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Research Article

POLYPHENOLIC CONTENT AND ANTIOXIDANT ANALYSIS OF *PSEUDOLACHNOSTYLIS MAPROUNEIFOLIA PAX* VAR *DIKINDTII* USED AS LIVESTOCK FEED BY FARMERS FROM EASTERN BOTSWANA

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ABSTRACT

In the present work, fresh fruits (FF),sun dried fruits (SDF), fresh leaves (FL) and sun dried leaves (SDL) of *Pseudolachnostylis maprouneifolia* var *dikindtii* were analyzed for total phenolic, total flavonoid content, presence of reducing sugars, proteins and lipids as well as free radical scavenging power. *P.maprouneifolia* is used as a dry season supplementary feed by livestock farmers in Eastern Botswana. The fruits and leaves of the studied plant tested positive for presence of reducing sugars and proteins. Lipids were detected only in fresh fruit samples. The order of total phenolic content (mg gallic acid equivalents/L) was SDF(1240.3±200.5)>FL(1097.9±154.6)>SDL(952.7±86.8)>FF(838.6±5.7). The order of the flavonoid content (mg Quercetin equivalent/L) was FL(384.9±5.2)>SDL(256.7±3.9)>SDF(159.9±8.3)>FF(139.1±2.6). Sun drying caused an increase in phenolic and flavonoid content in fruits, whilst in leaves the content of both phenols and flavonoids decreased. These changes in polyphenolic contents had no effect on the free radical scavenging potency of the extracts. At all tested concentrations, there was no significant difference between the radical scavenging powers of the fruits. Up to 100µg/ml, the scavenging power of the fruits was (85.6%) higher than that of leaves. Above 100µg/ml, the scavenging power of leaves was (87%) higher than that of the fruits. The current work is the first of its kind, providing new reference data regarding the polyphenolic and antioxidant analysis of this plant. In the light of the obtained result, the present study supports the use of the fruits and leaves of this plant as dry season supplementary feed to livestock. However, further investigations involving nutritional analysis and *in vivo* studies are required to ascertain the potential of *P.maprouneifolia* as a candidate for improving livestock health and production in Botswana.

Keywords: dry season feed, flavonoids, free radical scavenging activity, livestock. *Pseudolachnostylis maprouneifolia* Var *dikindtii,* total phenolics.

INTRODUCTION

Eastern Botswana, particularly Tswapong region, is mostly a rural area, dominated by small scale farming activities. Livestock rearing is considered as an occupation and source of income for the majority of resource-poor farmers in the area. Sustainable production of livestock usually involves efficient utilization of locally available resources. Indigenous plants play an important role in livestock diets particularly in remote areas. In Botswana, like in any other developing world, people living in rural areas, particularly the low income groups, rear livestock on diets consisting of high quantities of indigenous plants[1]. A major problem facing livestock producers in tropical areas is proper nutrition for their animals during the dry season when pastures, cereal residues are limiting in nutritional quality. Normally it is during this season when problems such as sickness and weight loss due to poor dietary profile arise[2].

As a major source of animal feeds in Africa, fodder trees and shrubs are highly valued by farmers. Browses have multiple roles in farming systems such as feed, firewood and as human and veterinary medicines[3]. These forage species contain appreciable amounts of nutrients that are deficient in other feed resources such as grasses during dry seasons and dry periods. Most browse plants have high crude protein content, ranging from 10 to more than 25% on a dry matter basis[4]. This reliable protein resource can be used to develop a sustainable feeding system and increase livestock productivity[5]. Although non-cultivated plants are used commonly by farmers, little is known of the nutritive and health improving value of many species which are very abundant in homestead areas[6]. Small scale livestock farmers in Seolwane Village, in eastern Botswana use *Pseudolachnostylis maprouneifolia* leaves and fruits as a dry season dietary supplement for their goats. *Pseudolachnostylis maprouneifolia* Pax has four varieties namely: Var. *dekindtii* (Pax) Radcl.-Sm, Var. *glabra* (Pax) Brenan, Var. *maprouneifolia*, Var. *polygyna* (Pax & K.Hoffm) radcl.-Sm. All these varieties are eaten by goats and antelopes. The most abundant varieties in eastern Botswana are Var. *dikindtii* and Var. *glabra*. A major advantage of this tree as a potential dry season browse feed is that it stays green and fruits in winter (dry season) when other browse plants have lost their leaves and fruits. It can therefore, be used as a sustainable dry season dietary supplement to increase livestock productivity in Botswana.

Antioxidants and animal health

A number of antioxidants and trace minerals have important roles in immune function and may affect health of livestock[7]. Strong evidence exist that polyphenolic compounds from plants play crucial role as antioxidants[8,9,10]. Many antioxidants are known to have amongst their many roles the following health benefits in animals; antihelmintic[11]; antiparasitic[12]; anti-inflammatory[13]. The damaging effects of free radicals such as superoxide (O_2^-), peroxide (O_2^{2-}), oxide (O^{2-}) and hydroxide (OH^-)ions on animal tissues have been reported to cause many pathological disorders[14]. There is strong evidence that some secondary metabolites in fruits and leaves possess properties to counteract the oxidative stress in animal tissues and these are antioxidants[15]. Amongst the diverse classes of secondary metabolites, polyphenolic compounds, such as tannins, flavonoids and phenolic acids have been found to play a role as antioxidants. Phenolic compounds are ubiquitous in the plant kingdom, being found in all fruits and in virtually all parts of the plant, but with quantitative distributions that vary between different organs of the plant[16]. Polyphenols intervene in colour changes during

ripening of fruits[17]. The interest in polyphenols has considerably increased mainly due to the discovery of their antioxidant effects and their possible role in prevention of several chronic diseases involving oxidative stress[18,19]. Antioxidant properties of *Pseudolachnostylis maprouneifolia* have not been investigated.

In the present study, total phenolic, flavonoid content and antioxidant properties of *Pseudolachnostylis maprouneifolia dikindtii* used by small scale livestock owners in eastern Botswana, were evaluated in order to validate its potential in improving livestock productivity.

Ethnobotanical data & species information

Species name: *Pseudolachnostylis maprouneifolia* Pax var. *dikindtii* (Pax) Radcl.-Sm

Family name: Euphorbiaceae

Common names: mosoto (setswapong); shiny kudu beery (English)

Part used and mode of preparation: Fresh green to pale yellow fruits are eaten by kudu and duiker. The fresh fruits, leaves (fresh and dry) are eaten by goats. Aphrodisiac: pulverized bark is drunk in beer[20]; throat troubles, tuberculosis and pneumonia; bark and root decoction is drunk[21].

Description: An attractive medium sized rounded tree that grows in deciduous woodland and on rocky outcrops over a wide range of altitudes, its habit varying very much according to the habitat. Leaves: simple, alternate, tendering to curl inwards, drooping ovate-elliptic with tapering apex[22]. Fruit: spherical 2cm or more in diameter, with 3-6 faint segments, indehiscent, falling off while still green and ripening on the ground to become yellowish brown and slightly wrinkled. This variety is very much similar to *maprouneifolia glabra* (Pax) Brenan (the latter being distinguished by its hairless leaves on both surfaces; male flower heads with stalks[23].

MATERIALS AND METHODS

Location of the study area

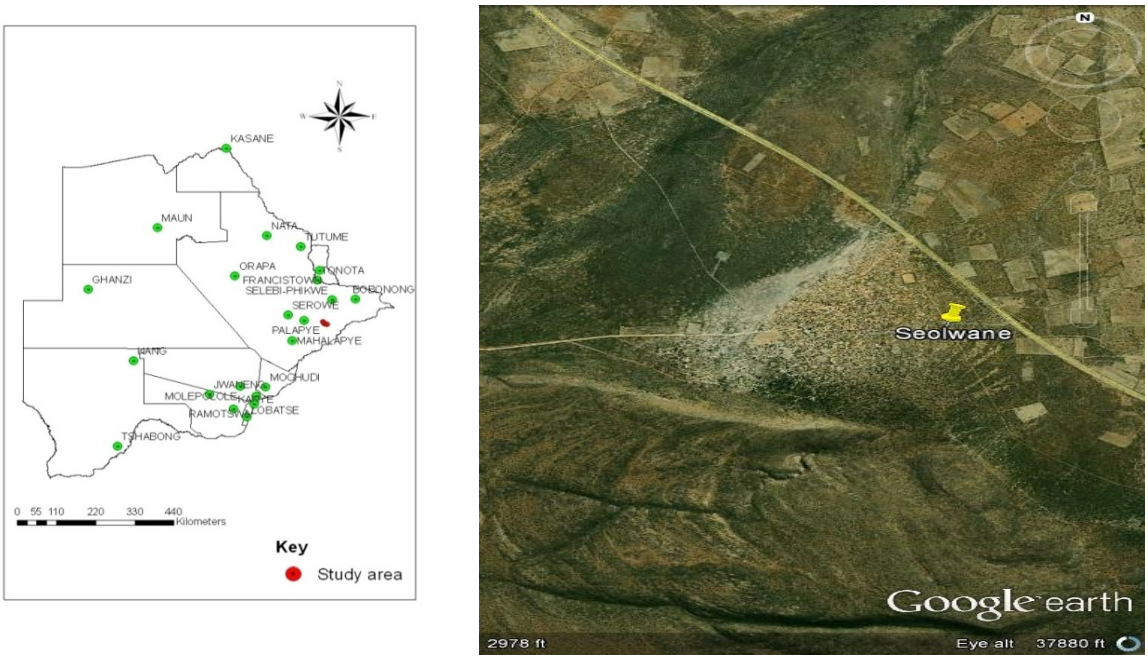

Figure 1:Map of the study area

Figure 2: Macroscopic characters of *P.maprouneifolia* var. *dikindtii* after pre treatment: (SDF)Sun dried Fruits (FF)Fresh fruits (SDL)Sundried leaves (FL)Fresh Leaves.

Collection of the plant

Fruits and leaves of *Pseudolachnostylis maprouneifolia* Pax *var. dikindtii* were collected in June 2012 from Seolwane Village (S22°39′12.0″; E027°42′13.9″) in eastern Botswana. The village is 370km from the capital city Gaborone. The fruits and leaves were harvested from 10 trees and pooled together to produce respective fruit and leaves samples. The fruits were separated from the branchlets and collected in black perforated polythene bags. The leaves were also removed from the branchlets and collected in separate perforated polythene bags. The samples were transported soon after their collection to the Botswana College of Agriculture Medicinal Plants Research laboratory for analysis. Genus and species of the plant were confirmed by comparison with herbarium reference materials at the Botswana National Herbarium and Gallery. Voucher specimen number (EBotMot34) has been deposited at the National Herbarium in Gaborone. Before analysis, visible dirt, soil and insect parts were removed from fruits and leaves.

Sample pre-treatment

The fruits were divided into four groups (SDF, FF, SDL, FL), where (SDF)were fruits sun dried for eight days, whilst (FF) were fresh fruits stored at 4°C until extracted. (SDL) were leaves which had been sun dried for eight days, whilst (FL) was fresh leaves which were stored at 4°C until extraction (Fig:2).

Preparation of the extract

The samples were ground into powders and separately extracted in methanol/acetone (50:50) by cold maceration with periodic agitation for 5days. The samples were then concentrated to dryness using a rotary evaporator (Buchii-Germany) under vacuum at 45°C. The obtained extracts were kept in light-protected containers at 4°C until total polyphenolic content and antioxidant activity were analyzed.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, ascorbic acid, were purchased from Sigma Chemical Co. (St Loius, MO,USA); Folin-Ciocalteus's reagent, sodium carbonate(ACE chemicals, RSA) were from Merck Chemical Supplies (Damstadt, Germany) Sudan III(Rochelle Chemicals, RSA), Biuret reagents (SKYLABS,RSA), Benedict's reagent (LNSLabs, RSA). Aluminium Chloride (SaarChemicals, RSA), Potassium acetate(Rochelle Chemicals, RSA), Quercetin (Fluka, Switzerland). All other reagents were of analytical grade.

Benedict test for reducing sugars

Powdered sample was dissolved in 5ml distilled water and vortexed. The suspension was then decanted to remove large particles. Then 2ml of the decanted liquid was added into a test tube and an equal volume of Benedict's reagent was added and the mixture vortexed. The test tube was left in a boiling bath for 5min or until colour of mixture did not change.

Biuret test for proteins

2ml of the decanted sample was added to a test tube. Then 2ml of 10%Sodium Hydroxide was added followed by 5ml of 1%Copper sulphate. The test tube was swirled to mix the contents. Purple-violet appearance indicates presence of proteins.

Sudan III test for lipids

To a test tube containing 2ml distilled water, 2ml of the decanted test sample was added. Then 3drops of Sudan III were added and the contents shaken. Then 5ml of distilled water was added and the test tube allowed to stand. Lipids will appear as a red ring above the water.

Determination of total phenolics

Total phenol contents in the extracts were estimated using the modified Folin-Ciocalteu method[24]. An aliquot (20µl) of the extract was mixed with 100µl Folin-Ciocalteu reagent in a clean cuvette and mixed well. Then 300µl of (0.2g/ml) Sodium carbonate was added. The tubes were vortexed for 15sec and allowed to stand for 30min at 40°C for colour development. Absorbance was then measured at 765nm using Hewlett Packard UV-Vis spectrophotometer. Samples of extracts were evaluated at a final concentration of 0.1mg/ml. The total phenolic content was determined from the calibration curve and presented as (Gallic acid equivalent (mg/L)).

Determination of Total Flavonoids

Flavonoid content in the acetone-methanolic extract of the plant was determined by Quercetin calometric method[25]. Briefly, 0.50ml of the extract of the sample was diluted with 1.50ml distilled water and 0.50ml of 10%(w/v) Aluminium chloride added along with 0.10ml of 1M potassium acetate and 2.80ml of distilled water. This mixture was incubated at room temperature for 30min. The absorbance of resulting reaction mixture was measured at 415nm using UV-Vis spectrophotometer (Hewlett Packard). Quantification of flavonoids was done on the basis of standard curve of quercetin prepared in 80% methanol and results were expressed in milligram quercetin equivalent (QE)/L.

DPPH radical scavenging assay

Free radical scavenging activity of the extracts was measured in terms of the hydrogen radical scavenging ability using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH)[26]. Briefly a 0.1mM solution of DPPH in methanol was prepared and 1.0ml of this solution was added to 0.5ml of samples in different concentrations. After 20min the absorbance was measured at 525nm. The % scavenging power of the extracts was calculated using the following relation:

DPPH radical scavenging activity (%) = $[(Abs_{blank}-Abs_{sample})]/(Abs_{blank})]x100$

where Abs_{blank} is the absorbance of DPPH radical +methanol; Abs_{sample} is the absorbance of DPPH radical+ sample/standard.

Statistical analysis

The experimental results were expressed as (mean± standard deviation) of three replicates.

RESULTS

Table 1: Analysis of proteins, reducing sugars and lipids in leaves and fruits of *P.maprouneifoilia*

Sample type	Proteins	Reducing sugars	Lipids
Fresh leaves	+	+++	0
Sun dried leaves	+	+++	0
Fresh fruits	+	+++	++
Sun dried fruits	+	+++	0

Key: +=weak response; ++moderate response; +++=strong response; 0=negative/no response.

All the samples tested positive for the presence of reducing sugars and proteins. There were no lipids detected except in fresh fruit samples.

Total phenolic and Flavonoid content

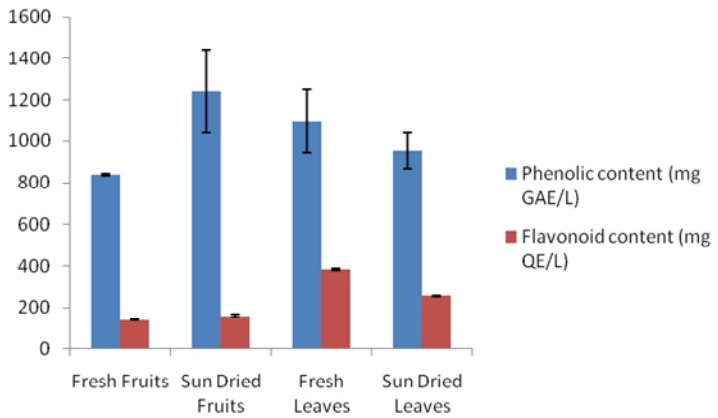

Fig 3: Total phenolic and flavonoid content of the acetone/methanolic extracts from fruits and leaves of *P.maprouneifolia*

Means of total phenols and flavonoid content ±Standard deviation (n=3)

From Fig.3, it is evident that there was an increase in total phenolic content of fruits following sun drying. A similar trend was observed in flavonoid content of the fruits following sun drying. In leaf samples, there was a noticeable decrease in both total phenolic and flavonoid contents following sun drying.

Regression analysis

The values for total phenolic and flavonoid concentrations were evaluated from the relation below:

Table 2: Linear regression analysis for phenol and flavonoid quantification

Analyte	Regression equation	R^2	P_{value}	SL
Total phenols	Y=0.0011x+0.1094	0.9884	<0.0001	*
Flavonoids	Y=0.0027x+0.3270	0.9870	<0.0006	*

*values are significant.

DPPH radical scavenging assay

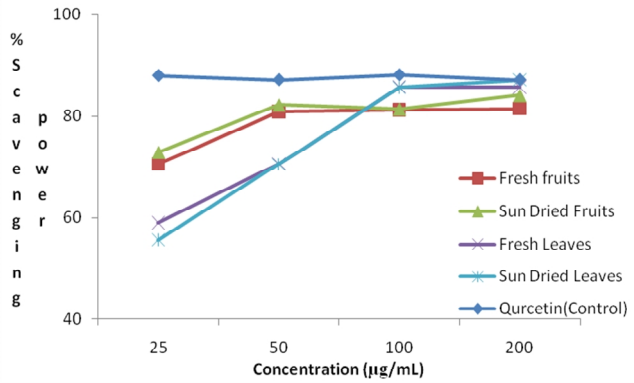

Figure 4: Free radical scavenging power of acetone/methanolic extract s from fruits and leaves of *P.maprouneifolia*

There was no significant difference in free radical scavenging activity between fresh fruits and sun dried fruits at all tested concentrations. Up to 100µg/ml, the scavenging power of fruits was (85.55%) higher than that of leaves. However, between 100 and 200µg/ml, the free radical scavenging activity of leaves was (≥87%), higher than that of fruits.

DISCUSSION

Macroscopic characters

As can be seen from Figure 2, during sun drying both fruits and leaves underwent observable morphological and physical changes. The fruits underwent ripening and became yellowish brown and slightly wrinkled. The leaves also lost their deep greenish colour and became pale brownish and brittle. These changes in colour and texture are associated with chemical composition changes within these plant parts. At molecular level, under the combined effects of heat, light and oxygen, the oxidative degradation of β-carotene in leaves and fruits results in the formation of β-cyclocitral and β-ionone. Other phenolic compounds are also degraded or produced under thermal exposure, giving rise to the observed colourations[27].

Presence of Proteins, reducing sugars and lipids

Analysis for reducing sugars and proteins revealed that all tested samples contained reducing sugars and proteins. Presence of lipids was detected in fresh fruit (FF) samples only (table 1).

Effect of sun drying on total phenolic and flavonoid content of the fruits

The amount of total phenolics (mg Gallic acid equivalents/L) in sun dried fruits (SDF) was as shown in (Fig. 3). SDF had the highest total phenolic content (1240.3±200.5). This was higher than the value obtained from fresh fruits (FF) (838.6±5.7). The majority of these fresh fruits have not fully ripened. Naturally the fruit of *P.maprouneifolia* falls off while still green and ripen on the ground. Sun drying of fruits is associated with an increase in TPC and is intricately linked to ripening of the fruit. With increasing degree of ripening, the content of phenolics such as quercetin and kaempferol increases significantly[28]. Similar to the present finding, an increasing trend in total phenolics of ripening fruits has been reported[29]. The above reported studies by different authors support the findings of the present study, which reveal that as ripening proceeds, the content of polyphenolics increases. In fresh fruit samples, the majority of which were not ripened, the TPC was comparatively low. The total flavonoid (mg Quercetin Equivalents/L) content of the sun dried fruits was higher (159.9±8.3) than that of fresh fruit sample (139.1±2.6). So the total flavonoid concentrations between (SDF) and (FF) showed a similar pattern to phenolic content. The content of polyphenols in fruits is affected by many extrinsic factors including storage, processing and extraction method[30]. However, their concentrations vary from plant to plant or even on different organs of the same plant at different ripening stages[31].

Effect of sun drying on total phenolic and flavonoid content of the leaves

From Figure 3, it can be deduced that the TPC of sun dried leaves was lower (952.7±86.8) than that of fresh leaves (1097.9±154.6). The decrease in the TPC of sun dried leaves (SDL) could be attributed to a number of factors including thermal interactions[32], light and exposure to air. On a thin tissue matrix like a leaf, with a huge surface area, temperature interactions become evident. Thus increased surface area of tissue in contact with heat may cause disruption of cell walls and breakdown of phenolic compounds in sun dried leaves. This may lead to the observed decrease in total phenolic content. The higher TPC in fresh leaves could also have been a result of thermal interactions. The fresh leaves were stored at freezing temperatures (4°C) before extraction in acetone/methanol solvent mixture. Over a thin leaf tissue matrix, freezing can lead to development of ice- crystals within the tissue matrix, leading to rupturing of cell structure, which may lead to better solvent access and release of polyphenolic compounds. Similar effects were observed in other studies investigating the effects of freezing on total phenolic content[33] in plant organs. The total flavonoid content of fresh leaves was higher (384.9±5.2) than that of sun dried leaves (256.7±3.9).The changes in flavonoid content after storage and sun drying follow similar patterns as phenolic content, suggesting that flavonoids contribute to the phenolic content of the leaves. It must be noted that other studies[34] have presented divergent results reporting heat treatment to cause an increase in levels of free flavonoids in vegetables. Flavonoids show different

behaviours depending on extent of heat exposure, processing and genetic factors of a species. It is therefore, likely to have ambiguous results between and within studies.

Phenolic compounds in plant tissues are present in both soluble forms and combined with cell wall complexes. Enzymatic/or non-enzymatic processes that may occur during drying process may also lead to significant changes in the composition of polyphenols[35].

Regression analysis of the calibration curves

The calibration curves of the standards (Gallic acid and Quercetin) were linear with R^2 values of 0.9884 at $P<0.0001$ and 0.9870 at $P<0.0006$, respectively.

Free radical scavenging activities of fruits and leaves of Pseudolachnostylis maprouneifolia

From Figure 4, it was observed that at 50µg/ml both fresh fruits and sun dried fruits exhibited very good scavenging power (≥80%). At a similar concentration both fresh and sun dried leaves exhibited scavenging power of just over 60%. At and above 100µg/ml, the fresh and sun dried leaves exhibited scavenging potencies higher (≥87%) than those of either fresh or sun dried fruits. At 200µg/ml, the order of scavenging potencies of the extracts were SDL(87%)>FL(86%)>SDF(84%)>FF(81%).

CONCLUSION

This study demonstrated the effects of sun drying and freezing on the free radical scavenging power and polyphenolic contents of fruits and leaves of *P.maprouneifolia* var *dikindtii*. The order of total phenolic contents were SDF>FL>SDL>FF, whilst that of total flavonoid content was FL>SDL>SDF>FF. The observed decrease in total phenolic and flavonoid contents of leaf extracts following sun drying had no effect on the free radical scavenging power. The increase in the total phenolic and flavonoid content of the fruits following sun drying also had no effect on the free radical scavenging power of the fruits. At all tested concentrations there was no difference in the free radical scavenging potencies between sun dried fruits and fresh fruits. Above 100µg/ml the scavenging potencies of the leaves were (≥87%), above those exhibited by the fruits. The current work is the first of its kind, providing new reference data regarding the polyphenolic content and antioxidant activity of *Pseudolachnostylis maprouneifolia* var *dkindtii*. The results have also revealed the presence of reducing sugars and traces of proteins in fruits and leaves of the studied plant. Low traces of lipids were detected in fresh fruit samples. In the light of the obtained results on the studied plant, the present study supports the use of the fruits and leaves of this plant as dry season supplementary feed to improve livestock production in Botswana. However, further investigations involving nutritional analysis and *in vivo* studies are required to ascertain the potential of *Pseudolachnostylis maprouneifolia* as a candidate for dry season supplementary feed to livestock.

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