INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 4, p. 157-165, 2015

RESEARCH PAPER OPEN ACCESS

# Qualitative and quantitative phytochemical analysis, essential element analysis, antibacterial and antifungal activities of leaves of *Typha angustata*

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**Key words:** *Typha angustata* leaves, phytochemical analysis, essential element analysis, antibacterial and antifungal activities.

http://dx.doi.org/10.12692/ijb/7.4.157-5 Article published on October 25, 2015

## Abstract

Different types of plants in Pakistan can be used as a medicine to cure many diseases**.** Since a long time ago, the plants have been traditionally used as a medicine due to its medicinal properties. In current study qualitative analysis of the leaves of *Typha Angustata* of family Typhaceae were collected which shows the presence of alkaloids, tannins, anthraquinones, glycosides, saponins, terpenoids, flavonoids, phlobotannins, carbohydrates, proteins and reducing sugars while quantitative analysis was carried out which shows that flavonoid, alkaloid and saponin were 0.03% , 0.029% and 0.25% respectively. The results of bio assay suggest that the leaves of *Typha Angustata* also show antimicrobial potential against gram positive and negative bacterial strains i.e. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and some fungal strains like *Aspergillus Niger* and *Aspergillus Flavus*. Similarly some essential elements were analysed by using atomic absorption spectrometer which shows essential elements i.e. Ca, Cu, Ni, Cr, Zn, Fe, Mn, Na, and K in different quantities. Moreover, the current research work will help to introduce new and cost effective antibiotic resources and will help to solve the problem of resistance develop by microbes against allopathic antibiotics.

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**Introduction**

Medicinal plants becoming more important with time due to the presence of potential drug compounds. Due to biologically active compounds like phytochemicals these practically play the role of medicines (Krishnaiah *et al*., 2009**)**. Phytochemicals have pronounced effect on the human body and combined with the nutrients and fibres they provide shield against certain diseases and conditions of stress (Afolabi *et al*., 2013). More than about 400,000 species of plant has been reported that it possess medicinal properties.

Phytochemicals are divided on the basis of function in plants metabolism, into two main categories (Krishna *et al*., 2009) primary one which include proteins, sugars, amino acids and chlorophyll etc. and secondary one consists Saponins, flavonoids, tannins, terpenoids, alkaloids, essential oils and phenolic compounds etc. ((Krishnaiah *et al*., 2007; Edeoga *et al*., 2005). Most of the phytochemicals have shown valuable therapeutic activities such as insecticidal (Kambu *et al*., 1982), antifungal, antibacterial, anticonstipative, spasmolytic, antiplasmodial and antioxidants activities etc.( (Kambu *et al*.,1982; Lemos *et al*., 1990; Ferdous *et al*., 1992; Santos *et al*., 1998; Benoitvical *et al*., 2001; Vardar-unlu *et al*., 2003). Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, ant-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. (Kappers IF *et al.,* 2005) Alkaloids are used as anaesthetic agents and are found in medicinal plants. (Hérouart D *et al*., 1998).

The plants species and herbs suggests the presence of anti-oxidative and antimicrobial constituents in their tissues.The human body can be protected against pathogens and cellular oxidation reactions by the antioxidant property present in many plant species, so, it is necessary to screened out the different species of the plant for the antioxidant activity. (Clyton *et al*., 2000). In the treatment of serious gram- negative and gram- positive infections is evident and is most required because of emergence of multidrug resistance in common pathogens, and the potential for use or multidrug – resistant agents in bioweapons (Kais *et al*., 2013).

*Typha Angustata* is a perennial plant and belongs to family Typhaceae. It is present in the marshes of the temperate regions of the world. The leaves are yellow and green and thick. Its leaves and stem standing straightly. (Clegg, J *et al*., 1986). The medicinal properties are to cure the nose bleeds, Uterine, Bleeding, Dysmenorrheal and Post-Partum Abdominal Pain. (Yeng Him-Che., 1985). It also has a flavonoid so it can be used as an antioxidant. Plants can be used for the treatment of Alzheimer's disease. (Pavar CR *et al*., 2011).

Plants need different types of elements which are essential for their growth. They need largely Nitrogen, Phosphorus and Potassium as a primary nutrient and these elements are supplied from soil and deficiency of soli can also be avoided by artificial fertilizers as well (Tisdale SL *et al*., 1993) While the Ca, Mg and S are three secondary nutrients which are required in small quantity and is supplied from fertilizer and Ca and Mg from the limiting materials.

Therefore the aim of current study is to perform the qualitative and quantitative investigation of phytonutrients of locally collected plants *Typha Angustata*. More ever this work was further extended to determine the antimicrobial activities of ethanolic extracts of this plant against some gram positive and gram negative bacterial as well some fungal strains. (Mahato *et al*., 1997). Similarly essential elemental analysis was carried by applying atomic absorption spectrometric technique.

**Material and methods**

*Plant materials*

Plant leaves were collected from the garden of Islamia College University Peshawar during month of March and April 2014 and identified in department of botany, islamia college university Peshawar.

*Preparation of sample*

The plant leaves were grinded into powdered form and about 5.0g of the ground leaves were soaked in ethanol for 48 hours and shaked it occasionally. The extract was filtered through filter paper and filtrate was used for further experiments.

*Preliminary qualitative phytochemical analysis*

For qualitative screening of ethanolic extracts of the leaves different standard procedure were applied. Flavonoid.

In 10mL ethyl acetate, small amount of powdered sample was dissolved. For some time it was warmed on water bath and then filtered. With small amount of filtrate 1ml of dil. ammonia solution was shaked. The presence of flavonoids was indicated through formation of yellow color.

*Reducing sugar*

In a test tube the test solution and 2.5 Benedict's reagent was mixed and on boiling water bath the mixture was heated for 5 minutes. The presence of reducing sugar was indicated through appearance of reddish yellow color.

*Tannins*

3ml of lead acetate was added to 3ml of extract. The presence of tannins was indicated through the appearance of white precipitate.

*Terpenoids*

5ml of plant extract was added with 2ml of $CHCl_3$ and carefully with 3ml of Conc $H_2SO_4$ to form a layer. Appearance of reddish brown coloration on upper side gives a positive indication for the presence of terpenoids.

*Alkaloids*

In a test tube 2% $H_2SO_4$was mixed with 2-3mL of extract and left for 2 minutes on water bath to make it warmed. After this it was filtered and then small amount of Dragon-dorff reagent was added. The presence of alkaloid was indicated through development of orange red ppt.

*Phlobotannins*

The test solution was mixed with 1% HCl and then warmed. The presence of phlobotannins was indicated through formation of red ppt.

*Steroids (Liebermann- Buchards test)*

Test solution was mixed with Chloroform then filtered. In test tube 1mL of extract, 3 drops of acetic anhydrides and 1 drop of concentrated $H_2SO_4$ was added. Test tube was warmed on water bath. The presence of steroid was indicated through development of brown ring at the higher joint with bluish color.

*Saponins*

5mL of distilled water was mixed with 10ml of test solution. The presence of saponin was indicated through appearance of pale yellow accumulation of minute foam.

*Cardiac glycosides*

2ml of glacial acetic acid having 1 drop of $FeCl_3$ was mixed with 5-6ml of test solution. Then 1mL of conc. $H_2SO_4$ was also added to the above mixture. The deoxysugar characteristic of cardinolides was indicated through interface of brown ring. Below the brown ring a violet ring may become visible while greenish ring may appear in acetic acid layer.

*Anthraquinone*

10mL of sulfuric acid was mixed with the small amount of test solution then boiled it. The mixture was filtered while it was hot.5mL of $CHCl_3$ was added to filtrate and then shaked well. In a new test tube the chloroform layer was pipette out and 1mL of dilute ammonia was added. The presence of Anthraquinone was indicated through the color changes of solution.
Anthraquinone glycosides.

To the test solution added 5% of $FeCl_3$ 5mL of dil. HCl. Heated for a minute on water bath , cooled and added benzene, shaked well, separated the organic layer, equal volume of ammonia was added, it turn pink or red.

*Carbohydrates*

To 2-3ml of ethanolic extract, added alpha-nepthol solution, shaked well and then added conc. $H_2SO_4$. The presence of carbohydrates was indicated through formation of Violet ring at the union of two liquids.

Protein.

5ml million's reagent was mixed with 3ml of extract due to which white ppt formed. The ppt turned brick red and by dissolving gave red color solution.

*Quantitative analysis*

Harborne (1973) method for determination of Alkaloid.

200ml of 10% acetic acid in ethanol and the sample of about five grams were taken in a beaker then enclosed it and were left for 4 hours. Through Whattman filter paper it was filtered and the filtrate was reduced to one quarter of the original volume. To this concentrated extract conc. ammonium hydroxide was added drop wise waiting for ppt was finished. Final extract was left for some time to settle down and the ppt was collected as total alkaloid. The collected ppt were washed with dilute ammonium hydroxide and filtered. After that it was dehydrated and weighed to obtain the quantity of alkaloids.

*Determination of Saponins*

Obadoni and Ochuko *et al.*, 2001) procedure was followed for quantitative determination the saponins. In a conical flask 100 mL of 20% aqueous ethanol and 20g of sample were added. About 4 hour the sample was warmed on a hot water bath at about 55°C with nonstop stirring. After filtration with 200 mL 20% ethanol the residue was re-extracted. Collective extracts were concentrated on water bath at about 90°C up to 40 ml. The concentrated extract was transferred to the separating funnel and then 20 ml of diethyl ether was added to it and shaked well.

The ether layer was leftover although aqueous layer was recovered. The purification process was done 3-4 times. Then n-butanol about 60ml was also added. The extract was washed with 10 ml of 5% aqueous NaCl two times. This solution was heated on a water bath for complete evaporation .The sample was dried in oven to have constant weight.

*Method of Bohm and Kocipai-Abyazan (1994) used for Flavonoid determination*

With 100 ml of 80% aqueous methanol the plant sample about 10g was extracted repeatedly. Then filtered it, after filtration the filtrate was transferred into crucible. It was dehydrated on a water bath for constant weighed.

*Antimicrobial activity*

*Test organisms*

All the bacterial and fungal strain was obtained from Sigma Aldrich. Two strains of gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, one strain of gram positive bacteria and two strains of fungus *Aspergillus Niger* and *Aspergillus Flavus* were selected for this study.

*Preparation of inoculums*

Different test microbes were transferred with the help of sterile inoculation loop to the 5ml sterile nutrient broth by adjusting McFarland standard to 0.5 ($10^8$cfu/ml) and then incubated at 37 °C for 24 hrs.

*Agar well diffusion method*

Agar well diffusion method was used to test the antimicrobial activity of the leaves of Typha angustata as described by Perez *et al.* [13] The bacterial and fungal cultures were inoculated by spreading method into nutrient agar in Petri dishes by adjusting McFarland standard to 0.5($10^8$cfu/ml). All the media plates were prepared and after solidification, the well was cut from each medium with the help of borer. Each well was filled with 50µl of sample to check its activity and 30 µl of standard was used as a control. Leave these agar plates for 15 to 20 minutes at room temperature then incubate these plates at 37°C for 24 hours in incubator. Next day all the plates were checked for zone of inhibition and its diameter was measured in millimeter.

*Essential element analysis*

First of all about 10g of powdered form of sample was taken in crucible. This sample was converted into ash form by igniting it for about three hours at 550-600°C. Then the sample in ash form was shifted to the beaker. Ten ml of nitric acid (conc.) was added to the sample. The beaker having reaction mixture was heated at about 120°C till 2 ml of acids left. Then at last distilled water was added to it and filtered to get the volume about 100ml. The solution was then analyzed using atomic absorption spectrophotometer.

**Results and discussion**

*Qualitative analysis*

The quantity of different constituents of the same plant is different in different solvent system which is totally dependent on solubility of different constituents in different solvents. The polarity and nature of phytochemical of different plants are also different from each other and that is the main factor in isolating these constituents from plant source. Due to different climatic conditions the same plant grown in different areas also differ in phytochemicals. In current study percentage, extractive value of leaves of *Typha Angustata* were determined in ethanol.

**Table 1.** Results of qualitative phytochemical screening of leaves of *Typha Angustata*.

| S.No | Phytochemical constituents | Ethanolic extract of leaves |
|---|---|---|
| 1 | Alkaloids | +ve |
| 2 | Flavonoids | +ve |
| 3 | Steroids | -ive |
| 4 | Saponins | +ve |
| 5 | Tannins | +ve |
| 6 | Reducing Sugar | +ve |
| 7 | Terpenoids | +ve |
| 8 | Anthraquinone | +ve |
| 9 | Anthraquinone Glycosides | +ve |
| 10 | Cardiac Glycosides | -ive |
| 11 | Phlobotannins | +ve |
| 12 | Carbohydrates | +ve |
| 13 | Protein | +ve |

This study revealed the presence of numerous phytonutrients as very important and active medicinal constituents of plants Leaves of *Typha angustata* and tells us about the medicinal importance of the mentioned plant leaves. The leaves show the presence of alkaloids, tannins, saponin, reducing sugars, anthraquinone, terpenoids, flavonoids, phlobotannins and glycosides while steroids and cardiac glycosides were found absent as shown in table 1. Quantitative analysis of the plant leaves showed that it has a measureable quantity of some of the important phytochemicals. The flavonoid content in the ethanolic extract of the leaves was found to be 0.030% and alkaloid and saponin was found to be 0.029 and 0.25% respectively as shown in table 2. The above discussion revealed that the leaves of *Typha angustata* are very rich with phytochemicals contains many medicinally important phytochemicals in it.

**Table 2.** Result of quantitative analysis of leaves of *Typha angustata*.

| Sl. No. | Phytochemical constituents | Yield (%) |
|---|---|---|
| 1 | Flavonoids | 0.030 |
| 2 | Alkaloids | 0.029 |
| 3 | Saponin | 0.250 |

*Antimicrobial activity*

Antimicrobial activity of the leaves of *Typha angustata* against different bacterial and fungal strains is summarized in Table 4. The results revealed good inhibitory effect when compared with different standards. The zone of inhibition of *Staphylococcus aureus* was maximum (10mm) similarly *Escherichia coli* and *Pseudomonas aeruginosa* zone of inhibition was (98±0.23mm) and (7±0.11mm) respectively. The ethanolic extract of *Typha angustata* shows interesting sensitivity against the fungal strains i.e. *aspergillus Flavus shows* lowest inhibitory activity and *zone of inhibition* is (06±0.3mm) whereas *aspergillus Niger* was not susceptible to the ethanolic extract of *Typha angustata* as shown in figure 2, 3 and table 4.

**Table 3.** Quantitative analysis of essential elements *Typha angustata*.

| Sample ID | Cu | Pb | Ni | Cr | Zn | Fe | Mn | Na | K | Ca |
|---|---|---|---|---|---|---|---|---|---|---|
| Mg/Kg or %age | 9.4 | 5.8 | 6.2 | 4.8 | 48.2 | 150.0 | 248.8 | 0.2% | 0.25% | 1.3% |

**Table 4.** Antibacterial and antifungal activity of leaves leaves of *Typha angustata* in paper disc method. Diameter of Zone of inhibition (mm).

| S. No | Strains | Ethanol Sample extract 50µg/disc (mm) | - Standard Nilidixic acid Fluconazole 30µg/disc and 25µg/disc (mm) |
|---|---|---|---|
| 1 | Escherichia coli | 8±0.23 | 13±0.05 |
| 2 | Staphylococcus aureus | 10±0.35 | 12±0.35 |
| 3 | Pseudomonas aeruginosa | 7±0.11 | 13±0.32 |
| 4 | Aspergillus Niger | 00 | 15±0.21 |
| 5 | Aspergillus Flavus | 06±0.3 | 14±0.11 |

The results have shown that this plant could be used as a drug to cure different diseases caused by different strains. In the present study the local medicinally used plant *Typha angustata* extract shows interesting activity against the gram positive pathogen of *Staphylococcus aureus* which is the major cause of the infection of upper respiratory tract. For this reason our study would help in copping the upper respiratory tract problems and more solely solved more complicated problems by isolating individual components of the plants extract. Similarly it also shows more effectiveness against gram negative pathogen i.e. *Escherichia coli*, which are the major cause of stomach problems, intestinal as well as lungs inflamations.so the current results of the plant tested will be helpful in future by designing more medical formulations against such infectious diseases.

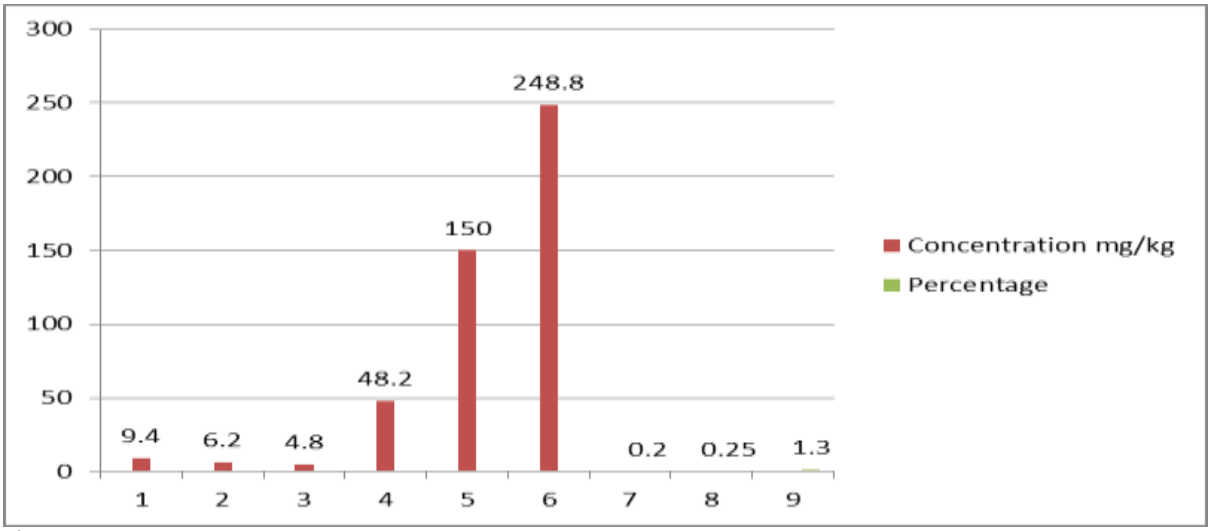


**Fig. 1.** Quantitative analysis of essential elements.

*Essential element analysis by AAS*

Different essential elements were quantitatively analyzed through AAS. The results of the leave extracts of *Typha angustata* has shown that the Cu, Ni, Cr, Zn, Fe, Mn, Na, K, Ca were present in different quantity. The concentration of selected elements has shown in table No 4.The results has shown that Mn is present in high quantity (248.8) in selected sample of *Typha angustata*. These all elements are mainly responsible for the growth of living things and treatment of many infectious diseases. These all the elements are helpful in the formation of secondary metabolites which are responsible for pharmacological actions in remedial plants.

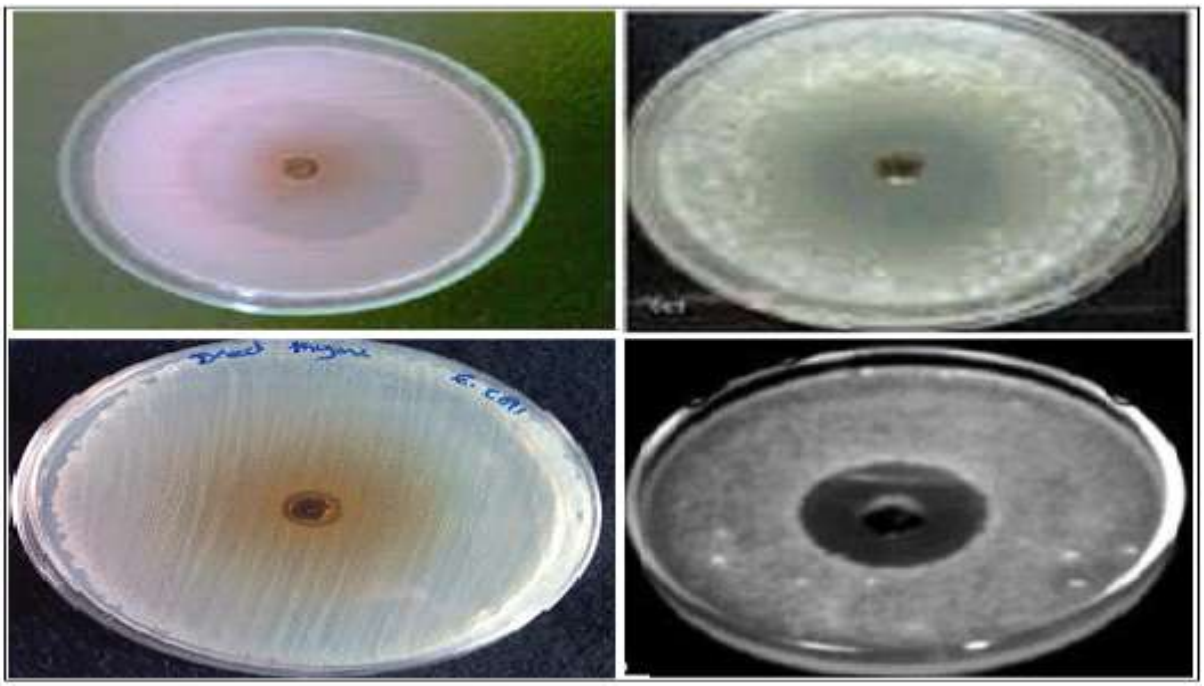

**Fig. 2.** Zone of inhibition against *S. aureus, P. Aeruginosa, E. coli and Aspergillus Flavus.*

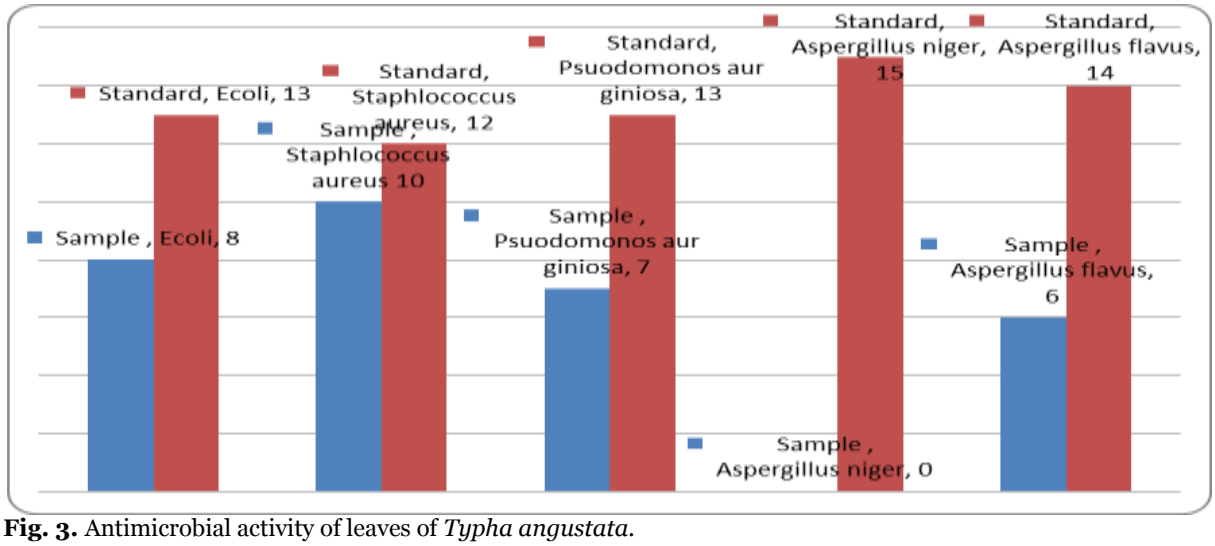


**Fig. 3.** Antimicrobial activity of leaves of *Typha angustata.*

**Conclusion**

The leaves of *Typha Angustata* shown the presence of different phytochemicals constituents so that its leaves can be used for the treatment of different diseases and can be used in drugs to give its medicinal effect. The results of bio assay suggest that the leaves of *Typha angustata* also show antimicrobial potential against different microorganisms and could be used

as natural antibiotic drugs. Leaves of *Typha Angustata* were found to accumulate a concentration of the elements Ca, Cu, Ni, Cr, Zn, Fe, Mn, Na, and K in different quantities. So it can be concluded that leaves of *T. angustata* is an important source of active ingredient having inhibitory effect and also a source of essential element so that it could be used in different drugs. Thus the phytochemical and antimicrobial analysis of medicinal plants are very crucial and have commercial interest in both research institutes and pharmaceutical companies for the synthesis of new drugs for the treatment of many diaseses.Thus we hope that important phytochemical and antimicrobial properties identifies of the *Typha Angustata* plants obtained from district Peshawar will help us in copping various diseases of our region.

**Acknowledgment**

We are thankful to Higher Education Commission of Pakistan and PCSIR Laboratories Complex Peshawar for providing us antimicrobial activities and essential element analysis facilities.

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