PHYSICO-CHEMICAL, PHYTOCHEMICAL AND ANTI-OXIDANT PROPERTIES OF BOTANICAL GALACTOGOGUES USED IN RUMINANT FEEDING

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ABSTRACT
Botanical galactogogues contained in Guiera senegalensis (Guiera), Tamarindus indica (Tamarind), Ficus thonningii (Blume) and Anogeissus leiocarpus (African birch) were evaluated for their physico-chemical, phytochemical and antioxidant properties. The samples were shade dried, ground to powder, stored and analyzed for the above-mentioned properties in the laboratory. The treatments were laid in a completely randomized design replicated thrice. The antioxidant properties were evaluated using the diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP). Data collected were analyzed using SAS (2000) and Tukey's test was used to separate means (P<0.05). Physico-chemical analysis of the browse plants showed that there was a significant (P<0.05) difference in their contents of moisture, ash, acid insoluble ash, water insoluble ash, sulphated ash, acid soluble extractive and water soluble extractive. Result further revealed that the extracts of G. senegalensis, T. indica, Ficus thonningii and A. leiocarpus possessed varied degree of free radical scavenging and antioxidant activities in a concentration-dependent manner. The values ranged from (0.220- 0.570) µg/ml for DPPH and (0.4715- 0.7185) µg/ml for FRAP. The extract of G. senegalensis was observed to be the most effective radical scavenger and antioxidant (5.124 µg/ml) and was closely followed by A. leiocarpus extract (1.841 µg/ml). Their high radical scavenging and antioxidant activity can be attributed to their high flavonoid and phenolic contents. It was concluded that G. senegalensis had the highest antioxidant property while F. thonningii had the highest ferric reducing antioxidant property. These findings supported the ethno-veterinary uses of these plants to produce effective intervention for free radical mediated diseases. It is thus reommended that antioxidant profile in milk of ruminants fed botanical galactogogues be further studied.

Keywords: galactogogues, antioxidant, physico-chemical, phytochemical

INTRODUCTION

Galactagogues are substances that boost established lactation, whereas the term galactopoietic is used independently to describe the hormone preparations which enhance milk production in an animal already in lactation (Asimov and Krouze, 1991). Phytochemical screening of plants extracts either with organic or aqueous solvents has revealed the presence of numerous active components including ascorbic acid, domperidone, metoclopramide, saponins, glycosides, essential oils, isoflavones, etc. which contribute to galactopoietic effect (Bharti *et al.,* 2012). Majority of these herbal preparations have however not been scientifically, systematically and thoroughly evaluated, but their traditional use suggests some safety and efficacy. The objectives of the study were to determine the physico-chemical, phytochemical and anti-oxidant properties of *Guiera senegalensis* (sabara), *Tamarindus indica* (tsamiya), *Ficus thonningii* (chediya) and *Anogeissus leiocarpus* (marke).

MATERIALS AND METHODS

The experiment was conducted at the Department of Biochemistry, Faculty of Science, Bayero University Kano, Nigeria.

Samples Collection and Preparation

Leaves of *Anogeissus leiocarpus* was collected at Tsohuwar Zara, *Ficus thonningii* at Tarauni, *Guiera senegalensis* at Dirbindai and *Tamarindus indica* at Magadawa in Kano State, Nigeria.

The leaves were air dried at room temperature and ground separately to powdered form. The extraction method used for dried samples involved adding 150 ml of methanol to 20g of the dried

sample. The mixture was then refluxed in a water bath at 50^0C for 1 hour and was then filtered and placed in fridge (Charalampos *et al*., 2013).

LABORATORY ANALYSIS

The total flavonoids content of each plant extract was assessed by a method employed by Zhishen *et al*., (1999). Each sample (0.5 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a $NaNO_2$ solution (15%).

The total phenol content (TPC) was measured using Folin-Ciocalteu assay (Kähkönen *et al*., 1999). A volume of 0.2 mL of the extract was introduced into test tubes followed by 0.5 mL Folin-Ciocalteu's reagent (diluted 10 times with water). Antioxidant activity of the extracts was evaluated using Phosphomolybdenum according to the procedure of Prieto *et al*., (1999). Then 0.3 ml of plant extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).

The antioxidant potential was measured using the stable radical DPPH. The experiments were carried out using Blois method (Blois, 1958).

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{(Absorbance of control–Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

A modified method of Benzie and Strain (1996) was used for the determination of total antioxidant activity (FRAP assay). The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution.

The Physicochemical analysis of United States Pharmacopoeia-National Formulary (2003) method was adopted for studying physicochemical properties which include;

Two grams of the powdered plant material was weighed in a tared china-dish and oven dried for 30 minutes at 105°C. The china-dish was put in desiccator to cool. It was then weighed on digital balance and the weight of empty dish was subtracted to get dried material. Two grams of powdered plant material was weighed in a tared china-dish and incinerated in furnace at a temperature of 675 ± 25°C for the duration of 48 hours, until the ash got free from carbon. After getting the desired form of ash, the china-dish content was cooled. At the end, the ash contents were weighed and the percentage of the total ash was calculated with reference to the sample weight.

The total ash contents were boiled in 25 mL dilute HCl for 5 minutes. It was then filtered through an ash less filter paper. The filter paper was then dried and ignited in tared china-dish until the ash got free from carbon and later cooled. The ash contents were weighed and the percentage of acid insoluble ash was calculated.

The total ash contents obtained from 2g of powdered plant material were boiled in 25 mL distilled water for 5 minutes and filtered through an ash less filter paper. The filter paper was then dried and ignited in tared china-dish until the ash got free from carbon and then cooled. The ash contents were weighed and the percentage of water insoluble ash was calculated with reference to weight of the total ash used in the test.

Sulphuric acid was mixed with 2g of the powdered plant material in a tared china-dish to make a paste like material. The china-dish was ignited and it was then cooled in a desiccator. The ash contents were

weighed and the percentage of sulphated ash was calculated with reference to the weight of the dried powdered plant material used in the test.

Five grams of powdered plant material was put in a tared flask. Ethanol 95% (100 mL) was poured on it and then macerated in a closed flask for 24 hours. The contents were filtered and 25 mL filtrate was evaporated to dryness in a china dish and the residue was dried in an oven at 105°C and weighed. The percentage of alcohol soluble extractives was calculated with reference to the weight of the sample. One gram of air-dried sample, coarsely powdered was macerated with 100 mL of distilled water in a closed flask for 24 hours shaking frequently. Solution was then filtered and 25 mL of filtrate was evaporated, further dried at 1000^0C and weighted. The percentage of water soluble extractive was calculated with reference to the air-dried samples (Momin and Kadam, 2012).

PHYTOCHEMICAL ANALYSIS

The powdered plant samples (50 g/250 mL) were extracted with petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus at 55-85 °C for 8-10 hours to remove the polar and non- polar compounds (Elgorashi and Van Staden, 2004). The extracts were air dried and then used. The solvents of the respective extracts were condensed and then stored at 4 °C for further use. A small portion of sample was added to 5mL of 1% aqueous HCl in a beaker. The mixture was then stirred, filtered and the volume was reduced to one quarter using a water bath. Few drops of Dragendorff's reagent was added to the filtrate. An orange-red precipitate results which shows the presence of alkaloids (Sofowara, 1993).

Total phenolic content was estimated using Folin-Ciocalteau reagent by the method of Sidduraju and Becker (2003). Twenty (20µg) of leaf extracts were taken separately and it was made up to 1 mL with distilled water. Then, 500 µL of diluted Folins-phenol reagent (1:1 ratio with water) and 2.5 mL of sodium carbonate Na_2CO_3 (20%) were added. The mixture was shaken and incubated in a dark condition for 40 minutes and the absorbance was measured at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 µg/mL. The total phenolics content in the plant extracts were expressed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

The total flavonoids content was estimated using the procedure described by Jia *et al*., (1999). A total of 1 mL of the plant extracts were diluted with 200 µL of distilled water separately followed by the addition of 150 µL of sodium nitrite (5%) solution. It was incubated for 5 minutes and 150 µL of aluminium chloride (10%) solution was added. Then, 2 mL of sodium hydroxide (4%) solution was added and made up to 5 mL with distilled water. The mixture was well shaken and the absorbance was measured at 510 nm. Appearance of a pink color showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

Tannins content was estimated by the method described by Siddhuraj and Manian, (2007). 0.5g of the extracts and 10mL of distilled water was mixed, stirred and filtered. Few drops of 1% ferric chloride solution was added to 2mL of the filtrate. Results showing blue-black, green, blue-green precipitate is positive (Trease and Evens, 2002).

Estimation of total saponins content was determined by the method described by Sofowara (1993). One gram of the plant extract was added with 5mL of distilled water. The mixture was then boiled and filtered. The filtrate and 3mL of distilled water was vigorously shaken for 5 minutes. Results showing persisting frothing on warming is positive.

STATISTICAL ANALYSIS

The experiment was laid in a completely randomized design (CRD) with three replicates in each treatment. Analysis of Variance (ANOVA) of the SAS (2000) package was the tool of analysis and the means were separated using the Tukey test ($p<0.05$).

Table 1: Antioxidant Activity of some Botanical Galactogogues

Treatment	Values(µg/ml)
Guiera senegalensis	5.124[a]
Tamarindus indica	1.530[c]
Ficus thonningii	1.329[d]
Anogeissus leiocarpus	1.841[b]

[a, b, c, d], means in the same row with different superscripts are significantly different (P<0.05)

Table 2: Antioxidant Activity of some galactogogues using the DPPH Method

Treatment (µg/ml) Concentration (mg/ml)	*Guiera senegalensis*	*Tamarindus indica*	*Ficus thonningii*	*Anogeissus leiocarpus*
0.2	0.255	0.365	0.360	0.255
0.4	0.220[b]	0.415[a]	0.400[a]	0.255[b]
0.6	0.230[c]	0.330[b]	0.390[a]	0.255[c]
0.8	0.265[c]	0.420[b]	0.515[a]	0.275[c]
1.0	0.320[b]	0.505[a]	0.570[a]	0.305[b]

[a, b, c], means in the same row with different superscripts are significantly different (P<0.05)

Table 3: Antioxidant Activity of some galactogogues using the Ferric Reducing Antioxidant Power Method

Treatment (µg/ml) Concentration (mg/ml)	*Guiera senegalensis*	*Tamarindus indica*	*Ficus thonningii*	*Anogeissus leiocarpus*
0.2	0.5020	0.4715	0.5420	0.5300
0.4	0.4875[b]	0.5345[ab]	0.6580[a]	0.5480[ab]
0.6	0.4860[d]	0.5740[b]	0.6915[a]	0.5410[c]
0.8	0.4830[c]	0.5705[b]	0.6995[a]	0.5575[b]
1.0	0.4820[c]	0.5940[b]	0.7185[a]	0.5670[b]

[a, b, c, d], means in the same row with different superscripts are significantly different (P<0.05)

Table 4: Physico-chemical characteristics of some Botanical Galactogogues

Variables (%)	Treatment			
	Guiera senegalensis	*Tamarindus indica*	*Ficus thonningii*	*Anogeissus leiocarpus*
Moisture Content	9.85[b]	8.50[c]	11.30[a]	8.15[c]
Ash	12.25[c]	10.85[c]	34.85[a]	23.85[b]
Acid Insoluble Ash	2.55[c]	2.56[c]	6.15[a]	3.55[b]
Water Insoluble Ash	4.55[d]	6.61[c]	11.60[a]	8.57[b]
Sulphated Ash	51.35[a]	32.84[b]	32.60[b]	23.94[c]
Acid Soluble Extractive	11.67[b]	8.77[c]	3.97[d]	13.73[a]
Water Soluble Extractive	2.63[b]	3.93[a]	2.67[b]	4.17[a]

[a, b, c, d], means in the same row with different superscripts are significantly different (P<0.05)

Table 5: Phytochemical Concentration of some Botanical Galactogogues

Variables (%)	Treatment	
	Guiera senegalensis	*Anogeissus leiocarpus*
Tannins	4.55[b]	6.91[a]
Saponins	3.64	3.47
Flavonoids	3.44[a]	2.28[b]
Alkaloids	8.53[b]	27.90[a]
Total phenolics content	159.68[b]	190.18[a]

[a, b], means in the same row with different superscripts are significantly different (P<0.05)

RESULTS AND DISCUSSION

The antioxidant activity of evaluated samples revealed a significant ($P<0.05$) difference. Results obtained ranged from 1.329-5.124(µg/ml) with *Guiera senegalensis* having the most while *Ficus thonningii* had the least. Table 2 presents the antioxidant activity of the botanical galactogogues using the DPPH method at different concentrations. However, *Ficus thonningii* (0.360- 0.570µg/ml) had the highest antioxidant activity while *Guiera senegalensis* (0.255-0.320µg/ml) and *Anogeissus leiocarpus* (0.255-0.305µg/ml) had the least among all the plants samples evaluated. Table 3 presents the antioxidant activity of the samples using the FRAP method at 0.2, 0.4, 0.6, 0.8, and 1mg/ml concentrations. At 0.2mg/ml, the result showed that the antioxidant concentration of the plant samples were the same and ranged from (0.4715-0.5420µg/ml). Result reveals that at 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml concentration, the values ranged from (0.4875-0.6580µg/ml), (0.4860-0.6915µg/ml), (0.4830- 0.6995µg/ml), (0.4820-0.7185µg/ml) respectively with *Ficus thonningii* having the highest while *Guiera senegalensis* had the least. Table 4 presents the result of physico-chemical characteristics of the plant samples evaluated. Moisture content was found to be highest in *Ficus thonningii* (11.30%) and lowest in *Anogeissus leiocarpus* (8.15%). Ash was also found to be highest in *Ficus thonningii* (34.85%) and lowest in *Tamarindus indica* (10.85%). Results of Acid insoluble ash and water insoluble ash revealed that *Ficus thonningii* had the highest (6.15/11.60%) while *Guiera senegalensis* had the least (2.55/4.55%) respectively. Sulphated Ash ranged from (23.94-51.35%) with *Guiera senegalensis* having the highest (51.35%) while *Anogeissus leiocarpus* had the least. Acid Soluble Extractive result showed that *Anogeissus leiocarpus* had the highest (13.73%) while *Ficus thonningii* had the least (3.97%). Water Soluble Extractive was also found highest in *Anogeissus leiocarpus* (4.17%) and lowest in *Guiera senegalensis* (2.63%). Table 5 shows the Phytochemical Concentration of the plant samples. Tannins content was found to be highest in *Anogeissus leiocarpus* (6.91%) and least in *Guiera senegalensis* (4.55%). *Anogeissus leiocarpus* and *Guiera senegalensis* were found to have the same saponin content which ranged from (3.47-3.64%). Result of flavonoids content was found to be highest in *Guiera senegalensis* (3.44%) and lowest in *Anogeissus leiocarpus* (2.28%). Alkaloids content reveals that *Anogeissus leiocarpus* has the highest (27.90%) while *Guiera senegalensis* had the least (8.53%). The Total phenolics content of the botanical galactogogues revealed that *Anogeissus leiocarpus* has the highest phenolics content (190.18%) while *Guiera senegalensis* has the least (159.68%).

CONCLUSION

The radical scavenging ability and antioxidant properties of these plants has been found to be adequate for enhancing milk production in lactating animals. Their high radical scavenging and antioxidant activity can be attributed to their high flavonoid and phenolic contents. Without any reservation, the plants extracts evaluated in the present study possess antioxidant properties which provides effective intervention for free radical mediated diseases that may affect lactation in mammals.

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