of roots of *Typha angustata*

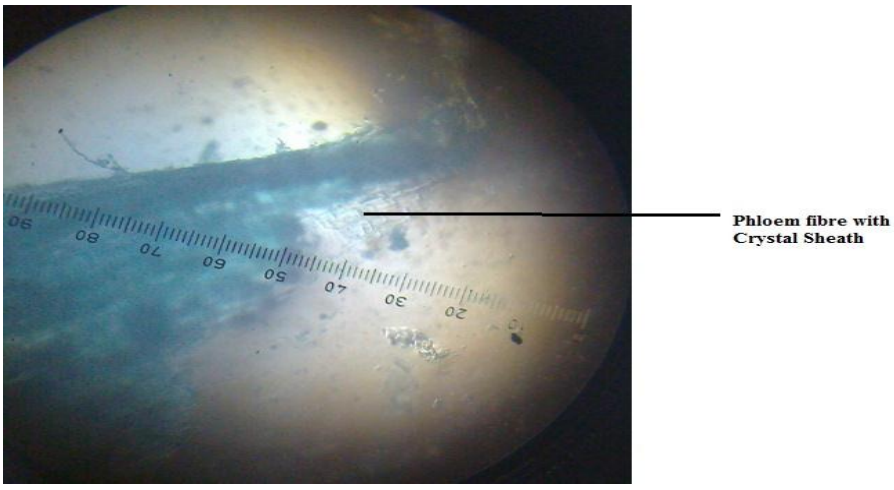


Figure 5 (f). Powder microscopy of roots of *Typha angustata*

Determination of length and width of phloem fibres

The average value of length and width of phloem fibres in *Typha angustata* root powder was found to be 8.2 µ in length and 25.9 µ.The details are represented in table 2.

ISSN: 0975-8585

Table 2. Determination of length and width of phloem fibres for the roots of *Typha angustata*

S. no	No. of eye piece micrometer divisions	
	length	**Width**
1.	8.2 µ	25.9 µ

Powder analysis with naked eye

Powder analysis with chemical reagents for the roots of *Typha angustata* showed the following different colours when observed with nacked eye. The details are produced in table 3.

Table 3. Powder analysis with chemical reagents for the roots of *Typha angustata*

S.NO	Reagents	Colour observed
1.	Powder as such	Light brown
2.	Powder + concentrated HCl	Pale yellow
3.	Powder + concentrated HNO_3	Brown yellow
4.	Powder + concentrated H_2SO_4	Reddish brown
5.	Powder + glacial acetic acid	Brown yellow
6.	Powder + 5% NaOH Solution	Brown yellow
7.	Powder + 5% KOH solution	Brownish yellow
8.	Powder + 5% $FeCl_3$	Brownish yellow
9.	Powder + picric acid	Yellow
10.	Powder + ammonia	Light brown

Fluorescence analysis

The fluorescence analysis for the root powder of *Typha angustata* on treatment with various chemical reagents showed the following different colours when observed under UV light. The details are produced in table 4.

Table 4. Fluorescence analysis of *Typha angustata* root powder

S.no	Treatment with chemical reagents	Fluorescence observed
1.	Powder as such	Light brown
2.	Powder + 1N NaOH in methanol	Green
3.	Powder + 1N NaoH in water	Light green
4.	Powder + 50% HCL	Light green
5.	Powder + 50% H_2SO_4	Colour less
6.	Powder + 50% HNO_3	Green colour
7.	Powder + petroleum ether	Colour less
8.	Powder + $CHCl_3$	Colour less
9.	Powder + picric acid	Colour less
10.	Powder + $FeCl_3$ solution	Colour less
11.	Powder + 5% iodine solution	Colour less
12.	Powder + methanol	Faded colour

Physico chemical analysis

The various Physico chemical parameters studied for the root powder of *Typha angustata* include total ash value, acid insoluble ash value, water soluble ash value, alcohol soluble extractive value, water soluble extractive value, and petroleum ether soluble extractive value, loss on drying or moisture content. The results were produced in table 5.

ISSN: 0975-8585

Table 5. Physico chemical Parameters *Typha angustata*

S.no.	Parameter	values % w/w
1.	Total ash value	25% w/w
2.	Acid insoluble ash value	5% w/w
3.	Water soluble ash value	10% w/w
4.	Alcohol soluble extractive	11.4 % w/w
5.	Water soluble extractive	12% w/w
6.	Petroleum ether soluble extractive	1.4 % w/w
7.	Loss on drying	3.8 % w/w

Quantitative determination

The Quantitative preliminary phytochemical screening was carried out for total flavonoid content,total phenolic content, total tannin content and total saponin content for the aqueous and alcoholic extracts of roots of *Typha angustata* . The results indicated that saponins, phenolics, and flavonoids were present in more amount in the aqueous extract of roots of *Typha angustata* The alcoholic extract of roots of *Typha angustata* contains more amount of tannins.The values were shown in table 6.

Table 6. Quantitative Phytochemical Screening of aqueous and methanolic extracts of *Typha angustata* roots

S.no	Phytochemical	AQERTA	MERTA
1.	Flavonoids (mg/g)	82.53±0.63	29.42±0.253
2.	Phenolics (mg/g)	107.94±0.70	82.7±0.67
3.	Tannins (mg/g)	50±2.5	92.5±2.5
4.	Saponins (mg/g)	108.5±0.7	90.7±0.7

Note:Values represented mean **± S.D.** of three parallel measurements. **AQERTA, MERTA**- aqueous extract of roots of *Typha angustata,* methanolic extract of roots of *Typha angustata.*

Antioxidant Activity

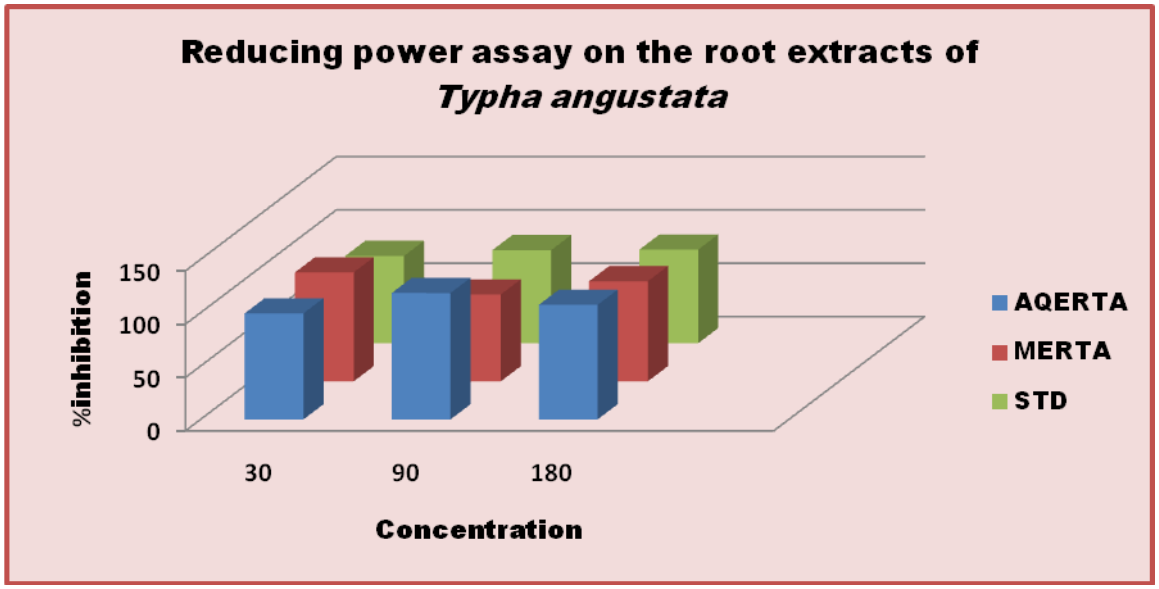
In vitro antioxidant activity determined as percentage inhibition of free radicals by reducing power assay, hydrogen peroxide radical scavenging method and nitric oxide radical scavenging method in both aqueous and methanolic extracts of roots of *Typha angustata* at various concentrations (30, 90, 180 µg/ml) was as followed. The results by reducing power assay were presented in table 7 and graphical abstract 1.The results by hydrogen peroxide assay are expressed in table 8 and graphical abstract 2. The results by nitric oxide radical scavenging method were presented in table 9 and graphical abstract 3.The root extracts of the plant were exhibiting potential antioxidant activity when compared with standard drug quercetin in the three methods.

Table 7. Antioxidant effect of different concentrations of aqueous and methanolic extracts of *Typha angustata* roots by reducing power assay

S.no	Sample	Concentration (µg/ml)		
		30µg/ml	**90µg/ml**	**180µg/ml**
1.	AQERTA	**% inhibition**		
		99.4 % ±0.020*	118.5%±0.027**	107.4%±0.034**
2.	MERTA	102.1%±0.0342*	81.4%±0.046*	93.8%±0.0062*
3.	STD	81.85%±0.018*	87.23%±0.084**	87.76%±0.017**

Note:The values are expressed as Mean ± **SEM, n= 6**. The values are significant,* **p< 0.05;** ****p < 0.01** when compared with standard**. AQERTA, MERTA, STD** – aqueous extract of roots of *Typha angustata,* methanolic extract of roots of *Typha angustata,* Standard.

ISSN: 0975-8585

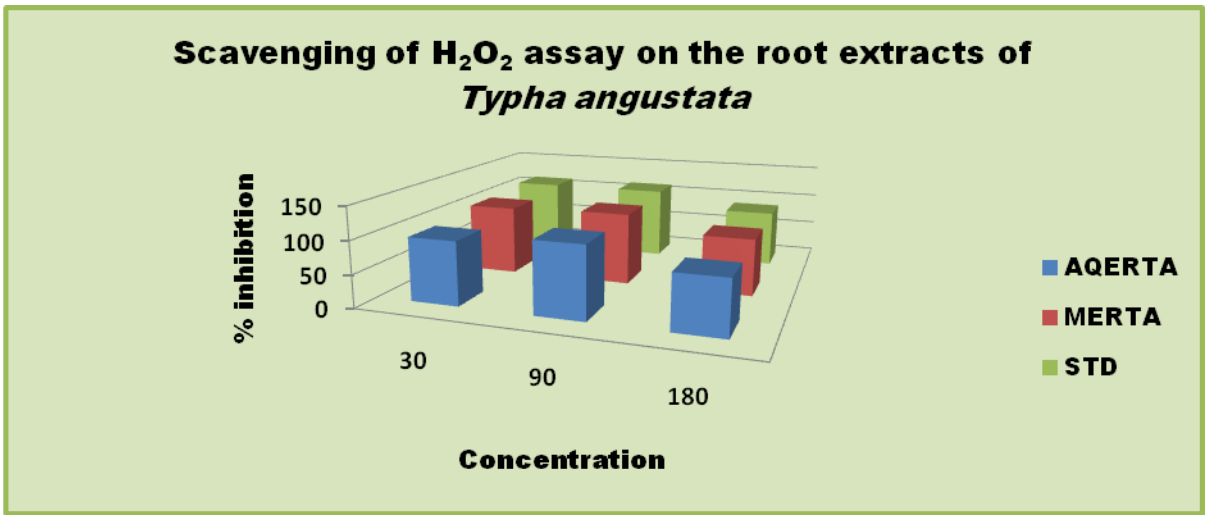


Graphical abstract 1. Reducing power assay on the root extracts of *Typha angustata*

Table 8. Antioxidant effect of different concentrations of aqueous and methanolic extracts of *Typha angustata* roots by scavenging of H_2O_2 assay method

S.no	Sample	Concentration (µg/ml)		
		30µg/ml	**90µg/ml**	**180µg/ml**
1.	AQERTA	**% inhibition**		
		97.1%±0.0329**	106.5%±0.082**	113.2%±0.078**
2.	MERTA	109.4%±0.082**	110.6%±0.063*	113.2%±0.044*
3.	STD	82.85%±0.0366*	87.23%±0.023*	87.56%±0.0242*

Note:The values are expressed as mean ± **SEM**, **n= 6**. The values are significant, * **p< 0.05**; ** **p < 0.01** when compared with standard. **AQERTA, MERTA**, **STD** – aqueous extract of roots of *Typha angustata,* methanolic extract of roots of *Typha angustata,* Standard.



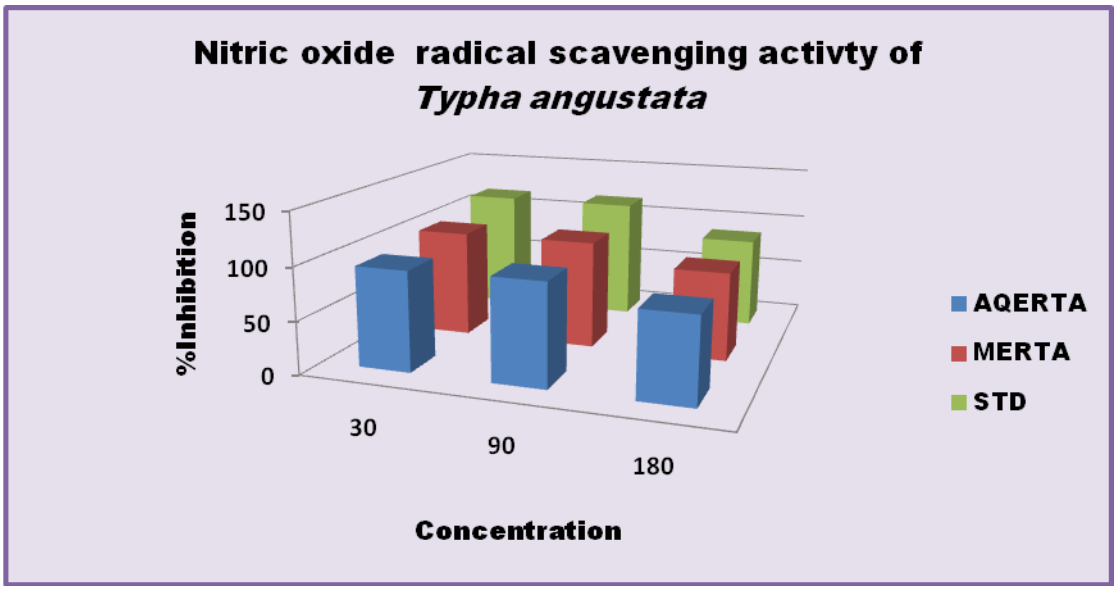
Graphical abstract 2. Scavenging of H_2O_2 assay on the root extracts of *Typha angustata*

ISSN: 0975-8585

Table 9. Anti oxidant effect of different concentrations of aqueous and methanolic extracts of *Typha angustata* roots by nitric oxide radical scavenging activity

S.no	Sample	Concentration (µg/ml)		
		30µg/ml	**90µg/ml**	**180µg/ml**
1.	AQERTA	**% inhibition**		
		95.1%±0.038**	102.1%±0.037*	116.1%±0.082*
2.	MERTA	96.9%±0.053*	102.6%±0.013*	116.1%±0.213*
3.	Standard	80.85%±0.200*	84.23%±0.003*	86.76%±0.059*

Note:The values are expressed as mean **± SEM, n= 6**. The values are significant, *** p< 0.05; ** p < 0.01** when compared with standard. **AQERTA, MERTA, STD** – aqueous extract of roots of *Typha angustata, m*ethanolic extract of roots of *Typha angustata,* Standard.



Graphical abstract 3. Nitric oxide radical scavenging activity of *Typha angustata*

CONCLUSION

The root extracts of the plant *Typha angustata* possess maximum amount of flavonoids, phenolics, tannins which are supposed to show antioxidant activity. The plant extracts showed potential freee radicalscavenging activity as compared to standard drug quercetin. This signifies the therapeutic utility of the plant in treating various chronic disorders. The root extracts of the plant can be further best studied for isolation , characterization and preclinical studies.

ACKNOWLEDGEMENT

The authors are thankful to Viaya institute of Pharmaceutical Sciences for Women for providing facilities to carry out the research work.

REFERENCES

[1] [WHO]World health organization. Quality control methods for medicinal plant material. WHO Library. 1998, pp. 110-115.

[2] Anderson KJ, Teuber S, Gobeille A Cremin P, Waterhouse A L, & Steinberg FM Walnut. Polyphenolics inhibit *in vitro* human plasma and LDL oxidation. Biochemical and molecular action of nutrients. J Nutr 2001; 131: 2837- 2842.

[3] Ansari MM, Ahmad J, Ahmad A, Ansari S. Pharmacognostic characterization and standardization of *Morus alba* stem bark. J med and Aromatic PI sci 2006; 28: 31-36.

[4] Brunner H. Direct spectrophtometric determination of saponins. Anal Chem 1984; 34: 1314-26.

[5] Chopra RN, Nayar SL and Chopra IC. Glossary of Indian Medicinal Plants Council of Scientific and Industrial Research, New Delhi, 1986.
[6] Duke JA, Wain KK. Medicinal plants of the world. 1999, pp. 3-107.
[7] Jagadish LK, Krishnan VV, Shenbhagaraman R, Kaviyarasan V. Comparative bisporus imbach before and after boiling. Afr J Biotech 2009; 654-61.
[8] Jude C Ikewuchi. Moderation of hematological and plasma biochemical indices of sub-chronic salt-loaded rats, by an aqueous extract of leaves of *Acalypha wilkesiana* 'Godseffiana' Muell Arg (Euphorbiaceae). Asian Pac J Trop Med 2013; 6(1): 37-42.
[9] Jung-Jin L, Yi H, Kim IS, Kim Y, Nhiem NX, Kim YH, Myung CS: (2S)-naringenin from Typha angustata inhibits vascular smooth muscle cell proliferation via a G0/G1 arrest. J Ethnopharmacol 2012; 139 (3): 873-878.
[10] Kirk H, Sawyer R. Frait Person. Chemical analysis of food. 8th ed. Edinburgh: Longman Scientific and Technical, 1998, pp. 211-2.
[11] Nishikawa M, Inoue M. Oxidative stress and tissue injury. Jpn J anaesthesiol 2008; 57(3):321–326.
[12] Nivati SA et al. A review *on Typha angustata.* Int J *G*reen Pharm 2013; 7 (1): 12-17.
[13] Olajire AA, Azeez L. Total antioxidant activity, phenolic,flavonoid and ascorbic acid contents of Nigerian vegetables. Afr J Food Sci Technol 2011; 2: 22-9.
[14] Pham-Huy L, and Pham-Huyc C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008; 4(2): 89- 96.
[15] Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989; 10: 1003-1008.
[16] Saski A.B.E van Acker, Guido R.M.M Haenen, Wini J.F. vander vijh, Aalt Bast. Flavonoids as scavengers of nitric oxide radical. Biophys. Res 1995; 214: 755-759.
[17] Willcox JK, Ash SL and Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 2004; 44**:** 275-295.
[18] Yen GH and Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric. Food Chem 1995; 43: 27-32.
[19] Yeng, Him Che. Handbook of Chinese herbs and formulas, Vol 2: Formulas. Institute of Chinese medicine. Los Angeles,CA, 1985.