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Phytochemical Screening and Quantitative Biochemical Assessment of Acacia Nilotica (Flowers and Leaves)

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Abstract: Acacia nilotica tree has multipurpose benefits known as the most exclusive source of life saving and herbal drugs worldwide. This plant is significant due to presence of useful natural organic groups like alkaloids, flavonoids, Tannins, phenols, Tannis, Saponins, proteins, carbohydrates and amino acids. The present study aim is to identify the presence of secondary metabolites like alkaloids, flavonoids, Tannins, phenols, Tannis, Saponins, proteins, carbohydrates and amino acids by ethanol extracts methods from local Acacia nilotica. The extract results revealed that aqueous extract of flowers showed maximum concentration of total protein (16.8mg/g) compared to leaves (3.2mg/g) whereas flowers of Acacia showed more concentration of carbohydrates i-e 80mg/g as compared to leaves (60mg/g). The present study also confirmed presence of alkaloids, flavonoids, Tannins, phenols, Tannis, Saponins and amino acids, Along heavy metals like Nickle (Ni),Chromium(Cr),Zinc(Zn),Cobalt(Co),Cadium(Cd),Copper(Cu),Manganese(Mn)and Iron(Fe). The most perilous metal like Fe (3.97ppm), Cd (below detection limit) and Mn (0.06ppm) were detected within the permissible limit in acacia flower. Concentration of other metals like Zn, Ni, Co, Fe and Cr found in acacia flower i-e 973ppb, 138ppb, 35ppb, 3.97ppm and 259ppb respectively. Thus phytochemical study of Acacia Nilotica indicates that the flowers and leaves are potential source of proteins and carbohydrate and rich in phytochemicals among both extracts but due to bioaccumulation of heavy metals in acacia, the plant usage should be limited as herbal medicines because it may leave hazardous effects upon human health.

Keywords: Acacia Nilotica, Phytochemicals, Screening, Assessment, Biochemical.

1. INTRODUCTION

This legume tree occurs in Asia, Australia, Africa and many parts of the world commonly. Almost all parts of Acacia nilotica like bark, root, gum, leaves and flowers have been used for diabetes, eczema, cough, astringent, dysentery and multipurpose diseases. This polyphenols plant contains many natural aromatic compounds including flavonoids, Tannis, phenols and acids (Sapna *et al.*, 2011; New, 1984).

Acacia plant tree belongs to Leguminosae with bright yellow flowers and bi pinnate leaves (5-7.5cm long). The other parts of plant are having tannis (gallic acid,tannic acid,ellagic acid), vitamin-C, Stearic acid, calcium, Selenium and Magnesium (Swain, 1965; Basu, *et al.*,1965; Bargali and Bargalis, 2009).

The tender leaves and pods of Acacia nilotica are considered very useful for diabetes and diarrhea treatment. The plant Carbohydrates, proteins and phenolic compounds are beneficial in reducing the syndrome of orthopnea and hypertensive dyspnea by decreasing cardiac workload and blood pressure (Gilani *et al.*, 1999). Even twigs are used as tooth brushes and bark for bronchitis, cold, bleeding piles and leukoderma (Del, 2009). This could be used as antimicrobial agents for dental caries prevention and loss of teeth against streptococcus mutants (Deshpande and Kadam, 2013)

The aim of the conceptualizing this present study was to find out phytochemical presence in local Acacia nilotica in flowers and leaves grown in Jamshoro, Sindh, Pakistan, comparative study were done in both leaves and flowers for presence of protein, carbohydrates and phenolic compounds.

2. MATERIALS AND METHODS

Sample Collection. Samples of Acacia (Flowers and leaves) were collected in the month of October 2014 from the premises of the Institute of Biochemistry, University of Sindh, Jamshoro.

Plant extract Preparation. Samples were brought to laboratory for experimental procedure and fresh flower and leaves were washed first with tape water and than washing was done with the double distilled water to remove dust and dirt properly. After washing samples was let to dry at room temperature and after 10-15 days the dried plant (flower and leaves) were ground into fine powder (Harborne,1983), this plant powder were preserved in air tight bags for further analysis.

Sample Extraction. Sample extract was prepared in two solvents one in concentrated ethanol and other in distilled water by dissolving 10 grams of powdered sample and kept for 48 hours for ample extraction. Both extract were filtrate through Whatman no: 1 filter paper and stored at 4^{O}C for phytochemical analysis.

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Phytochemical analysis. The phytochemical screening of the Acacia nilotica plant was carried out by following methods with extra pure chemicals and reagents.

1. Test for Alkaloids

Hager's Test. 2ml of plant extract was added in 1ml of picric acid and presence of alkaloid were confirmed by the formation of yellow color precipitates (Lanjwani *et al.*, 2015).

Wagner's Test. 2ml of plant extract (both flowers and leaves separately) was mixed with 1ml of Wagner's reagent and formation of brown reddish precipitates indicates presence of alkaloids (Wagner, 1993).

2. Test for Phenols

Ferric Chloride Test. Few drops of $FeCl_3$ was added in 2 ml of plant extract and blue green or red color indicates positive test for phenol (Mace, 1963).

Lead acetate Test. In 2ml of plant extract few drops of lead acetate was added and vigoursly shaken. A brownish precipitates indicates the phenol test positivity (Mace, 1963).

3.Test for Saponins.

Foam Test. 2ml of plant extract and 4ml of distilled water was dissolved and shaken vigorously. Formation of foam indicates the presence of saponins (Kokate, 1999).

4.Test for Carbohydrates

Benedict's Test. Reagent preparation: Sodium citrate and sodium carbonate was dissolved in distilled water and boil till clear, copper sulphate ($CuSO_4$) was dissolved in 100ml of distilled water (Lanjawani *et al.*, 2015).

Method: 2ml of extract and 4ml of Benedict reagent were mixed. Boil the mixture for about two minutes, until the red precipitates appears which indicates the presence of reducing agent.

Molisch's Test: 2ml of extract and 1ml of Molisch's reagent was mixed thoroughly. A violet color ring appears between the two layers that confirmed the presence of carbohydrates (Lanjawani *et al.*, 2015).

5.Test for Amino acids and Proteins

Ninhydrin test. 1ml extract and 1ml of ninhydrin reagent was dissolved and kept in water bath. Blue color appears and indicates presence of proteins (Yasuma and Ichikawa, 1953).

6. Flavonoids Test.

Sodium Hydroxide (NaOH) Test: 1ml of extract and 1ml of NaOH were mixed in test tube and shaken vigorously. Formation of dirty yellowish brown precipitates test positive for Flavonoids (Mace, 1963).

7. Xanthoprotein Test. In 2ml of extract 1ml HNO_3 (nitric acid) was added and kept in water bath and then Sulphric acid (H_2SO_4) was added, until the orange color appeared.

8. Terpenoids Test. 1ml of the plant extract was mixed in 2ml of chloroform and 1ml concentrated H_2SO_4 was added to form a layer. A reddish brown colour of the interface was formed to show the presence of terpenoids.

9. Glycoside Test. 2ml plant extract was taken and 1ml of concentrated H_2SO_4 and 2ml Glacial acetic acid and 1 drop of $FeCl_3$ was mixed, until the brown ring appeared (Lanjawani *et al.*, 2015)..

Phytochemical Quantitative analysis of Acacia nilotica:

The phytochemical quantitative screening of the plant extract (leaves and flowers separately) was carried out by following methods.

Estimation of Carbohydrates by Anthrone Method.

Extraction of Sample: 100 mg of powdered sample and 5 ml of 2.5 N Hydrochloric acid (HCl) was added and kept in boiling water bath for one hour. After cooling at room temperature neutralized it by Sodium carbonate (Na2CO3), until the effervescence stop. Diluted this with 100ml of distilled water and centrifuged to get supernatant.

Method: About 0.5ml of the supernatant filtrate was taken and 4ml of anthrone reagent was added to each tube. Kept in a boiling water bath for 8-10minutes. Rapidly cool and measure the absorbance against blank at 630 nm (Hodge and Hofrieter, 1962). The standard curve was prepared ranges (0.2, 0.4, 0.6, 0.8, 1.0) ug/ml for calibration curve.

Estimation of Phenolic compound. Extraction of sample: 1gm of powdered plant sample and 50ml of 80% methanol was mixed and incubated at 37^{O}C and kept on shaking water bath for 1hour. Then filtered was collected.

Folin Reagent.

Gallic acid (standard): 1000 µg of Gallic acid was dissolved in 80% methanol.

Method: 1.0 ml of plant extract was taken and 1ml of Folin reagent was added, then 2ml of Sodium carbonate (Na_2CO_3) mixed and incubated for about 1hour to measured absorbance at 660nm by Folin Cioacaltea method (Kim *et al.*, 2003).

Estimation of proteins. Sample extraction: 1gm of powdered sample (leaves and flowers both) dissolved in 10ml buffer. Bovine Serum albumin (BSA) 2mg/ml in distilled water was prepared as standard. 0.1ml of extract (leaves and flowers both separately) and 0.1ml of NaOH was taken in test tube. Boiled for 10minutes in water bath and diluted in distilled water up to 2ml by

adding 1ml reagent. After 10minutes keeping at room temperature 0.1ml Folin reagent was added and measure the absorbance at 550nm after 50 minutes of incubation time.

Acid Digestion method for heavy metals detection. Acid digestion method was used to digest the dried plant powder 500mg (acacia flowers) for mineral analysis (Hina *et al,* 2011). After complete digestion the sample was cooled, diluted with distilled water up to final volume of 25 ml. The estimation of important heavy metals i.e. Ni, Cr, Zn, Co, Cd, Cu, Fe and Mn was done on Perkin Elmer's Atomic absorption spectrometer Analyst 800.

Table.1. The qualitative analysis of aqueous and ethanolic extracts for both leaves and flowers

Test	Aqueous leaves Extract	Ethanolic leaves extract	Aqueous flower extracts	Ethanolic flower extract	Color observation
1.Test for alkaloids:					
Wagner's Test	_	_	+	_	Brown red ppts
Hager's Test					Yellow ppts
2.Test for Phenolic compounds and Tannis					
Ferric Chloride Test	+++	+++	+++	+++	Red color
Lead acetate Test	++++	++++	++++	++++	Brown ppts
3.Test for Flavonoids					
Sodium hydroxide Test	_	++	++	++	Yellow brown ppt
4.Test for Saponins Foam Test	++	+	+++	++	Foam appears
5.Test for Proteins					
Ninhydrin Test	++++	++++	++++	++++	Blue color
Test	Aqueous leaves Extract	Ethanolic leaves extract	Aqueous flower extracts	Ethanolic flower extract	Color observation
6.Test for Carbohydrates					
a. Molish's Test	++++	++	++++	++	Violet ring
b.benedict Test	+++	++	++++	++++	Red ppts
7.Test for Amino Acid					
Xanthoprotein test	+++	+++	+++	+++	Orange colour
8.Test for fixed oils and Fats					
a. Terpenoids	++	_	++	++	Reddish brown
b. Glycoside Test	++	+	++	+++	Brown ring

(Note: + present**,** ++ prominent,+++ most prominent,- means absent and ppt means precipitates)

3. RESULTS AND DISCUSSION

Table-1 shows the phytochemical screening of leaves and flowers extracts (aqueous and ethanolic) of Acacia nilotica. Both extracts contained abundant amount of protein, amino acid, carbohydrates, phenolic compounds, tannis and saponins and flavonoids were also present. Whereas alkaloids were present only in aqueous flower extracts, and were found absent in extracts of aqueous leaves extract. Fixed oils and fats were found prominently in all except ethanolic leaves extracts by Terpenoids test.

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The phytochemical quantitative determination of acacia leaves and flowers for presence of proteins, carbohydrates and phenolic compounds are given in table-2. The present quantitative determination of total carbohydrates, total proteins and total phenolic compounds indicates Acacia nilotica flowers have greater amount of these three components compare to leaves. Although, previous studies showed that this plant is a rich source of biomolecules and important medicinal properties and it has protective effects against cancer and other serious ailments (Meena *et al*., 2006). Acacia nilotica leaves and flower extracts may contain valuable safe therapeutic agents including alkaloids, tannins and glycosides and acting as antimicrobial activity against microorganisms (Adebajo,1983). Many research studies on Acacia nilotica reported that this plant is rich source of natural antioxidants and is capable against free radical diseases like diabetes, cancer and inflammation (Amos,1999).

Table.2. Phytochemical quantitative determination of acacia leaves and flowers

Part of Plant Acacia nilotica	Carbohydrates	Total phenol content	Protein
Leaves	60 mg/g	10 mg/g	3.2 mg/g
Flowers	80mg/g	12.5 mg/g	16.8mg/g

Table-3 shows determination of trace heavy metals of acacia flower extract assessment of results indicates that only Manganese (0.06ppm) and Cadmium values were detected below the detection limit and these value

were within the acceptable limit for raw herbal medicines by WHO standards (WHO, 2007). Nickle was found in level of 138ppb and Ni is required for many biological processes including for coordination by proteins whereas EPA has recommended Ni should be less than 1 mg beyond which Ni is known to be toxic (McGrath and Smith, 1990). Cobalt was found in concentration i-e 35ppb, Co is well known as vital component of Vitamin B-12. The other essential metal part of proteins in plants is Zinc (Zn) and was found 973ppb which may become toxic, because acceptable limit of Zn in medicinal plants is 100 ppm (Jones, 1987). Iron (Fe) concentration was recorded 3.97ppm and value was within the permissible limits as 10-60mg of iron intake in a day was recommended (Kaplan *et al*., 1993). Chromium (Cr) was noted 259 ppb in acacia flower whereas US National Academy of Sciences has recommended daily intake of Cr 50-200 µg (Waston, 1993). Although, copper concentration (162ppm) is higher in flower although recommended limit of Cu is20 µg/mg body weight per day (FDA, 1993 .

Table.3. Concentration of Heavy metals in flower acacia nilotica

Element	Zn (ppb)	Ni (ppb)	Co (ppb)	Cd (ppb)	Cr (ppb)	Cu (ppm)	Mn (ppm)	Fe (ppm)
Concentration	973	138	35	BDL	259	162	0.06	3.97

***BDL= Below detection limit**

4. CONCLUSION

The present study revealed that the phytochemical screening and biochemical assessment proved that the Acacia nilotica flowers and leaves could be useful for low cost medicine, but bioaccumulation of heavy metals in medicinal plants like acacia is increasing due to environmental pollution and urbanization. Although, the herbal drugs from medicinal plants are extensively used but may cause hazardous effects upon humans. Therefore, the quality of herbal medicines need heavy metals assessment before producing safe drugs in future.

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