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VARIABILITY IN CONDENSED TANNINS AND BITTERNESS IN SPIDER PLANT GENOTYPES

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ABSTRACT

Spider plant *(Cleome gynandra* L.) contributes considerably to the nutrition and medicines of communities in southern Africa. However, its utilisation is limited by its bitterness caused by condensed tannins. Unfortunately, processing options that reduce the bitterness also remove nutritionally and medicinally useful compounds. The objective of this study was to assess the genetic variability of condensed tannins and their association with bitterness in *C. gynandra* so as to devise variety recommendations for either direct utilisation or for breeding programmes in Zimbabwe. Total phenolic compounds and condensed tannins were quantified in five genotypes. The amount of total phenolic compounds were not significant, but quite significant (P<0.001) for condensed tannins. Genotype CGSKGP had the highest (0.49 mg g^{-1}) and twice as much condensed tannins as CGKEX and CGSKP. Bitterness was positively correlated with the concentrations of condensed tannins (r=0.94, P<0.05), but not with total phenolics. We identified two genotypes, CGKEX and CGSKP with less bitterness (condensed tannins) that can be used in breeding for less bitterness to reduce excessive processing that lowers the nutritional and medicinal properties of *C. gynandra*.

Key Words: *Cleome gynandra*, indigenous vegetable, nutrition, phenolic compounds

RÉSUMÉ

La plante araignée (*Cleome gynandra* L.) contribue de façon considérable à la nutrition et à la médecine traditionnelle chez les communautés en Afrique du Sud. Néanmoins, son utilisation est limitée en raison de son goût amer causé par la concentration en tanins. Malheureusement, le mode de transformation qui y réduit le goût amer réduit également les nutriments et principes actifs utilisés en médecine traditionnelle. L'objectif de cette étude était dévaluer la variabilité génétique du taux de tanins concentré et la relation entre ce taux et le goût amer de *C. gynandra*, ceci pour recommander des variétés appropriées soit pour l'utilisation directe, soit pour des programmes d'amélioration variétales en Zimbabwe. Les taux globaux de composés phénoliques et de tanins concentrés ont été estimés dans cinq variétés. L'étude a montré une différence significative pour les tanins concentré (P<0.001), mais pas pour les composés phénoliques. La variété CGSKGP avait la concentration la plus élevée (0.49 mg g^{-1}) et était deux fois plus concentrée en tanins que les variétés CGKEX et CGSKP. Le gout amer était positivement corrélé avec la concentration en tanins (r=0.94, P<0.05), mais pas avec les composés phénoliques. Nous avons identifiés deux variétés ayant un faible gout amer (tanins concentrés), CGKEX et CGSKP, qui peuvent être utilisées pour développer des variétés améliorées ayant un faible gout amer afin de réduire les transformations excessives qui réduisent les propriétés nutritionnelles et médicinales de *C. gynandra*.

Mots Clés: *Cleome gynandra*, légume indigène, nutrition, composes phénoliques

INTRODUCTION

Spider plant (*Cleome gynandra* L.), also called cat's whiskers is an erect annual herbaceous plant, belonging to the family *Capparaceae* and genus *Cleome* (Chweya and Mnzava, 1997; Orchard and Ngwerume, 2003; USDA, 2013). Since ancient times, this African indigenous vegetable has been used as a relish (Chweya and Mnzava, 1997; Orchard and Ngwerume, 2003; USDA, 2013). Of paramount importance are the antioxidant and therapeutic characteristics of phenolic compounds found in *C. gynandra*. These compounds make this vegetable a suitable herb to treat a number of non-communicable diseases, such as hypertension and other cardiovascular diseases, diabetes, cancer and rheumatism, and boost immunity as well as retard ageing (Kumar and Sadique, 1987; Sankaranarayanan *et al*., 2010). In this regard, this naturally growing weed commonly found in wastelands, has been semi-domesticated (Chweya and Mnzava, 1997; Orchard and Ngwerume, 2003).

The wide utilisation of *C. gynandra* is limited by its bitter taste (Mnzava and Chigumira, 2004). Processing methods that involve boiling (Chweya and Mnzava, 1997) and draining water for several hours can reduce bitterness, but may lead to loss of nutrients, especially the thermo-labile vitamins and other medicinally useful phenolic compounds. Mathooko and Imungi (1994) reported up to 81% loss of ascorbic acid within 15 minutes of boiling, and significant leaching of nutrients when water was drained.

The bitterness in *C. gynandra* is caused by condensed tannins, which are a fraction of phenolic compounds (Mathooko and Imungi, 1994). Phenolics are compounds with one or more aromatic rings and hydroxyl groups (Dai and Mumper, 2010). The phenolic compounds can be partitioned into flavanoids and non-flavanoids (Bolling *et al*., 2009). The flavanoids are polyphenolic compounds which comprise of 15 carbon atoms and 2 aromatic rings. Although all phenolic compounds are responsible for the palatability properties of foods, the flavanoids in general contribute more to the bitterness. Flavanoids are further subdivided into anthocyanidines, flavanols, condensed tannins, flavones, flavanones and isoflavanones (Robbins, 2003; Wilfred and Nicholson, 2008) and these sub-groups are very common in *C. gynandra* (Dai and Mumper, 2010; Pereira *et al*., 2010; Castellano *et al*., 2011).

The different flavanoids have different roles, for example flavanols are antioxidants (Dai and Mumper, 2010); while flavanones have anti-cancer, anti-inflammatory and liver protectant properties (Wilfred and Nicholson, 2008). The condensed tannins are mainly responsible for protection against pests due to their undesirable palatability. Therefore, the presence of condensed tannins in vegetables meant for human consumption is not desirable. This raises the need to reduce the condensed tannins that cause the bitter taste; while increasing other types of useful flavanoids such as flavanols and flavanones.

Crop improvement techniques can be used to lower the concentrations of condensed tannins that will eventually reduce the bitterness without compromising the nutritional and the medicinal properties of *C. gynandra*. However, this depends on the existence of genetic variability in the quantity of condensed tannins among *C. gynandra* genotypes and a strong association of bitterness with the amounts of condensed tannins present. Chweya and Mnzava (1997) noted that most of the phytochemical research done on *C. gynandra* was on isolation and identification of individual compounds; but little on quantification of these phytochemical compounds and assessment of genetic variability among members of the same species. This study aimed at assessing the status of genetic variability in the quantity of condensed tannins and the relationship between bitterness and the condensed tannins in five *C. gynandra* genotypes.

MATERIALS AND METHODS

Description of plant materials. Five African *C. gynandra* genotypes were used in this experiment; three of these were native to Zimbabwe. One genotype (CGMRR) was collected from the Horticultural Research Institute in Marondera; while the other two genotypes (CGSKGP and CGSKP) were previously collected from the communal areas in Seke district in Zimbabwe. Two genotypes, CGTZX and CGKEX, were introductions from Tanzania and Kenya, respectively.

Experimental design and crop management. The five *C. gynandra* genotypes were raised in a greenhouse at the Department of Crop Science, University of Zimbabwe. They were planted in two litre capacity pots arranged in a randomised complete block design, with three replications. Vegefert fertiliser (6N: $15P_2O_5$: $12K_2O$ + 0.1%B) was applied during planting at a rate of 300 kg ha^{-1} (7 g pot^{-1}). Leaf eating pests were controlled by spraying with an insecticide known as karate®, at a rate of 0.5 l ha^{-1}.

Data collection

Extraction and quantification. Leaves for each of the *C. gynandra* genotypes were collected from the five genotypes and oven-dried, and then ground into powder using a pestle and mortar. The Folin-Ciocateu method as described by Makkar *et al*. (1993) and Dai and Mumper (2010), was used to quantify the phenolic compounds and the condensed tannins. The phenolic compounds and condensed tannin concentrations were multiplied by the total leaf dry mass to obtain the contents per gram of leaf dry weight.

Organoleptic tests. Fresh tender leaves were plucked off from each potted plant and boiled for an hour using a metal pot and an electric stove. A panel of five women and five men, between ages of 25-35 years, was selected from the local community based on their strong history of consuming this vegetable. The panel was supplied with three types of cards, which they placed on a sample based on its taste. The cards were green (mild taste), yellow (bitter) and red (very bitter). Each person put the cards at a time and an independent enumerator recorded the type of cards placed per sample. After tasting each of the samples, the participants rinsed their mouths with distilled water and waited for some 5 minutes before tasting the next sample.

Data analyses. Analysis of variance (ANOVA) was performed for the total phenolic compounds and condensed tannins. Least significant difference at 5% productivity level was used to separate the means, where significant differences were recorded from the ANOVA. Pearson correlation analysis was done on the mean organoleptic scores, total phenolic compounds and condensed tannins contents.

RESULTS

The total phenolic compounds were not significantly different among the *C. gynandra* genotypes and the total phenolic compounds ranged from 1.51 to 1.76 mg g^{-1} (Table 1). However, the five genotypes showed significant

TABLE 1. Variability of total phenolics, condensed tannins and organoleptic test responses among the five *C. gynandra* genotypes

C. gynandra genotype	Total phenolic compounds (mg g^{-1})	Condensed tannins (mg g^{-1})	Organoleptic test mean score
CGTZX	1.51	0.29	1.67
CGKEX	1.60	0.23	1.33
CGSKGP	1.71	0.49	2.33
CGMRR	1.76	0.31	2.00
CGSKP	1.60	0.23	1.33
Grand mean	1.64	0.31	1.33
Entry mean square	0.03	0.03	0.57
Error mean square	0.02	0.001	0.22
Repeatability (%)	95.8	99.8	35.0
P-value	0.25	<.001	0.12
LSD (5%)	-	0.08	-
CV (%)	8.0	14.0	26.9

TABLE 2. Correlation of organoleptic tests with total phenolics and condensed tannins

	Organoleptic score	Total phenolics	Condensed tannins
Organoleptic score	1.00		
Total phenolics	0.66	1.00	
Condensed tannins	0.94*	0.51	1.00

*Significant at 5% probability level, r^2 value = 0.88

differences (P<0.001) in the amount of condensed tannins (Table 1). The condensed tannins ranged from 0.23 - 0.49 mg g^{-1}. Genotype CGSKP had the highest level of condensed tannins; while genotypes CGKEX and CGSKP had the lowest amounts. Genotype CGSKP had twice as much condensed tannins (0.49 mg g^{-1}) as the CGKEX and CGSKP (0.23 mg g^{-1}). Interestingly, the genotypes with high levels of condensed tannins had high organoleptic scores (very bitter taste); while those with little amounts of condensed tannins had the least organoleptic scores (mild taste) (Table 1).

The organoleptic test responses were not correlated with total phenolics, but were highly and positively correlated ($r = 0.94$, $P < 0.05$) with the amount of the condensed tannins present (Table 2). Interestingly, the amount of the condensed tannins was not related to the total phenolic compounds (Table 2).

DISCUSSION

The high amounts of total phenolic compounds observed in all *C. gynandra* genotypes are expected given the fact that this crop has a natural bitter taste when consumed. However, the most important observation is the significant variability in the amounts of the condensed tannins noted in the five *C. gynandra* genotypes studied (Table 1). Since the condensed tannins are involved in biochemical plant defense systems, this variation could result from evolutionary history in terms of pathogen/pest pressure in areas of origin of these genotypes. Evolutionary adaptations usually result in mutations and shifts in the genetic makeup of individuals (Falconer, 1989). Such evolutionary adaptations are often genetically controlled, highly heritable and amenable to breeding.

Irrespective of the cause of the variability in tannin amounts, the occurrence of this variation is essential since it raises the potential to select and promote genotypes such as CGKEX and CGSKP that proved to have low levels of condensed tannins. Such genotypes will be less bitter and may not require extensive processing that cause massive loss in the nutritive and therapeutic value. However, consumers may not be aware that some genotypes have less bitterness and thus process all genotypes the same way, hence, causing nutritive and therapeutic value loss. Therefore, such genotypes may need to be accompanied by consumer education in processing. Furthermore, the huge variability in condensed tannins observed among *C. gynandra* genotypes permits breeding to lower these compounds and improve the crop quality.

The quality of phenolic compound is governed by the composition of the medicinally useful phenolic compounds in relation to the total phenolic compounds (Dai and Mumper, 2010). Understanding the genetic variability of *C. gynandra* genotypes in phenolic compounds will help to identify the source of plant materials for use in various genetic studies including breeding aimed at lowering the bitterness; while enhancing the anti-oxidants. This can be done by lowering the contents of condensed tannins while maintaining or increasing the levels of other types of useful flavanoids (Muchuweti *et al.*, 2007). This is possible since the bitterness was correlated with the concentrations of condensed tannins, but not with the total phenolics. Furthermore, the amount of the tannins present was not related to the total phenolic compound present, suggesting that the constituents of the total phenolics can be manipulated independently. To this end, the condensed tannins can be lowered without altering the other phenolic compounds that are nutritionally and medicinally helpful.

In this study, samples with high levels of tannins were rated extremely bitter (Table 1). According to Dai and Mumper (2010), the bitterness of crops such as *C. gynandra* is as a result of a precipitatory reaction between the procyanidin (condensed tannin) and glycoproteins found in saliva. By definition, condensed tannins are high molecular weight phenolic compounds with sufficient hydroxyl and other suitable groups, such as carboxyl which form complexes with protein and other macromolecules (Sengbusch, 2004; Wilfred and Nicholson 2008; Klepacka *et al*., 2011; Kumari and Jain, 2012). Bitterness and astringency are mechanisms for reducing the palatability of plants so as to deter its feeders (Singleton *et al*., 1999). For this reason, the condensed tannins responsible for this action may be medicinally non-essential and must be lowered. Therefore, the bitterness can be a good measure of the amount of condensed tannins present in a sample, where samples with more levels of tannins will be bitterer.

In line with the observations from this study, Chweya and Mnzava (1997) and Muchuweti *et al*. (2007) noted that phenolic compounds constitute a significant proportion of *C. gynandra* phytochemistry. High levels of total phenolics observed in *C. gynandra* genotypes are desirable because some of them are useful anti-oxidants. The antioxidant capacity of phenolics found in *C. gynandra* is far more pronounced than that of the vitamins (Dai and Mumper, 2010). Therefore, there is need to exploit this indigenous vegetable for health reasons, especially for the resource poor communities who cannot afford expensive fruits and vegetables. *Cleome gynandra* seemed to be a potentially high quality vegetable for southern Africa. A vegetable of high quality should have high content of therapeutically important phenolic compounds with the ability to scavenge the reactive oxygen species. Therefore, *C. gynandra* can be easily made a high quality vegetable by lowering the bitterness while increasing the contents of other flavanoids with theraupetic effects. Furthermore, *C. gynandra* is a nutritionally helpful vegetable since it was reported to have significant amounts of amino acids (glutamic acid, arginine and leucine), essential fatty acids (oleic acid, palmitic acid and stearic acid), mineral ions (iron, magnesium and calcium) and antioxidants (vitamin E and phenolic compounds) (Chweya and Mnzava, 1997). The results from this study are a key step towards the massive exploitation of the medicinal and nutritional properties in *C. gynandra* in southern Africa.

CONCLUSION

There is genetic variability in the condensed tannins (bitterness) in *C. gynandra*. This study identified two genotypes (CGKEX and CGSKP) with low levels of condensed tannins. We recommend their direct use since they will require less processing that causes loss of essential nutrients. Furthermore, these materials can be used in breeding new varieties with even much lower bitterness.

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