



## Research Article



### Effect of physical treatments on phenolics and tannins in *Ficus roxburghii* and *Quercus leucotrichophora* leaves

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#### ABSTRACT

Inadequate availability of straws and conventional concentrates is one of the major constraints for rearing large and small ruminants. Alternative or unconventional fodder resources play a supplementary role in meeting the demand of livestock owners; but these tree leaves contain some antinutritional factors. Tremal (*Ficus roxburghii*) and Oak (*Quercus leucotrichophora*) are among the traditional tree fodder sources available in the North Western Himalayan region. Their use as a feed stuff for livestock is limited due to presence of tannins. So, the study was undertaken to assess the effect of different physical treatments on total phenol and tannin fractions of Tremal and Oak leaves. Fresh leaves lopped from Tremal and Oak trees of Kangra district of Himachal Pradesh were subjected to physical treatments like chopping and sun drying after chopping. The phenol and tannin fractions of these leaves were estimated before and after treatment. Total phenols, total tannin and hydrolysable tannin contents of the *F. roxburghii* and *Q. leucotrichophora* leaves were reduced significantly ( $P < 0.0001$ ) by both the physical treatments. However, both the treatments increased ( $P \leq 0.0001$ ) the condensed tannin content of *F. roxburghii* leaves, whereas chopping followed by sun drying also increased the non-tannin phenol contents. Both the treatments were effective in reducing tannin contents (both hydrolysable and condensed) of *Q. leucotrichophora* leaves. It may be concluded that physical treatments, chopping, chopping and sun drying, were though effective in reducing hydrolysable tannins, but were not effective in reducing condensed tannin contents of *F. roxburghii* leaves; whereas chopping, chopping and sun drying were effective in reducing tannin contents in case of *Q. leucotrichophora* leaves.

**Keywords:** Physical treatments, *Ficus roxburghii* leaves, *Quercus leucotrichophora* leaves, Tannins, Antinutritional factors.

#### INTRODUCTION

Lack of adequate year-round feed resources is one of the most important factors contributing to low animal production (Bhar *et al.*, 2014; Kawas *et al.*, 2010). A well-known constraint to livestock rearing in the North West Himalayan Region of India is inadequate availability of conventional feed resources like concentrates, straw and cultivated green fodder. In Himachal Pradesh there exists a shortage of dry and green forages to the extent of 35.0 and 57.0%, respectively. In this context, fodder trees are of great importance as a major feed resource, especially, during lean period (Jiban, 2000 and Bhar *et al.*, 2017).

Development of alternate complete feeds out of locally available feeds may partially alleviate the problem of animal feeding. Locally available processed tree leaves with optimum level of anti-nutritional factors could be incorporated in complete feed mixture of small and large ruminants. However, most of the tree leaves available in

the region contain varying amounts of anti-nutritional factors viz. tannins, saponins, alkaloids etc. which limits their use as feedstuff for livestock.

Tremal (*Ficus roxburghii*) is one of the tree forages, which is available in plenty in upland areas. *F. roxburghii* leaves contain 87.64% Organic Matter (OM), 13.81% Crude Protein (CP), 4.33% Ether Extract (EE), 13.08% Crude Fibre (CF), 56.42% Nitrogen Free Extractives (NFE), 12.36% ash; and 5.10% Total Phenols (TP), 4.50% Total Tannins (TT), 0.90% Hydrolysable Tannins (HT) and 4.20% Condensed Tannins (CT) (on DM basis) (Devarajan, 1999 and Bharatbhushan M, 2012). Sharma, *et al.*, 2000 reported the values of CP, EE, CF, TA and NFE in *F. roxburghii* on DM basis as 12.70, 4.79, 13.59, 21.74 and 47.18%, respectively.

Oak (*Quercus leucotrichophora*) is one of the tree forages and evergreen tree found in the Himalayas.

Leaves have dense white-woolly hairs on the underside with a greenish-white underside. The species name *leucotrichophora* means carrying white hairs. The flowers come out in catkins (slim cylindrical flower clusters). The acorns are said to contain a peanut like core when broken (Parmar and Kaushal, 1982). Leaves contain 9.56% CP, 4.8% EE, 31.30% CF, 18.40% NFE and 5.2% total ash on DM basis (CSIR, 1969). The values of OM, CP, EE, CF, NFE and total ash (on DM basis) in *Q. leucotrichophora* have been reported to vary from 91.89-95.43, 8.09-10.73, 2.45-5.38, 13.46-36.67, 48.40-49.50 and 4.76-20.19%, respectively (Sen et al., 1978; Sinha et al., 1989; Singh et al., 1999; Devarajan, 1999; Anandan and Dey, 2000; Bharatbhusan., 2012; Ajith, 2012; Ajith et al., 2014). The TP, TT, HT and CT in *Q. leucotrichophora* in per cent (on DM basis) have been reported as 5.00, 3.70, 1.30 and 4.25, respectively (Devarajan, 1999).

Tannins reduce voluntary Dry Matter intake, cell wall digestibility, growth rate and true digestibility of protein. Tannins also exert inhibitory effects on growth and activity of rumen microbes. These polyphenols are reported to bind with epithelial proteins and proteins in the gut wall causing liver damage and preventing nutrient uptake. All these factors inhibit growth and productivity of animals grazing tannin rich forages. Therefore, the present study was undertaken to study the effect of different physical treatments on total phenol and tannin fractions of *F. roxburghii* and *Q. leucotrichophora* leaves (Devarajan, 1999, Anandan and Dey, 2000, Bhar et al., 2017).

## MATERIALS AND METHODS

The fresh mature tree leaves of *F. roxburghii* and *Q. leucotrichophora* were manually lopped from local forest area of Kangra District. Efforts were made to collect the leaves from the same tree to avoid the variability of leaves in stage of maturity, chemical composition and tannin content. Leaves were packed in gunny bags, brought to the Institute for various physical treatments. Leaves were divided into 4 parts as Fresh Untreated leaves [Control], Chopped [CL], Chopping and sun drying (for 3 days) [CSD] and chopping, sun drying (for 3 days) and grinding [CSDG] each containing 2.5 kg, for physical processing. The total phenol and tannin fraction of fresh leaves and treated leaves were estimated. The chemical composition of the leaves was determined by the methods of AOAC (2000), while fibre fractions were analyzed as per Van Soest et al. (1991). Condensed tannin and other fractions of tannins were estimated as per the method described by Makkar (2003). The data obtained were analysed by using SAS software (SAS, 2003).

## RESULTS AND DISCUSSION

**Chemical composition of tree leaves:** The values of different proximate principles, cell wall and mineral constituents are presented in the table 1. The OM (Organic Matter), CP (Crude Protein), EE (Ether

extract), TCHO (Total Carbohydrate), NDF (Neutral Detergent fibre), ADF (Acid Detergent Fibre), cellulose, hemi cellulose, total ash, calcium and phosphorous contents of *Q. leucotrichophora* (% on DM basis) were 94.02, 10.53, 4.02, 79.47, 48.89, 31.51, 20.54, 17.38, 5.98, 1.89 and 0.27, respectively; of *F. roxburghii* (% on DM basis) were 92.38, 13.86, 4.34, 74.18, 60.78, 52.72, 36.80, 8.06, 7.62, 2.38 and 0.22, respectively.

**Table1.** Chemical composition of tree leaves:

Attributes	Tree leaves	
	<i>Q. leucotrichophora</i>	<i>F. roxburghii</i>
<b>Proximate Composition</b>		
OM	94.02	92.38
CP	10.53	13.86
EE	4.02	4.34
TCHO	79.47	74.18
<b>Cell wall constituents</b>		
NDF	48.89	60.78
ADF	31.51	52.72
Cellulose	20.54	36.80
Hemi cellulose	17.38	8.06
<b>Minerals</b>		
Ash	5.98	7.62
Calcium	1.89	2.38
Phosphorous	0.27	0.22

### Effect of physical treatments on different polyphenols of *F. roxburghii*:

Effect of physical treatments on different polyphenols of *F. roxburghii* leaves is presented in Table 2. Total phenol (TP), non-tannin phenol (NTP), total tannin (TT), condensed tannin (CT) and hydrolysable tannin (HT) content (% DM basis) of fresh *F. roxburghii* leaves were  $6.27 \pm 0.17$ ,  $1.12 \pm 0.01$ ,  $5.15 \pm 0.17$ ,  $1.63 \pm 0.04$  and  $3.52 \pm 0.18$ , respectively. In chopped leaves these polyphenols were 5.18, 1.08, 4.10, 1.86 and 2.24 %; in CSD leaves 5.40, 1.29, 4.11, 1.78 and 2.32 %; where as in CSDG leaves these were 5.34, 0.38, 4.95, 1.62 and 3.33 %, respectively.

All the polyphenols were reduced ( $P < 0.0001$ ) in all the physical treatments (chopping, CSD and CSDG). However, the extent of reduction differed from one polyphenol to other and from one treatment to other physical treatment. TP was reduced to the maximum extent (17.35 %) due to chopping, followed by the other two treatments, CSD and CSDG, difference between which were not significant. The NTP and CT were reduced to the maximum extent due to CSDG; whereas the extent of reduction in TT and HT was maximum due to chopping and followed by CSDG, differences between the treatments chopping and CSD being non-significant. Both the physical treatments, chopping ( $T_2$ ) and chopping & sun drying ( $T_3$ ), significantly ( $P < 0.0001$ ) reduced total phenol, total tannin and hydrolysable tannin content (% DM basis) of *F. roxburghii* leaves (Table 2). However condensed tannin content significantly ( $P < 0.0001$ ) increased in both the treatments. Though chopping reduced ( $P < 0.0001$ ) non-

tannin phenol content, chopping and sun drying increased it.

**Table 2.** Effect of physical treatments on different phenol contents of *Ficus roxburghii* leaves.

Physical Treatment	Total phenol	Non tannin phenol	Total tannin	Condensed tannin	Hydrolysable tannin
Fresh	6.27 <sup>A</sup> ± 0.17	1.12 <sup>B</sup> ± 0.02	5.15 <sup>A</sup> ± 0.17	2.33 <sup>A</sup> ± 0.01	2.82 <sup>B</sup> ± 0.17
Chopped	5.18 <sup>B</sup> ± 0.02	1.08 <sup>B</sup> ± 0.01	4.10 <sup>B</sup> ± 0.02	1.86 <sup>B</sup> ± 0.01	2.24 <sup>C</sup> ± 0.02
Chopped sun dried (CSD)	5.40 <sup>B</sup> ± 0.02	1.29 <sup>A</sup> ± 0.01	4.11 <sup>B</sup> ± 0.02	1.78 <sup>C</sup> ± 0.003	2.32 <sup>C</sup> ± 0.01
Chopped sun dried grinded (CSDG)	5.34 <sup>B</sup> ± 0.01	0.38 <sup>C</sup> ± 0.04	4.95 <sup>A</sup> ± 0.05	1.62 <sup>D</sup> ± 0.12	3.33 <sup>A</sup> ± 0.05
Overall mean ± SE	5.55 ± 0.12	0.97 ± 0.09	4.58 ± 0.13	1.90 ± 0.07	2.68 ± 0.12
P Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Per cent reduction on physical treatment					
Chopped	17.35 <sup>a</sup> ± 0.38	3.81 <sup>b</sup> ± 0.47	20.29 <sup>a</sup> ± 0.37	20.03 <sup>c</sup> ± 0.06	20.51 <sup>a</sup> ± 0.73
Chopped sun dried	13.90 <sup>b</sup> ± 0.37	-15.32 <sup>c</sup> ± 0.64	20.26 <sup>a</sup> ± 0.46	23.51 <sup>b</sup> ± 0.23	17.57 <sup>a</sup> ± 0.75
Chopped sun dried ground (CSDG)	14.83 <sup>b</sup> ± 0.23	65.63 <sup>a</sup> ± 0.68	3.79 <sup>b</sup> ± 1.01	30.27 <sup>a</sup> ± 0.65	-18.09 <sup>b</sup> ± 1.60
Overall mean ± SE	15.36 ± 0.47	18.04 ± 10.48	14.78 ± 2.37	24.60 ± 1.30	6.66 ± 5.32
P Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

\*Means bearing different superscripts with in a column (A, B, C/a,b,c etc,) differ significantly (P < 0.0001). All the polyphenols were reduced (P < 0.0001) in all the physical treatments (chopping, CSD and CSDG). However, the extent of reduction differed from one polyphenol to other and from one treatment to other physical treatment. TP was reduced to the maximum extent (17.35 %) due to chopping, followed by the other two treatments, CSD and CSDG, difference between which were not significant. The NTP and CT were reduced to the maximum extent due to CSDG; whereas the extent of reduction in TT and HT was maximum due to chopping and followed by CSDG, differences between the treatments chopping and CSD being non-significant. Both the physical treatments, chopping (T<sub>2</sub>) and chopping & sun drying (T<sub>3</sub>), significantly (P<0.0001) reduced total phenol, total tannin and hydrolysable tannin content (% DM basis) of *F. roxburghii* leaves (Table 2). However condensed tannin content significantly (P<0.0001) increased in both the treatments. Though chopping reduced (P<0.0001) non-tannin phenol content, chopping and sun drying increased it.

**Effect of physical treatments on different polyphenols of *Q. leucotrichophora* leaves:**

Effect of physical treatments on different polyphenols of *Q. leucotrichophora* leaves is presented in Table 2. Fresh *Q. leucotrichophora* leaves contained 7.26 % total phenols (TP), 1.12 % non-tannin phenols (NTP), 6.15 % total tannins (TT), 1.62 % condensed tannins (CT) and 4.52 % hydrolysable tannins (HT). All the physical treatments [chopping, chopping and sun drying (CSD), chopping sun drying and grinding (CSDG)] were effective (P < 0.0001) in reducing all, the phenolic contents in oak leaves (Table 3).

**Table 3.** Effect of physical treatment on phenolic contents (% on DM) in *Q. leucotrichophora* leaves (Comparision between 3 treatments)

Physical Treatments	Total phenol	Non tannin phenol	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	7.26 <sup>A</sup> ± 0.03	1.12 <sup>A</sup> ± 0.01	6.15 <sup>A</sup> ± 0.04	1.62 <sup>A</sup> ± 0.02	4.52 <sup>A</sup> ± 0.06
Chopped	4.96 <sup>C</sup> ± 0.02	1.08 <sup>B</sup> ± 0.01	3.88 <sup>C</sup> ± 0.02	1.41 <sup>B</sup> ± 0.01	2.47 <sup>C</sup> ± 0.02
Chopped sun dried (CSD)	5.16 <sup>B</sup> ± 0.02	1.04 <sup>C</sup> ± 0.01	4.12 <sup>B</sup> ± 0.02	1.39 <sup>B</sup> ± 0.003	2.73 <sup>B</sup> ± 0.01
Chopped sun dried ground (CSDG)	4.30 <sup>D</sup> ± 0.05	1.02 <sup>C</sup> ± 0.005	3.27 <sup>D</sup> ± 0.05	1.38 <sup>B</sup> ± 0.002	1.89 <sup>D</sup> ± 0.05
Overall mean ± SE	5.42 ± 0.23	1.06 ± 0.008	4.35 ± 0.23	1.45 ± 0.02	2.90 ± 0.21
P Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Per cent reduction on physical treatment					
Chopped	31.73 <sup>b</sup> ± 0.25	3.77 <sup>b</sup> ± 0.46	36.93 <sup>b</sup> ± 0.30	12.86 <sup>b</sup> ± 0.50	45.42 <sup>b</sup> ± 0.52
Chopped sun dried (CSD)	28.91 <sup>c</sup> ± 0.24	7.26 <sup>a</sup> ± 0.46	32.96 <sup>c</sup> ± 0.27	14.03 <sup>a</sup> ± 0.17	39.60 <sup>c</sup> ± 0.33
Chopped sun dried ground (CSDG)	40.76 <sup>a</sup> ± 0.68	8.18 <sup>a</sup> ± 0.45	46.79 <sup>a</sup> ± 0.75	14.83 <sup>a</sup> ± 0.13	58.13 <sup>a</sup> ± 0.1
Overall mean ± SE	33.80 ± 1.25	6.40 ± 0.52	38.89 ± 1.44	13.90 ± 0.26	47.72 ± 1.91
P Value	< 0.0001	< 0.0001	< 0.0001	< 0.002	< 0.0001

\*Means bearing different superscripts with in a column (A, B, C) differ significantly (P < 0.0001)

**Comparision of effects in polyphenols of *F. roxburghii* and of *Q. leucotrichophora* leaves:**

All the physical treatment was effective in reducing all the polyphenols (Table 2 and 3). However, present reduction varies from one polyphenol to other. In *F. roxburghii* maximum overall reduction was of CT (ie 24.60%) followed by NTP (18.04%), TP (15.36%), TT (14.78%) and was lowest of HT (6.66%). Whereas in case of *Q. leucotrichophora* maximum reduction on physical treatment was of HT (47.72%) and lowest was of NTP (6.40%). This was obviously due to the difference in the nature of polyphenols between the plant species. Both the nature and quantity of polyphenols differ greatly between plant species (Synge, 1975).

Chopping significantly reduces all the polyphenols; chopping followed by sun drying further reduces CT and HT. Whereas, NTP was increased ( $P < 0.0001$ ) on sun drying of chopped leaves, but initial reduction of TT and HT of chopped leaves was further increased on sun drying and grinding. It seems that rate of oxidation of polyphenol differs from one polyphenol to other; which in turn changes the proportion of polyphenols on DM basis. Moreover, there was respiratory loss of Organic matter or sugars till the plant cells were dried. Among the polyphenols, the percentage of NTP in fresh *F. roxburghii* leaves was lowest and the quantitative proportion was only 1.12%, which increased to 1.29% on chopping, followed by sun drying. This increase was probably due to reduction of dry matter or soluble sugars due to respiratory loss (Mullen and Koller, 1988). The percentage increase or decrease of respective polyphenols, in respective physical processing of leaves may be due to differences in the rate of losses between polyphenols and soluble sugars or other organic matter/dry matter. Rate of losses of organic matter either as polyphenols or other soluble sugars or dry matter may vary between the three processes of losses. i.e., Due to oxidation of polyphenols; Due to respiration till drying and Due to physical processing (Mullen and Koller, 1988; Makkar, 2003; Bensalema et al., 2005; Vitti et al., 2005).

It is revealed that the reduction of polyphenols is proportional to the particle size of the leaves. Chopping facilitated the oxidative enzymes. Grinding increases the surface area, which in turn accelerated the oxidation and de-naturation of the poly phenols. Moreover, it was revealed that the degree of susceptibility to oxidative enzyme varies from one polyphenol to other. It seems that the degree of susceptibility of HT to oxidative enzyme is relatively more than the other polyphenols (Haslam, 1966; Bagheripour et al., 2008). Effect of physical treatment on reduction of polyphenols, however, reported to be variable from one feed to other, and also from one polyphenol to other. Makkar and Singh (1993) did not observe any effect on TP and CT on drying of mature oak leaves. However, Makkar and Singh (1991) reported that drying was effective for the feed stuffs having high moisture content. Bensalema et al (1999) reported that sun drying was more effective in reducing CT in acacia foliage than shade drying.

## CONCLUSION

It may be concluded that all the physical treatments- chopping, chopping & sun drying, chopping, sun drying and grinding were effective in reducing hydrolysable tannin, were not effective in reducing phenolic constituents like Total phenol, non-tannin phenol, Total tannins, Condensed tannins and Hydrolysable tannins. Hence after the physical treatment, these tree leaves can be used as a fodder resource for livestock sector in scarce and draught areas.

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