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# Chemical Composition and *in vitro* Dry Matter Digestibility of Leaves of *Julbernardia globiflora*

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**Abstract:** An *in vitro* dry matter digestibility study of leaves of *Julbernardia globiflora* at four different post-sprouting stages was conducted to determine usefulness as dry season feed for ruminants. Dried leaf material were used as substrates. The leaves from the early stage of leaf sprouting had significantly higher levels of crude protein (22.57%), IVDMD (61.04%) and total tannins (6.21) but lower in terms of crude fibre (16.90%), NDF (32.83%), ADF (12.01%), lignin (8.4%), hemicelluloses (14.01%) and cellulose (10.42%) ($p<0.05$). Leaves from the dry stage were lowest in terms of crude protein (10.29%), IVDMD (38.11%) and total tannins (1.37%) but the highest in terms of NDF (59.83%), ADF (24.72%), lignin (24.32%) and hemicelluloses (17.26%). There was a general decrease in crude protein with increase in maturation of the leaves. There was also a general decrease in total tannins with maturation of the leaves. The amount of tannins in early stage of leaf sprouting (6.21%) differed significantly to the amount in the late stage (4.14%) and in dry leaves (1.37%) but was similar to the amount in the medium stage ($p<0.05$). This study suggests that the leaves of *Julbernardia globiflora* have high feeding value for ruminants in the dry season. However, use of the leaves in the early stages of leaf sprouting could be limited by high tannin levels.

**Key words:** Tannins, sprouting, dry season, maturation, leaves, protein

## INTRODUCTION

In Zambia as in various parts of the tropics, most small-scale farmers rely on grasses as a major feed source for the grazing animals. However, forage quality (low digestibility and low nutrient content) declines during the dry season. The major factor that limits animal production from these grasses is the fact the animals lose weight due to the nutritional imbalance in the available feed.

To mitigate the problem of feed availability, use of browse plants would be regarded as a good option (Haile and Tolemariam, 2008). Use of indigenous browse tress is important because browse species have high persistence in their respective areas (Woodward and Coppock, 1995). Herders can rely on fodder trees in the dry season because the foliage retain sufficient crude protein, minerals and energy due to the deep root system of these species (Speed and Pugliese, 1992; Paterson *et al*., 1998; Upreti and Shresta, 2006). In Zambia *Julbernardia globiflora* is one of the most important browse species that is utilized by pastoralists in the dry season (Chileshe and Kitayi, 2002). New flush of leaves are produced at the beginning of the dry season which are an important source of browse in the dry season. Although, leaves of this browse tree are an important source of forage for ruminants during the critical period when quality and quantity of pasture herbages are limited; there is little information about the nutritive value of leaves of indigenous browse trees. Leaves at four stages from sprouting namely; early stage, medium late stage and dry stages were analyzed. The early stage is the beginning of the sprouting which occurs mainly in August/September; the medium stage is around October/November when the sprouted leaves are beginning to mature; the late stage is around December when the leaves have fully matured and the dry stage in June/July when the matured leaves have dried and are dropping on the ground. Therefore, it is important to

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evaluate the potential nutritive value of leaves based on their chemical composition and *in vitro* dry matter digestibility.

Chemical composition can be known by carrying out proximate analysis and parameters such as Dry Matter (DM %), Crude Protein (CP %), Neutral Detergent Fibre (NDF %), Acid Detergent Fibre (ADF %) and key minerals such as Calcium (Ca%) and Phosphorus (P%) can be used to measure forage quality. *In vivo* digestibility of many different herbages have shown that digestibility could be an important index of the relative feeding value of a herbage. However such measurements are costly when carried out with animals; a technique for the prediction of digestibility by a laboratory method is therefore desirable. Chemical analysis of herbages alone is not adequate for such prediction purposes. *In vivo* digestibility studies are labour intensive, costly and require sophisticated equipment and animal holding facilities (Khan *et al*., 2003). Therefore, *in vitro* digestion is a biological method in which under conditions which simulated those within the rumen of a ruminant (anaerobic, near neutral pH and blood heat), small samples of herbage could be digested with crude rumen liquor rich in microorganisms. This laboratory technique for determining the digestibility of dried forages involves incubation first with rumen liquor and then with acid pepsin solution. The rumen inocula is capable of dealing with the digestible structural carbohydrates while the digestible protein could however, be readily removed by a second-stage treatment with acid-pepsin. With this method *in vitro* and *in vivo* results are in very close agreement over a very wide range of herbage samples. Methods based on the use of enzymes rather than rumen liquor have subsequently been used.

It is therefore, important measure the chemical composition of browse trees and to carry out *in vitro* digestibility studies in order to determine the level usefulness and effectiveness of the browse species as feed for the animals in extreme weather conditions.

## MATERIALS AND METHODS

The samples (leaves) for this study were collected from Choma district in Southern Zambia during the 2011 dry season. Leaf samples from the four stages from sprouting namely; early stage, medium late stage and dry stages were collected and then they were dried in the shade. The dried samples were ground to pass through a 2 mm screen and analysed for Dry Matter (DM %), Crude Protein (CP %), Crude Fiber (CF %), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF %), Lignin (%), Cellulose (%), Hemicellulose (%), Ash (%) and key minerals such as Calcium (Ca%) and Phosphorus (P%).

For *in vitro* digestibility analysis (1 g) of each replicate sample was weighed with 0.1 mg accuracy in a stoppered 120 mL culture vials bottles. Phosphate buffer (pH 7.35) (35 mL, 0.1 M) containing 1% sodium lauryl sulphate and 1% 2-mercaptoethanol (w/v) was added and the resultant mixture homogenized by gentle stirring. Pronase solution (5 mL, 5 mg $mL^{-1}$ in 0.1 M phosphate buffer, pH 7.35) was added and mixture placed in an oscillating water bath (40°C) for 2 h. The mixture was rinsed with 15 mL distilled water and the washings were filtered and the proteolysis supernatant was discarded. The residue or pellet from proteolysis was suspended in hot (96.8°C) 90% dimethly sulfoxide (10 mL) and transferred into a stoppered 120 mL culture vials bottles. The mixture was homogenized by gentle stirring, placed in a boiling water bath (96.8°C) for 30 min. Boiling 0.1 M acetate buffer (pH 5.66, 30 mL) was added and the resultant solution mixed thoroughly; 0.5 M of Termamyl 60 L was added and the resultant solution was mixed and allowed to remain in the boiling water bath for an additional 15 min. The Erlenmeyer flask was withdrawn and left on the bench to cool to 40°C. Aminoglucosidase solution (5 mL, 20 mg $mL^{-1}$ in 0.1 M acetate buffer (pH 5.66) was added and the flask placed in water bath (40°C) for 2 h. The contents were filtered and the amolysis supernatant was discarded. The final residue (pellet) was washed extensively with distilled water, re-suspended in 50 mL of absolute ethanol and filtered through a weight fritted glass crucible (porosity 4, o.d. pores 10-16 um). It was dried by acetone and then diethyl oxide (50 mL) each placed in a vacuum oven (0.50 psi) overnight at 70°C, cooled in a dessicator and weighted with 0.1 mg accuracy. Finally, the pellet was incinerated at 550°C for 4 h and weighed with 0.1 mg accuracy. The undigested material (insoluble cell walls %) was calculated as follows:

$$\text{Insoluble cell walls (\%)} = \frac{\text{Weight of residue-Weight of ashes}}{\text{Weight of sample}}$$

$$\text{Digestibility (\%)} = 100\% - \text{Insoluble cell walls (\%)}$$

For determination of total tannins, dried (finely ground; passed through a 0.5 mm screen) of the leaves (100 g) was taken into a glass beaker of approximately 25 mL capacity. About 5 mL of aqueous acetone (70%) was added and subjected to ultrasonic treatment for 20 min at room temperature. The contents of the beaker were transferred to centrifuge tubes and subjected to centrifugation for 10 min at approximately 3,000 g. The supernatant was collected and the pellet left in the

centrifuge tube was transferred to the beaker using two portions of 5 mL each of 70% aqueous acetone and again subjected the contents to ultrasonic treatment for 20 min. The contents were centrifuged for 10 min at approximately 3,000 g. Then, 0.01 mL of aliquots of tannin containing were put in tests tubes and the volume was made up to 0.5 mL with distilled water. About 0.25 mL of the Folin-Ciocalteu reagent and 1.25 mL of sodium carbonate solution were added. The mixture in the tubes was vortexed and absorbance was read at 725 nm after 40 min. The total amount of phenols as tannic acid equivalent were calculated from the calibration curve and the total phenolic content was expressed on dry matter basis.

The data were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) of Minitab Reference Manual Release 13 (Minitab, 2000).

## RESULTS AND DISCUSSION

The data for the chemical content (Table 1) indicates that the leaves from the early stage of leaf sprouting had the highest levels of crude protein (22.57%), IVDMD (61.04%) and total tannins (6.21) but the lowest in terms of Crude fibre (16.90%), NDF (32.83%), ADF (12.01%), lignin (8.4%), hemicelluloses (14.01%) and cellulose (10.42%). Leaves from the dry stage were lowest in terms of crude protein (10.29%), IVDMD (38.11%) and total tannins (1.37%) but the highest in terms of NDF (59.83%), ADF (24.72%), lignin (24.32%) and hemicelluloses (17.26%).

There was a general decrease in crude protein with increase in maturation of the leaves. This is similar to what Khazaal *et al.* (1993) found. This decrease in crude protein as the leaves mature could make nitrogen the limiting factor to intake and digestibility. However, the protein range is still above the adequate range (10-13%) for maintenance and growth for cattle, sheep and goats (Kearl, 1982). Calcium content was within the required levels for ruminant growth while phosphorus content was lower than the ruminant animal requirement. Similar results were obtained by Olsson and Welin (1989) and Bhalahenda (2001). It is therefore important to supplement minerals to browsing animals. The increase in NDF and ADF are the major determinants of forage quality. The NDF content ranged from 32.83-59.83% while that of ADF ranged from 12.01-24.72% and compared well with those reported by Romero *et al.* (2000), Bhalahenda (2001), Kuria *et al.* (2005) and Kamalak (2005).

NDF was also negatively correlated with IVDMD ($R^2$ = -0.96) (Fig. 1). This is similar to what Solorio-Sanchez *et al.* (2000) found in leaves of fodder trees in South East Mexico. ADF was also negatively correlated with IVDMD ($R^2$ = - 0.90) (Fig. 2) while crude protein was positively correlated with IVDMD ($R^2$ = 0.96) (Fig. 3). There was a general decrease in total tannins with maturation of the leaves. The high amount of tannins in young leaves (6.21%) differed significantly to the amount in mature leaves (1.37%). Koukoura and Nastis (1994) also found general decrease in total tannins with maturation of the leaves in selected fodder trees in the mediterranean zone. The red colour of the leaves in early sprouting is probably an indicator of the high tannins levels.

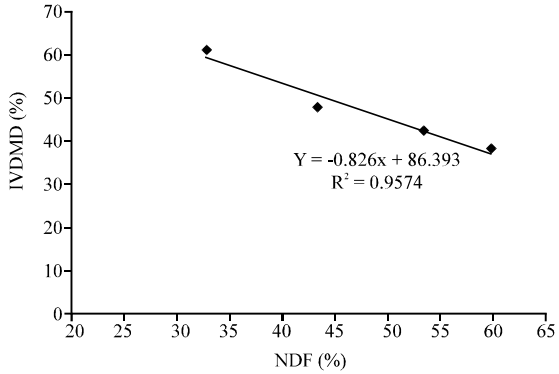


Fig. 1: Relationship between IVDMD and NDF content of the leaves of *Julbernardia globiflora*

Table 1: Nutritive value and *in vitro* digestibility of four different re-growth stages of *Julbernardia globiflora*

| Parameters | Early stage | Medium stage | Late stage | Dry stage |
|---|---|---|---|---|
| Crude protein (%) | 22.57±1.11$^{a}$ | 14.30±0.10$^{b}$ | 14.09±0.14$^{b}$ | 10.29±0.99$^{c}$ |
| Ether extract (%) | 1.23±0.05$^{a}$ | 3.27±0.05$^{b}$ | 4.94±0.11$^{c}$ | 1.03±0.01$^{a}$ |
| Crude fiber (%) | 16.90±1.58$^{a}$ | 23.75±2.96$^{ab}$ | 32.05±2.28$^{b}$ | 25.86±1.51$^{ab}$ |
| NDF (%) | 32.83±2.87$^{a}$ | 43.34±0.13$^{b}$ | 53.40±0.84$^{c}$ | 59.83±0.06$^{c}$ |
| ADF (%) | 12.01±1.64$^{a}$ | 15.66±0.23$^{ab}$ | 20.74±0.47$^{bc}$ | 24.72±1.54$^{c}$ |
| Lignin (%) | 8.40±1.26$^{a}$ | 10.87±0.20$^{a}$ | 14.42±2.02$^{a}$ | 24.32±0.68$^{b}$ |
| Hemicellulose (%) | 14.01±0.55 | 16.48±0.57 | 16.81±0.33 | 17.26±1.29 |
| Cellulose (%) | 10.42±1.04$^{a}$ | 16.00±0.08$^{b}$ | 19.68±1.00$^{b}$ | 18.31±0.67$^{b}$ |
| Ash (%) | 4.23±0.22$^{ab}$ | 4.88±0.42$^{a}$ | 3.41±0.06$^{b}$ | 3.20±0.38$^{b}$ |
| Calcium (%) | 0.98±0.08$^{bc}$ | 1.28±0.03$^{a}$ | 0.93±0.02$^{c}$ | 1.14±0.03$^{ab}$ |
| Phosphorus (%) | 0.52±0.01$^{a}$ | 0.32±0.01$^{b}$ | 0.20±0.01$^{c}$ | 0.28±0.01$^{b}$ |
| IVDMD (%) | 61.04±1.84$^{a}$ | 47.71±0.49$^{ab}$ | 42.26±4.86$^{b}$ | 38.11±0.06$^{b}$ |
| Tannins | 6.21±0.09$^{a}$ | 5.06± 0.25$^{ab}$ | 4.14±0.16$^{b}$ | 1.37±0.62$^{c}$ |

Figures with a different superscript are significantly different ($p<0.05$)

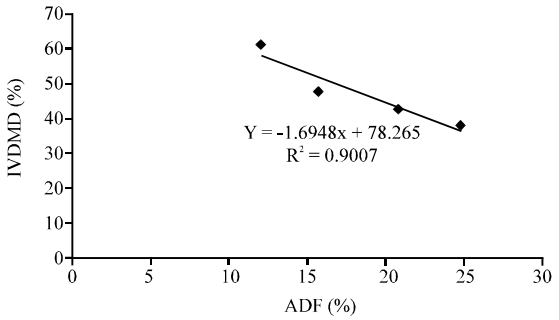


Fig. 2: Relationship between IVDMD and ADF content of the leaves of *Julbernardia globiflora*

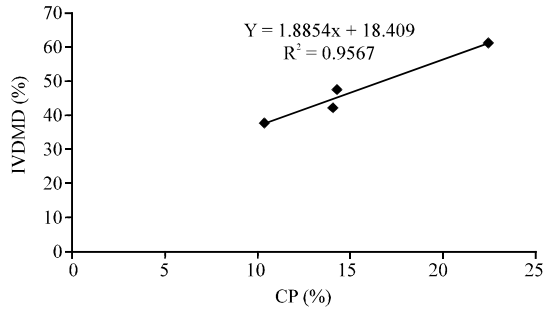


Fig. 3: Relationship between IVDMD and CP content of the leaves of *Julbernardia globiflora*

These high tannins levels may play an important role in prevention of predation (Rebecca *et al*., 2004) and regulation of plant growth. It has been speculated that tannins are an ecologically developed defense mechanism. Provenza and Malechek (1983) have reported that plant parts that which are more succeptible to herbivory damage contain higher concentration than their counterparts which have been developed at heights beyond the animal reach.

Tannins therefore play an important role in affecting forage preference and quality. They can bind both proteins and carbohydrates. Their binding ability varies according to their chemical structure. Tannins bind to proteins and modify the rate and extent of their digestion (Feeny, 1970). Feed intake is depressed when feed contains relatively high levels of tannins (5%).

The limited intake may result partly also from reduces digestion since condensed tannins seem to depress digestion. The leaves in the early stages of sprouting though they contain very high crude protein and high IVDMD and very low crude fibre may not necessarily be consumed in high amount and utilised well by the animals because of the high levels tannins.

## CONCLUSION

This study has shown that the leaves have a good potential to supply highly digestible feeds suitable for ruminants in the dry season. The high protein and digestibility levels in the early stages of leaf sprouting provides a good opportunity for use as browse for the animals at a time when grass resources are dwindling in quantity, quality and digestibility. However, the high tannin levels could cause a serious limitation to feed intake. There is need for more research on how to reduce tannin levels to ensure better utilization of the browse material.

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