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The content, profile and biological activity of tannins in some tanniniferous plants

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ABSTRACT

The present study was carried out on the quantification, chromatographic profiling and biological activity assessments of selected tanniniferous forages viz. leaves of oak/ban (Quercus leucotrichophora), robinia (Robinia pseudoacacia), khirk (Celtis australis), kachnar (Bauhinia variegata), siris (Albizia lebbeck), pakar (Ficus infectoria), tremal (Ficus roxburghii) and buince (Salix alba), and leaves and fruits of bhera (Terminalia bellerica) and harad (Terminalia chebula). The total tannin content was found high in harad and bhera fruits & leaves. Kachnar, robinia and pakar leaves were rich source of condensed tannins. However, hydrolysable tannins were the predominant component of the total tannin phenols of oak, harad and bhera leaves. The extraction of tannins was followed by TLC fingerprinting. TLC fingerprint analysis of selected plants showed remarkable difference in the overall complexity and the polarity of the tannins. Prospections were also carried out on the protein precipitating capacity and free radical scavenging activity of these tannins. The antioxidant activity of the plant samples was done using 1,1-Diphenyl-2-picryl-hydrazyl spray. The protein precipitation capacity and antioxidant activity – the major indicators of biological activity of tannins, suggested that a fine balance has to be struck between the beneficial and deleterious effects of these tannins by maintaining a control over their concentration vis-à-vis their intake by the animals.

INTRODUCTION

In the tropics and subtropics, forage consists mainly of grain and forage residue which is generally high in fiber and low in protein content. This results in reduced animal performance, particularly in the dry season. A great number of forage legumes, particularly the fodder trees, are available as supplement and are characterized by relatively high protein content digestibility as compared to grasses. However, many fodder trees have high content (up to 50% in DM) of secondary ingredients, particularly tannins [10], which may bind with the

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protein, thus rendering it unavailable to the animal and thereby having a negative effect on nutritional value.

Tannins form a highly diverse group of natural products with promising nutritional, veterinary and environmental effects [8]. The methods available to estimate total phenolics and tannins cannot be considered as predictive of their nutritive value. Mueller-Harvey et al. [7] have developed a chromatographic method which may be of use in predicting the nutritive value of tanniniferous forages. The study on the chromatographic analysis of tanniniferous feeds and forages could be of help in the development of their tannin fingerprints. These may be of use in developing protocols for safe and effective use of tanniniferous feeds in ruminant diets. The present studies were envisaged to develop appropriate feeding strategies for tanniniferous feeds and forages based on the profile and bioactivity of their tannin content.

MATERIALS AND METHODS

The tanniniferous trees whose leaves and fruits were used for the study were: leaves of oak/ban (*Quercus leucotrichophora*), robinia (*Robinia pseudoacacia*), khirk (*Celtis australis*), kachnar (*Bauhinia variegata*), siris (*Albizia lebbeck*), pakar (*Ficus infectoria*), tremal (*Ficus roxburghii*) and buince (*Salix alba*), and leaves and fruits of bhera (*Terminalia bellerica*) and harad (*Terminalia chebula*). The mature leaves and fruits of different tanniniferous trees were obtained during October to December 2013 from areas in and around Palampur (HP) and Bareilly (UP), and were dried at 37^0C. The leaves were ground to form powder of 2 mm particle size. The powder was used for extracting tannins by the method of Dhar et al. [3]. The filtrate obtained after extraction were used for tannin estimation. Total phenols (TP), non-tannin phenols (NTP), total tannin phenols (TTP), condensed tannins (CT) and hydrolysable tannins (HT) were estimated in freshly prepared extract of leaves and fruits by the method of Makkar [6]. The extraction of tannins was followed by removal of the solvent and lypholization of the aqueous solution to the powder form, and a TLC fingerprint analysis was done on aluminum plates coated with silica gel 60 [12]. The plates were detected with vanillin-sulfuric acid spray followed by heating at 110^0C for 5 and 15 min, or spraying with 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) reagent and visualizing after 10 min [11] for determining the correlation of tannin fingerprints with their biological activity. The interaction of tannins was studied with bovine serum albumin (BSA), a standard protein, for investigating their protein precipitation capacity [1]. Studies were also carried out to determine the free radical scavenging activity of tannins and their standard monomers catechin and epicatechin, which was evaluated by the methods of Kordali et al. [5] and Sharma and Bhat [11].

The percentage of DPPH free radical scavenging activity (% inhibition) was calculated with the help of following equation:

$$\% \text{ Inhibition} = \frac{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{test})} \times 100}{\text{Abs}_{(\text{control})}}$$

IC_{50} value (the amount of antioxidant necessary to decrease the initial DPPH free radical concentration by 50 per cent) was calculated from the regression line obtained from the plot of per cent inhibition against concentration of each solution using the following equation:

$$IC_{50} \text{ value} = \frac{(50 - \text{y intercept})}{\text{Slope}}$$

RESULTS

Extraction and determination of tannins from leaf and fruit samples

The tannin content of various plant samples of leaves and fruits given in Table 1. The total tannin content was high (>6%) in harad and bhera fruits & leaves, medium (3-6%) in oak, robinia, kachnar and pakar and low (<3%) in siris, khirk, tremal and buince. Kachnar, robinia, pakar, oak and tremal leaves were rich in Condense tannins (CT; 1.32-2.82 g%) while harad and bhera leaves & fruits, siris, buince and khirk leaves were poor in CT content (0.02-0.83 g %). However, hydrolysable

tannins whose content was negligible in khirk, siris, kachnar, pakar and buince leaves (0.16-0.49 g %), were comparatively high in bhera and harad fruits and leaves (5. 75-15.30 g %), and low in oak leaves (2. 86 g %)

Chromatographic fingerprinting of tannins obtained from various leaf and fruit samples

The TLC profile in a standardized solvent system of wattle tannins and tannin content of leaf samples obtained after partition chromatography is shown in Figure 1 and Figure 2. The band pattern of tannin content in all the samples was different. The heating of the TLC plate for 15 min at 110^0C resulted in the appearance and visualization of more number of bands as compared to TLC plates heated for 5 min at 110^0C.

Correlation of chromatographic profiles of tannins with their biological activity

The tannins of mature leaves of oak (*Q. leucotrichophora*), robinia (*R. pseudoacacia*), khirk (*C. australis*), kachnar (*B. variegata*), siris (*A. lebbeck*), pakar (*F. infectoria*), tremal (*F. roxburghii*), buince (*S. alba*), bhera (*T. bellerica*) and harad (*T. chebula*), and fruits of bhera (*T. bellerica*) and harad (*T. chebula*) were used in this study.

The thin layer chromatogram of tannin content of the mature leaves and fruits of these trees gave a variable band pattern (Figure 1, 2). The band pattern of tannins of each tree leaf was uniquely different from the tannin band pattern of other tree leaves and potentially could be used for identification of a tanniniferous tree. As is seen in the TLC plates, the condensed tannins do not seem to move from their origin. This is evident from the band patterns of harad and bhera fruits where no band is visible at the origin and which have negligible CT content of 0.04 and 0.08 g %, respectively (Table 1). The movement and position of their band patterns indicate the presence of hydrolysable tannins. However, the band patterns of other leaf extract tannins and wattle tannins indicate that they have both CT and hydrolysable tannins. These band patterns may be used to develop a unique TLC tannin fingerprint for each plant and for studies on correlation of thin-layer chromatographic profiles with biological activity.

Table 1: Tannin content (g percent dry matter) of the leaves and fruits of various plants

S. No.	Sample Name	TP	NTP	TTP	CT	HT
1.	Oak	5.03	0.86	4.18	1.32	2.86
2.	Robinia	4.38	1.32	3.06	2.64	0.42
3.	Khirk	1.07	0.66	0.41	0.14	0.27
4.	Kachnar	3.72	0.62	3.10	2.82	0.28
5.	Siris	1.09	0.91	0.18	0.02	0.16
6.	Bhera leaves	9.90	1.72	8.19	0.83	7.36
7.	Harad leaves	7.90	1.41	6.49	0.73	5.75
8.	Pakar	4.59	1.78	2.80	2.31	0.49
9.	Tremal	2.88	0.30	2.57	1.74	0.83
10.	Buince	1.33	0.90	0.42	0.18	0.24
11.	Harad fruit	16.86	1.54	15.33	0.04	15.30
12.	Bhera fruit	13.51	1.44	12.07	0.08	11.98

TP-total phenols; NTP-non-tannin phenols; TTP-total tannin phenols; CT-condensed tannins; HT-hydrolysable tannins

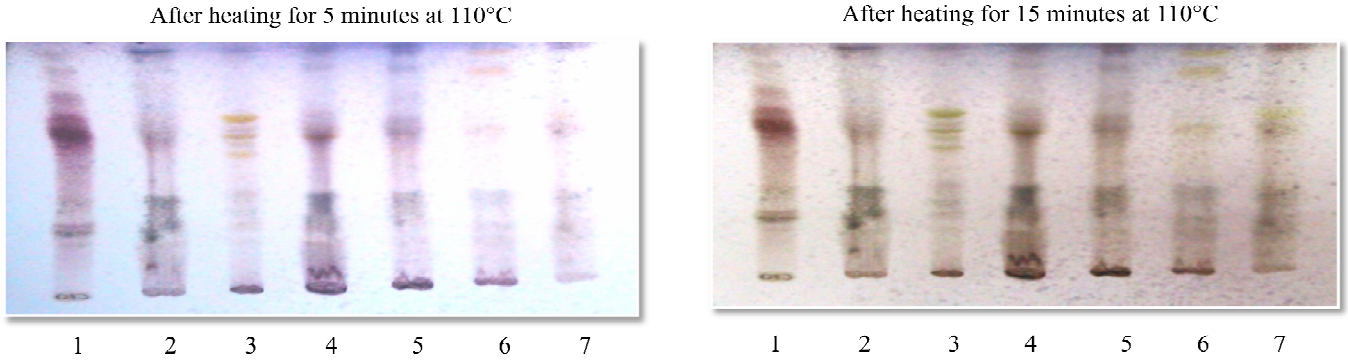

Figure 1: Thin layer chromatographic profile of standard wattle tannins and tannin extracts of different plants (1-7)
Standard: wattle tannins (50 mg/mL); Sample: 50 mg/mL each of the samples; Lane 1: Wattle tannins; Lane 5: Pakar; Lane 2: Oak; Lane 6: Tremal; Lane 3: Robinia; Lane 7: Buince; Lane 4: Kachnar; Solvent System: Acetonitrile: water: chloroform: formic acid (60: 15: 10: 5); *Detection: By Vanillin-H_2SO_4* spray

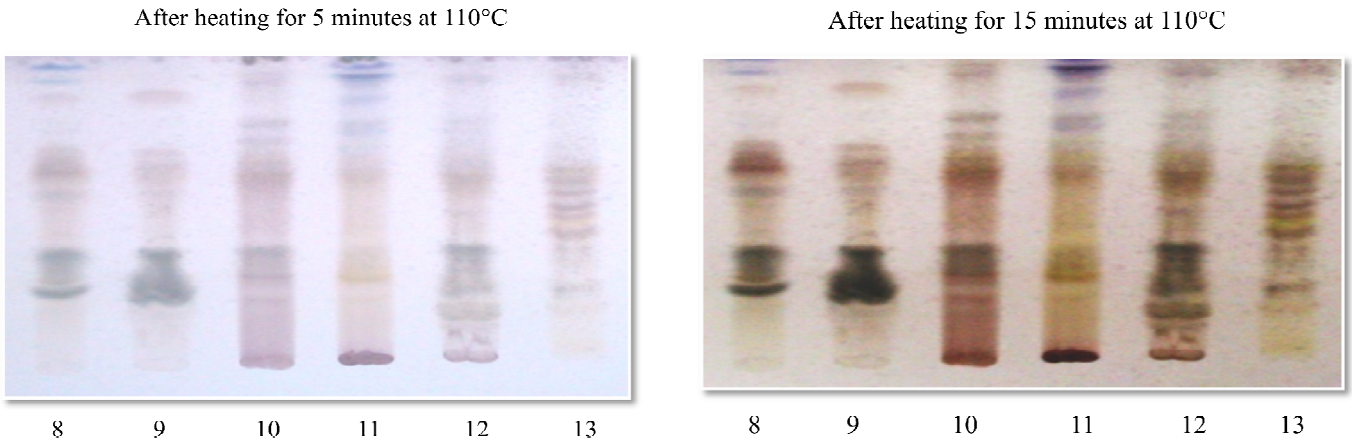

Figure 2: Thin layer chromatographic profile of tannin extracts of different plants (8-13); Lane 8: Harad fruit; Lane 11: Harad leaves; Lane 9: Bhera fruit; Lane 12: Khirk; Lane 10: Bhera leaves; Lane 13: Siris; Solvent System: Acetonitrile: water: chloroform: formic acid (60: 15: 10: 5); *Detection: By Vanillin-H_2SO_4* spray

Free radical scavenging activity

The harad, bhera fruit and leaves showed very high free radical scavenging activity because the percent inhibition of oxidation was observed to be very high at a very low concentration. On the other hand, khirk, buince and siris showed very low radical scavenging activity. A comparative picture of the free radical scavenging activity of all the leaf and fruit samples based on their IC_{50} value is summarized in Table 2. Antioxidant activity of tannins is based on their ability to donate hydrogen atoms to free radicals. The antioxidant and radical scavenging activity of harad, bhera was very high and that of khirk, siris and buince was very low. TLC fingerprint analysis of antioxidant activity of the plant samples in the standardized solvent system is shown in Figure 3.

Protein precipitating capacity

The protein precipitating capacity (PPC) of all the leaf and fruit samples is shown in Table 3. It ranged from negligible (0.002) in khirk and siris to high (0.661) in bhera fruit. The PPC of other leaf and fruit tannins ranged between these two values.

DISCUSSION

Tannins, because of their protein-binding properties, are known to be strongly astringent [4]. CT bind with protein by hydrogen bonding at near neutral pH (pH 4.0-7.0) to form CT-protein complexes, but dissociate and release protein at

Table 2: Antioxidant activity of the leaves and fruits of plant samples on the basis of IC^{50} values

S. No.	Sample Name	IC_{50} value (µg)
1	Oak	6.32
2	Robinia	11.28
3	Khirk	26.95
4	Kachnar	6.13
5	Siris	159.49
6	Bhera	2.86
7	Harad	2.66
8	Pakar	7.40
9	Tremal	10.82
10	Buince	37.65
11	Harad fruit	2.38
12	Bhera fruit	2.85

pH ~ 3. The protein precipitation by the CTs may be more responsive to the relative molecular mass of the CT, and to a lesser extent, affected by the chemical structure. Tannins inhibit the activity of enzymes of rumen microbes. CTs are known to inhibit several digestive enzymes, including proteases, pectinases, amylases, cellulases, and lipases [2]. Enzyme inhibition is believed to be caused mainly by non-specific binding of tannins with the enzyme protein, but may also occur when tannins bind with the substrate.

There are many factors which may influence the extent of digestive enzyme inhibition by tannins [8]. They way include : (a) amount of protein in the diet, (b) relative amounts of various enzymes in

the diet and the order in which they are encountered, (c) formation of tannin-protein complexes prior to and following ingestion, and (d) how various enzymes are affected by pH, type of tannin, and species and age of the animal. When tannins complex with protein in an animal's gut, they are believed to be responsible not only for growth depression, but also for low protein digestibility and increased faecal nitrogen concentrations [9]. Thus, once they have been consumed, their adverse effects seem to be related to their binding of dietary protein. Tannins, however, can also have a positive effect due to their antioxidant activity and this depends upon the type and concentration of tannins in a plant. In the present study, the interaction of tannins was studied with BSA (standard protein) for investigating their protein precipitation capacity. Studies were also carried out to determine the free radical scavenging activity of tannins. The advantage of PPC method is that it measures potential biological activity of tannins in foods and feeds. It has been observed that reduction in digestible protein of the feeds containing tanniniferous tree leaves and fruits (antinutritional quality) is proportional to the PPC of the plant tannins. PPC of tanniniferous leaves and fruits is therefore useful in predicting the nutritional quality of foods and feeds. The PPC was higher in harad, bhera and lower in khirk, siris and buince. A similar picture was shown by the tannins of these plants for antioxidant activity, indicating that they can have both beneficial and deleterious effects, depending upon their concentration.

CONCLUSION:

The main findings that emerged from the present investigation are as follows:

- Total tannin content of harad and bhera was high and low in khirk, siris, tremal and buince while other leaves were moderate in their amounts. Hydrolysable tannins also showed the same pattern as that of total tannins. The condensed tannin content was high in kachnar, medium in oak & tremal and low in khirk leaves, harad & bhera fruit.
- TLC fingerprint analysis of selected plants showed remarkable difference in the overall complexity and the polarity of the tannins. Tannins from oak leaves and bhera & harad fruit were more polar as compared to the other plant samples.
- TLC profile of leaf samples showed the presence of tannins after heating for 5 minutes at 110°C which were visualized better after heating for 15 minutes at 110°C.
- A comparison of the TLC fingerprint with the antioxidant fingerprint was also made. It appears that there are many minor tannin components which remained undetected by vanillin-sulfuric acid spray but could be detected by DPPH spray.
- The protein precipitation capacity of tannins was high in harad and bhera leaves & fruits which indicated antinutritional activity of these tannins in animals as they may cause decreased protein availability.

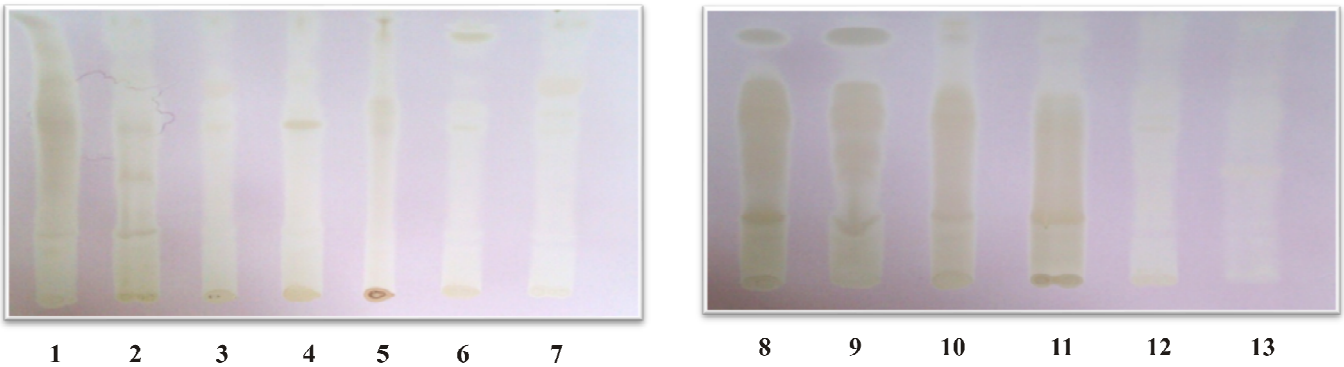

Figure 3: Thin layer chromatographic profile of *in situ* biological activity of standard wattle tannins and tannin extracts of different plants Lane 1: Wattle tannins; Lane 2: Oak; Lane 3: Robinia; Lane 4: Kachnar; Lane 5: Pakar; Lane 6: Tremal; Lane 7: Buince; Lane 8: Harad fruit; Lane 9: Bhera fruit; Lane 10: Bhera leaves; Lane 11: Harad leaves; Lane 12: Khirk; Lane 13: Siris; Solvent System: Acetonitrile: water: chloroform: formic acid (60: 15: 10: 5); Detection: By DPPH spray, drying and visualization after 10 min

Table 3: Protein precipitating capacity (mg BSA precipitated/mg powder) of the leaves and fruits of plant samples

S. No.	Sample Name	PPC (mg BSA pptd./ mg leaf / fruit powder)
1	Oak	0.414
2	Robinia	0.174
3	Khirk	0.002
4	Kachnar	0.246
5	Siris	0.002
6	Bhera	0.490
7	Harad	0.407
8	Pakar	0.026
9	Tremal	0.074
10	Buince	0.004
11	Harad fruit	0.575
12	Bhera fruit	0.661

- The antioxidant activity of tannins of harad and bhera leaves & fruits was high which indicated the positive effect of these plant tannins due to their free radical scavenging activity.
- The protein precipitation capacity and antioxidant activity – the major indicators of biological activity of tannins suggest that a fine balance has to be struck between the beneficial and deleterious effects of these plant tannins by maintaining control over their concentration vis-à-vis their intake by the animals.
- Further work on the chemistry of these compounds like education of their molecular structure, active groups, degree of polymerization etc. can elucidate the interrelationship of their structure with their biological activity.

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