INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 6, p. 78-84, 2018

RESEARCH PAPER OPEN ACCESS

Qualitative and quantitative evaluation of tannins in bark extracts of some indigenous plants of Pakistan

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Key words: Tannins, Indigenous plants, Bark extracts, Polyphenols, Non-tannins.

http://dx.doi.org/10.12692/ijb/12.6.78-84 Article published on June 14, 2018

Abstract

Plant barks contain more tannin than any other plant part. In the present study two extraction techniques were applied to extract tannins from bark of some indeginous plants of Pakistan. The tannins were extracted from the barks of plants by maceration & ultrasonic extracion. Folin-Ciocalteu method was conducted to measure the total phenolic content in bark extracts of some plants. Polyvinylpyrrolidone (PVPP) solution was used to bind the tannins, so that the non-tannins (NT) content value can be detected. Thus, the total tannins (TT) content was measured. Qualitative tests for the determination of the presence of polyphenols/tannins were also conducted. It was seen that highest tannin content was present in plant *Cassia fistula* whereas the lowest tannin content was present in *Quercus dilatate*.

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Introduction

Almost all of the assays of polyphenolics and tannin content in current chemical Codexes and Pharmacopoeias are based on polyphenol-protein binding. For that purpose hide-powder and casein are normally used as protein substrates (Farmacopia Brasileira 1988; British Pharmacopoeia 1999; European Pharmacopoeia 2002; Soares *et al.* 2006). On a following step the assays are accomplished with a spectrophotometric quantitation by the Folin-Ciocalteu method (AOAC 1975; FAO/IAEA 1975). It was recently demonstrated that methods using hide-powder and casein are non-specific when flavonoids are also present in the reaction milieu (Soares *et al.*, 2006; Verza *et al.*, 2007).

The capacity of insoluble cross-linked povidone (PVPP) to bind polyphenols arises in this context as a seldom explored analytical alternative (Soares *et al.*, 2006; Horn *et al.*, 1982; Makkar *et al.*, 1995). One example of this is the FAO/IAEA method for the *Quantification of Tannins in Tree Foliage* Monograph (FAO/IAEA 1975).

Materials and methods

Collection of plant material

Plant barks of fifty plants were collected from the selected areas of Northern & Central Punjab by using the criteria viz. easy approachable, abundant available, cosmopolitan, easy to grow and easy to maintain.

Extraction of plant material

Two extraction techniques *i.e.*, maceration and ultrasonic extraction was used for the extraction of plant material (Sarkar *et al.*, 2005). Extraction of tannins was followed by the concentration and spray drying to get powder extract. (Musa and Gasmelseed 2012).

Extraction of tannins

All plant samples (bark) were dried under shade to remove excess moisture. The over drying of samples was done for two days. Dried samples of plants were grounded into a fine powder and placed in air tight jar for further use.

Maceration:5.0gm of powder sample was mixed with 250ml of ethanol in a beaker. The prepared solution was macerated under room temperature for 48 hours with agitation at rate of 360 rpm. After required time in the solution was filtrated under vacuum and concentrated by a rotary evaporator under 40°C and a low pressure. Finally extracts obtained were stored in the refrigerator for further analysis (Bandar *et al.,* 2013).

Ultrasound-assisted Extraction (UAE): In Ultrasound-assisted extraction, 5.0 g of bark was mixed with 200 ml of methanol as extracting solvent in a 500 ml of beaker. The beaker was immersed in ultrasound cleaning bath at 40°C.

The amplitude, time of extraction and frequency were set accordingly. Temperature of sample was continuously controlled manually by using water bath. The solution was then filtered. The solution was then mixed with 70% methanol/ethanol for different samples. Centrifuged and supernatant was collected for further use.

Qualitative test for the determination of the presence of polyphenols/tannins:

Phytochemical analysis of plant bark extracts.

Following test were performed to check the presence of tannins.

Ferric chloride test: To 3 ml of extract, 3 ml of 5% w/v ferric chloride solution was added. The blue – black colour indicated the presence of tannins and phenols (Ukoha *et al.* 2011).

Lead acetate test: To 3ml of extract, 3 ml of lead acetate solution was added. The occurrence of white precipitates indicated the presence of tannins and phenols (Ukoha *et al.* 2011).

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of

white precipitate indicated the presence of tannins.

Confirmation of tannins

Two tests were performed for the confirmation of tannins.

Match stick test: A match stick is dipped in aqueous plant extract, dried near burner and moistened with concentrated hydrochloric acid. On warming near flame, the matchstick wood turns pink or red due to formation of phloroglucinol.

Potassium Dichromate test: If on an addition of a solution of potassium dichromate in test filtrate, dark color is developed, tannins are present.

Quantification of tannins/phenolics content

The method for total phenol is useful in order to know the efficiency of extraction of phenolics in solvents. This method can be coupled with the use of insoluble matrix, polyvinyl polypyrrolidone (PVPP; binds tannin-phenolics) for measurement of tannins. The results can be expressed as tannic acid equivalent. The nature of tannic acid varies from one commercial source to the other.

Total tannin content in each plant extract was determined by using Folin-Ciocalteau method (Sadasivam and Manickam, 2008; Makkar *et al.* 1993).

Analysis of total phenols

To calculate the tannin and non-tannin content in the plant sample, following procedure was adopted. 50 µml tanning containing extract of plant sample was transferred with micropipette into a labelled test tube; 0.25ml Folin- Ciocaltue reagent and 1.25ml sodium carbonate solution was added in it. All tubes were placed in the Vortex for five minutes and then the tubes were kept at room temperature for 40 minutes. The absorbance of sample was recorded at 725nm with the help of spectrophotometer (UV-2800) Hitachi.

Removal of tannins from extract

PVPP function to binds tannins. 100mg PVPP was taken in a test tube. 1.0 ml distilled water and 1.0 ml tannin-containing extract were added to it. Vortex it and then placed the tube at 4 ˚C for 15 min, again vortex it and centrifuged (3000rpm for 10 min) and the supernatant was collected. Phenolic content of the supernatant was calculated (took at least double the volume used for total phenol). Measured the Non-tannin content of phenols on dry matter (y%).

Estimation of extraction yield of tannin

The dried bark material was extracted with extracting solvent at room temperature by maceration & ultrasonic extraction methods. After removing residues by filtration, methanol extract was concentrated on rotary evaporator and water extract was dried using spray dryer.

The amount of product which was obtained by drying was divided by weight of original sample and the extraction yield was calculated (Tonon *et al.,* 2008*).*

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the spray -dried extract x 100}}{\text{Weight of the original sample}}$$

Results and discussion

In order to determine the tannin contents in bark extracts of plants, qualitative and quantitative tests are carried out.

Table 1. List of collected plants.

Sr.No.	Family	Botanical name of plant/Local name
1.	Pinacae	*Pinus roxburghii* *Pinus wallichiana* *Abies pindrow* *Cedrus deodara*
2.	Myrtaceae	*Eucalyptus globulus* *Callistemon citrinus* (Bottle Brush) *Eugenia jambolana* (jamun) *Psidium guajava*

		Syzygium cumini
3.	Cupressaceae	*Cupressus funebris* *Taxodium mucronatum*
4.	Fabaceae	*Acacia niloctica* *Acacia modesta* *Crateva religiosa* *Dalbegria sisso* *Tarmindus indica* *Millettia ovalifolia* *Butea frondosa*
5.	Fagaceae	*Quercus dilatata* *Quercus incana*
6.	Araucariaceae	*Arucaria angustifolia*
7.	Mimosaceae	*Albizzia lebbek*
8.	Apocynaceae	*Alstonia scholaris*
9.	Caesalpiniaceae	*Bauhinia variegata* *Saraca declinata*
10.	Bombacaseae	*Bombax malabaricum*
11.	Caricaceae	*Carica papaya*
12.	Bignoniaceae	*Jacaranda mimosifolia* (Guleneelum) *Spathodea campanulata*
13.	Simaroubaceae	*Alianthus altissima* (Tree of Heaven)
14.	Combrataceae	*Angeissus acuminata*
15.	Rosaceae	*Eriobotrya japonica*
16.	Moraceae	*Ficus bangalensis* *Ficus infectoria* *Ficus elastic (rubber plant* *Morus alba*
17.	Meliaceae	*Melia azedarach*
18.	Rutaceae	*Murraya koenigii* (curry leaf)
19.	Anacardiaceae	*Magniferaindica*
20.	Salicaceae	*Populas alba*
21.	Phyllanthaceae	*Pyhllanthusemblica* (amla)
22.	Oxalidaceae	*Averrhoa carambola (star fruit)*
23.	Malvaceae	*Chorisia insignis* *Brachychiton rupestris*
24.	Rhamnaceae	*Ziziphus jujube*
25.	Euphorbiaceae	*Sapium sebiferum*
26.	Magnoliaceae	*Magnolia grandiflora*
27.	Juglandiaceae	*Juglans regia*

The confirmation tests for the presence of tannins and polyphenols showed the presence of these compounds in all the 10 different plant species selected for the study as seen in the table 2.

Table 2. Confirmation of tannins and phenols.

Sr.No.	Plant	Tannins	Polyphenols
1.	*Pinus roxburghii*	+++	+++
2.	*Pinus wallichiana*	+++	+++
3.	*Eucalyptus globulus*	+++	+++
4.	*Cassia fistula*	+++	+++
5.	*Quercus dilatata*	+++	+++
6.	*Melia azadirachta*	+++	+++
7.	*Acacia nilotica*	+++	+++
8.	*Abies pindrow*	+++	+++
9.	*Cedrus deodara*	+++	+++
10.	*Quercus incana*	+++	+++

*+++=Extremely present, ++=Moderately present, + = Present, ---- = Absent

Table 3. Calibration curve (Absorbance of tannic acid at 725nm).

Test Tube No.	Gallic acid 0.1mg/ml	Distilled water	Folin Reagent	Sodium carbonate solution	Tannic acid absorbance at 725nm	Gallic/Tannic acid
	M1	M1	M1	M1	Nm	µg
Blank	0.00	0.50	0.25	1.25	0.00	0.00
T1	0.02	0.48	0.25	1.25	0.08	2.0
T2	0.04	0.46	0.25	1.25	0.156	4.0
T3	0.06	0.44	0.25	1.25	0.23	6.0
T4	0.08	0.42	0.25	1.25	0.31	8.0
T5	0.10	0.40	0.25	1.25	0.38	10.0

Table 4. Percentage of extraction yield of selected plant samples.

Sr.No	Plant samples	Extraction yield (%)
1	*Acacia nilotica*	12%
2	*Cedrus deodar*	12%
3	*Cassia fistula*	23.3%
4	*Eucalyptus globulus*	18.6%
5	*Abies pindrow*	20%
6	*Quercus dilatata*	15%
7	*Quercus incana*	15%
8	*Melia azdarichta*	16%
9	*Pinus wallichiana*	25%
10	*Pinus roxburghii*	16.6%

The percentage of extraction yield was found maximum i.e. 23.3% in *cassia fistula* plant whereas the minimum extraction yield was seen in *Acacia nilotica* and *Cedrus deodar* which were 12% each as seen in table 4.

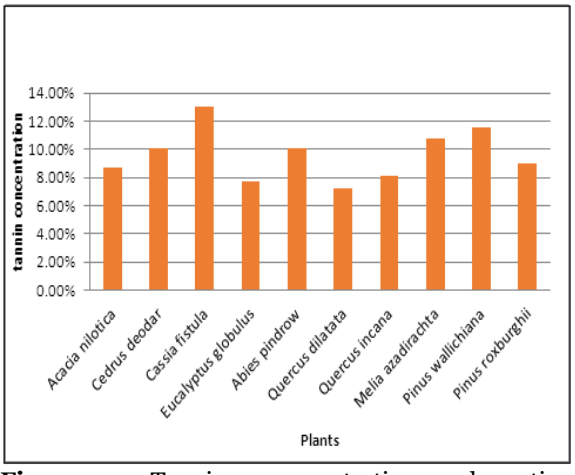

Fig. 1. Tannin concentration absorption Spectrophotometer at 725nm.

Tannin concentration of bark extracts of all the plants was determined using UV spectrophotometer.

The total tannin contents at 725 nm were found maximum in *Cassia fistula* as seen in figure 1.

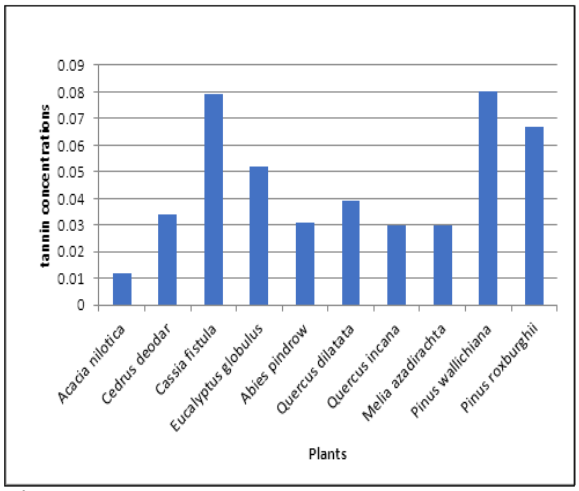

Fig. 2. Concentration of tannin absorption at 725nm after PVPP treatment.

On the other hand, concentration of tannin absorption at 725 nm after PVPP treatment was found maximum in *Pinus wallichiana* and minimum in *Acacia nilotica* as shown in figure 2. PVPP method is

considered to be a simple, inexpensive, rapid and efficient method and can be used to determine the presence of tannins and polyphenols in different plant materials.

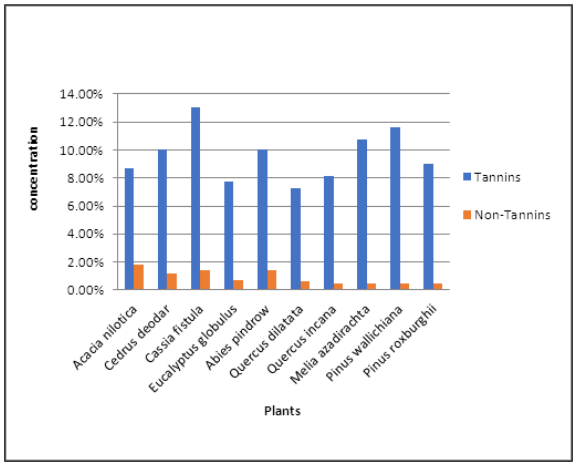

Fig. 3. Tannin and non-tannins content in plants.

It was seen that all the bark extracts contained higher concentration of tannins as compared to the non tannin contents. According to the figure 3, maximum tannin contents were found in *Cassia fistula* whereas, maximum non-tannin contents were found in *Acacia nilotica*.

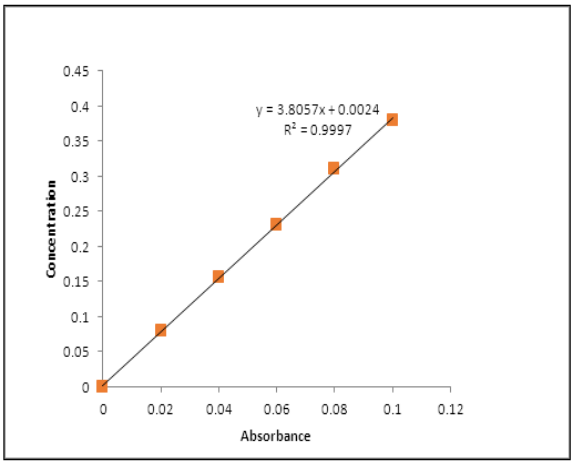

Fig. 4. Standard calibration curve.

The present study shows that relevant amount of tannins can be found in all the plants mentioned and further studies can be done to use them as an eco-friendly approach for various activities.

Acknowledgement

The authors thank Lahore College for Women University, Lahore for providing all the facilities to carry out this research.

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