Phytochemical composition and cytotoxicity of ethanolic extracts of some selected plants

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ABSTRACT
This study determines the phytochemical contents of selected medicinal plant extracts quantitatively using spectrophotometry and to evaluate their safety on mammalian cells in vitro using tetrazolium-based colorimetric cytotoxicity assay. Twenty-two ethanolic plant extracts were assessed, and the result showed that Psidium guajava had the highest concentration of alkaloids (219.06±11.50mg/g dry-weight (dw)). In contrast, Acacia nilotica leaf extract with 191.60±9.07mg/gdw had the highest level of flavonoids. Vernonia amygdalina and Moringa oleifera contained more steroids than the other plant extracts while condensed tannin was highest in Carya illinoinensis (21.72 ± 0.84 mg/g dw). Coffea arabica, Acacia nilotica leaf, Vernonia amygdalina, Carya illinoinensis and Psidium guajava had more saponins than the other plant extracts. Regarding cytotoxicity evaluation, Allium cepa and Tulbaghia violacea with concentration killing 50% of cells (LC50) values of 0.5182±0.40 and 0.4909±0.034 mg/ml, respectively, were the safest of all the plant extracts. Acacia nilotica pod and Camella japonica leaf extracts were the most cytotoxic with LC50 values of 0.0101±0.016 and 0.0151±0.005 mg/ml, respectively, which are well below the recommended toxic cut-off point (0.03mg/ml). The best selectivity index (SI) value of 1.061 was obtained with Aloe ferox against S. aureus, which is a Gram-positive bacterium.

Introduction

The importance of ruminant production to humanity cannot be over-emphasized, due to their significant contribution to animal protein sources in the form of milk, meat and other by-products. However, for successful ruminant animal production, the science of rumen fermentation is invaluable. In ruminants, unproductive end-products such as methane, excess ammonia and heat have been identified as part of the inherent output of rumen fermentation, which leads to a significant loss of digestible nutrients (Callaway et al. 2003). Hence, animal nutritionists have realized that optimization of rumen efficiency is a goal for successful ruminant production.

Over the years, different strategies of improving rumen fermentation have evolved, through feeding strategies to systematic strategies such as the use of urea blocks and defaunation, to the use of chemical additives and antimicrobial agents (Martin et al. 2010). All these strategies have been identified to have one or more setbacks in their adoption globally, ranging from cost implications, competition with man for grains used in concentrate formulation (Steinfeld and Opio 2010), to toxicity of chemical residues in animal products, the ‘feed-to-food carry over’ (Mantovani et al. 2010) and ultimately antibiotic resistance (Lammie and Hughes 2016). Despite all the issues raised against these strategies, optimizing rumen fermentation towards improved production efficiency cannot be compromised especially in the tropics where these animals are produced mainly using poor roughtage.

Another smart approach to improving rumen efficiency is through mitigating rumen methane output by inhibiting methanogenesis within the rumen environment. Methane mitigation approaches that have been reported are; the use of ionophores (Callaway et al. 2003), oils (Nur Atikah et al. 2018), direct-fed microbials (Jeyanathan et al. 2014), halogenated agents (Denman et al. 2015), propionate enhancers (Newbold et al. 2005) and defaunation (Newbold et al., 2015). The most successful and commercially accepted of all is that of ionophores such as monensin which itself is a macrolide antibiotic. Unfortunately, it tops the list of feed additives that are no longer permitted in the European Union (EC 2005). This prohibition has led to an increased interest in the research area of alternative means of rumen manipulators. And one of the alternatives to antibiotics identified is the use of plant secondary metabolites (natural plant products/phytochemicals) (Greathead 2003; Seal et al. 2013).

Some of these plant secondary metabolites responsible are saponins, tannins, alkaloids, flavonoids, essential oils, glycosides among others, most of which are non-nutritive but are functional (Marriott 2000). They have been identified to have the potential to be used in ruminants and non-ruminants to manipulate their gut function. There have been several reports on the beneficial uses of these phytochemicals either as crude extracts, pure compounds or as dietary plants...
(forage) on ruminant performance in vivo and in vitro (Hart et al. 2008; Gemeda and Hassen 2015; Akanmu and Hassen 2018).

Interestingly, with the inclusion of phytochemicals in ruminant rations in various forms, some of the approaches to improve rumen efficiency have been achieved (Anmut et al. 2008). Kim et al. (2012) reported the ability of plant extracts to cause defaunation which eventually results in a decrease in methanogenesis (Bhatta et al. 2009). Overall improvement in animal performance was achieved with animals fed with condensed tannin-rich legumes (Ramirez-Restrepo et al. 2005; Gemeda and Hassen 2015).

Meanwhile, some of these phytochemical compounds may exhibit toxicity against animal cells (Greathed 2003). The common belief that treatment with medicinal plants and their products is generally safe is a great misconception. Medicinal plants are known to contain pharmacologically active agents that in an overdose, may cause a harmful effect. For instance, an overdose of aqueous extract of garlic bulb becomes lethal in experimental rabbits (Mikail 2010). The existence of powerful natural poisons like atropine and nicotine in many plant species indicates that the assumption that products from natural sources are safe is not true (Tanha 2017). McGaw et al. (2014) also stated that almost any chemical substance is capable of becoming a toxin and this could be related to the quote of Paracelsus (father of toxicology), who said that ‘All things are poison, and nothing is without poison; only the dose makes a thing not a poison’. Hence, the need for toxicity evaluation of plant extracts before adoption as animal feed additive is appropriate. Cytotoxicity testing provides an initial indication of whether or not a substance is potentially toxic in vivo.

Marriott (2000) indicated that incorporating knowledge from various fields into the research of medicinal plants is imperative to use an integrative approach to meet the challenges ahead or else some results in this area of science will be misinterpreted or directionally flawed. Given this, this study was conducted to evaluate and examine the phytochemical and cytotoxicity activity of some selected medicinal plant extracts that have earlier been studied for their antibacterial activity by some of the authors (Abd’Quadri-Abojukoro et al. 2022). This study is a part of an ongoing project, and it serves as a preliminary screening of the plant extracts for their phytochemical and cytotoxicity activity. Hence, the hypothesis of this study is that medicinal plant extracts are rich in phytochemicals that have potential use in ruminant nutrition, and they are not cytotoxic to normal mammalian cells.

Materials and methods
Collection of plant material
Plants used in this study (Table 2) were collected from the University of KwaZulu-Natal (UKZN) Botanical Garden, Pietermaritzburg with geographical coordinates 29°37’S and 30°24’E at an altitude of 659 m and mean annual rainfall of 695 mm and from Ukulinga research farm of the University (UKZN), Pietermaritzburg, with coordinates 29°39’S and 30°24’E at an altitude of 700 m and mean annual rainfall of 735 mm. While Persea americana Mill., Vernonia amygdalina Delile, Carya illinoinensis (Wangen) K. Koch and Psidium guajava L. were collected from residences around UKZN, Pietermaritzburg. Allium sativum L., Zingiber officinale Roscoe and Allium cepa L. samples were purchased from a supermarket. All plant species used were properly identified and collected with the assistance of a botanist at the Department of Botany, University of KwaZulu-Natal, Pietermaritzburg.

Plant species used in this study (Table 1) were selected based on the result obtained from their anthelmintic activity in the same lab (Ahmed et al. 2013; Sserunkuma et al. 2017) and their antimicrobial activity by the same author (Abd’Quadri-Abojukoro et al. 2022). Moreso, their usage in ethnoveterinary medicine in this part of the world and almost all of them has been enlisted in the WHO monographs on selected medicinal plants series (WHO 1999; 2009).

Preparation of plant extracts
Plant parts collected were washed immediately with tap water, cut into small pieces, and air-dried. Then oven dried (LABCON oven EFDO type, Chamdor, South Africa) at 40°C for 5–7 days depending on the moisture content of individual plant material.

The oven-dried plant materials were then milled into a fine powder using an electric blender (RETSCH, GmbH & co. ZM 200, Haan, Germany) fitted with a 1 mm diameter sieve. Powdered samples of the plant materials were preserved in different well labelled airtight plastic containers and kept at room temperature.

Powdered samples of each plant were extracted using 80% ethanol in ratio 1:10, according to the method described by Ahmed et al. (2013). The extracts were placed in a glass jar and kept in a water bath at 60°C to dry. The dried extracts were then powdered and sieved to pass 100 mesh. The dried extracts were weighed and then transferred to airtight plastic containers for storage. The extraction procedure was repeated until the plant material was exhausted. Table 1. List of plant species evaluated for their phytochemical content and cytotoxicity.

| Scientific name | Common name | Family name | Part used |
|-----------------|-------------|-------------|-----------|
| Acacia nilotica L. | Gum Arabic | Fabaceae | Leaves |
| Acacia nilotica L. | Gum Arabic (pod) | Fabaceae | Pods with seeds |
| Acacia sieberiana DC. | Paperbark | Fabaceae | Leaves |
| Allium cepa L. | Onions | Amaryllidaceae | Bulbs |
| Allium sativum L. | Garlic | Amaryllidaceae | Bulbs |
| Aloe ferox | Bitter Aloe | Asphodelaceae | Leaves |
| Ananas comosus (L) Merr. | Pineapple | Bromeliaceae | Leaves |
| Camellia japonica | Tea | Theaceae | Leaves |
| Carica papaya L. | Pawpaw | Caricaceae | Leaves |
| Carya illinoinensis (Wangenh.) K.Koch | Pecan | Juglandaceae | Kernel shell |
| Cichorium intybus L. | Chicory | Asteraceae | Leaves |
| Citrus limon (L.) Osbeck | Lemon | Rutaceae | Leaves |
| Coffea arabica L. | Coffee | Rubiaceae | Leaves |
| Ficus benjamina L. | Weeping fig | Moraceae | Leaves |
| Ficus natalensis Hochst. | Natal fig | Moraceae | Leaves |
| Moringa oleifera Lam. | Drumstick | Moringaceae | Leaves |
| Morus nigra L. | Mulberry | Moraceae | Leaves |
| Persea Americana Mill. | Avocado | Lauraceae | Leaves |
| Psidium guajava L. | Guava | Myrtaceae | Leaves |
| Balhagia violacea Harv. | Wild garlic | Amaryllidaceae | Whole plant |
| Vernonia amygdalina Delile | Bitter leaf | Asteraceae | Leaves |
| Zingiber officinale Roscoe | Ginger | Zingiberaceae | Rhizomes |
were then stored in well-labelled airtight glass bottles and kept at room temperature until required for analysis. Plant extract yields were calculated using the following formula (Ahmed et al. 2013):

\[
\text{Plant crude extract yield (\%)} = \frac{\text{weight of dried extract}}{\text{weight of dried plant powdered extracted}} \times 100
\]

**Phytochemical analysis**

Phytochemical evaluation of the 22 medicinal plant extracts was done quantitatively. Flavonoids, saponins, steroids and tannins were determined using spectrophotometry (Cavalcanti de Amorim et al. 2012), while total alkaloid was determined using the method described by Ezeonu and Ejikeme (2016), and the crude fat proportion of the plant extracts was extracted using Soxhlet apparatus.

Chemicals and reagents: 5% sodium nitrite, 10% aluminium chloride, 1M sodium hydroxide, FeCl₃, potassium ferricyanide, vanillin, hydrochloric acid, sulphuric acid, ethanol, 10% acetic acid in ethanol, conc. and diluted ammonium hydroxide, and petroleum ether (Merck). Standards were quercetin, beta-sitosterol, catechin and diosgenin (Sigma-Aldrich, Saint Louis, USA). All chemicals and reagents were of analytical standard.

Reconstitution of plant extracts: – the dried plant extracts were reconstituted in 80% ethanol (the extracting solvent) to 5 mg/ml and used for flavonoid, steroid, tannin, and saponin determinations.

**Estimation of flavonoids using aluminium chloride**

In a 10 ml volumetric flask, 1 ml of plant extract (5 mg/ml) and 4 ml of distilled water were added. After 5 min, 0.3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminium chloride was added. The solution was allowed to stand at room temperature for 6 min, and then 2 ml of 1M sodium hydroxide was added to the mixture. Immediately, the final volume in the volumetric flask was made up to 10 ml with distilled water. The absorbance of the mixture was then measured at 510 nm against a blank in a spectrophotometer (SHIMADZU UV-1800, SHIMADZU Corporation, Kyoto Japan). Quercetin was used as the standard and was serially diluted in two folds (1.0, 0.5, 0.25, 0.125, 0.0625 and 0.0312 mg/ml) to create a standard curve. Flavonoid concentration of the plant extracts was expressed as quercetin equivalent (mg/g dw plant extract).

**Estimation of saponin**

One ml of plant extract (5 mg/ml) was diluted with 1 ml of 80% ethanol, 2 ml of vanillin in ethanol (10% vanillin) was added and it was well mixed and then 2 ml of 72% sulphuric acid was then added. The mixture was heated on a water bath at 60 °C for 10 mins. Then the absorbance was measured at 544 nm in the spectrophotometer (SHIMADZU UV-1800, SHIMADZU Corporation, Kyoto Japan) against a blank. Diosgenin was used as the standard and was serially diluted in two folds (0.5, 0.25, 0.125, 0.0625, 0.0312 and 0.0156 mg/ml) to create a standard curve. Saponin contents were determined from the standard curve and expressed as catechin equivalent (mg/g of plant extract). Catechin was used as the standard and was serially diluted in two folds (0.5, 0.25, 0.125, 0.0625, 0.0312 and 0.0156 mg/ml) to create a standard curve. Tannin concentration of the plant extracts was expressed as catechin equivalent (mg/g dw plant extract).

**Estimation of total alkaloids**

Total alkaloids were determined as described by Ezeonu and Ejikeme (2016). Briefly 5 ml of 10% acetic acid in ethanol was added to 2 ml (500 mg/ml) of plant extract, covered and allowed to stand for 30 min. The solution was then concentrated on a water bath to get half of its original volume. Concentrated ammonium hydroxide was added in drops to the extract until precipitation was complete. The whole solution was allowed to settle, and supernatant was discarded, the precipitate was later washed with 20 ml of 1% ammonium hydroxide and then filtered using filter paper. The residue (alkaloid) was oven dried and weighed. The percentage of the alkaloid was expressed mathematically as follows (it was then presented as mg/g dw plant extract) (Ezeonu and
weight of alkaloid
weight of the plant extract × 100

Estimation of lipids

The crude fat and oils content of all the 22 plant extracts was extracted with petroleum aether in Soxhlet apparatus and it was refluxed for 4 h. The crude fat and oil content of the plant extracts was presented in mg/g dw of the plant extract.

In vitro cytotoxicity study

All the 22 plant extracts were also subjected to cytotoxicity evaluation against African monkey kidney (Vero) cells using the MTT assay of Mosmann as described by Sserunkuma et al. (2017).

Reagents: Minimum essential medium (MEM, Whitehead Scientific), foetal calf serum (FCS, Highveld Biological), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) (Sigma), dimethyl sulfoxide (DMSO), and gentamicin (Virbac), doxorubicin hydrochloride (Pfizer Laboratories), phosphate buffered saline (PBS, Whitehead Scientific).

Preparation of stock (plant extract): Reconstituted plant extracts prepared above were serially diluted individually in growth medium (MEM) at concentrations of 100, 75, 50, 25, 12.5 and 7.5 µg/ml.

Preparation of MTT: MTT solution was prepared by dissolving 5 mg of MTT in 1 ml of sterile PBS at room temperature shortly before use. It was then filter-sterilized and stored in a dark container at 4°C.

Cell line culture

Vero cells were maintained in MEM supplemented with 0.1% gentamicin and 5% FCS. They were kept in a humidified 5% CO₂ incubator at 37°C for 24 h until the cells reached confluence. Following trypsinization with trypsin-EDTA, cultured cells were distributed in 96 well microtitre plates at 1×10⁴ cells in 100 µl per well; 200 µl MEM were added to blank wells. Plates were incubated for 24 h at 37°C in a 5% CO₂ incubator until the cells were in exponential phase of growth. After that, the growth medium was removed completely from the cells and was replaced with 200 µl of plant extracts at differing concentrations that were serially diluted (100–7.5 µg/ml) and doxorubicin (60–62 µM) (Pfizer Laboratories). All plates were incubated at 37°C in a 5% CO₂ incubator for 48 h. Each concentration for all plant extracts and the controls (positive and negative) were tested in triplicate.

MTT assay

Cell viability was determined using the MTT assay of Mosmann, after 48 h of incubation, the culture medium in all the wells was removed from plates and replaced with 100 µl of fresh culture medium. Ten µl of 5 mg/ml MTT in PBS was added into each well and then incubated for 3 h at 37°C. The medium was carefully removed, the cells washed with phosphate buffered saline and fresh MEM added to the wells. After that, 50 µl of DMSO was added to each well to dissolve the formazan crystals and then incubated for 1 h or until the formazan dissolved. The optical density (OD) was read at 570 nm on a microplate reader (BioTek Synergy, BioTek Instruments, Vermont USA) and the absorbance was recorded.

The percentage cell viability was calculated from the OD values using Microsoft Excel.

\[
\%\text{cell viability} = \frac{A_{570} \text{of treated cells}}{A_{570} \text{of control cells}} \times 100
\]

The results of the percentage cell viability were plotted against the logarithms of concentrations. The regression equation obtained from the graphs was used to calculate the concentration of plant extract required to inhibit 50% of cell growth (LC₅₀) which is defined as the concentration killing fifty percent of the cells.

Selectivity index (SI) was calculated using the minimum inhibitory concentration (MIC) data obtained from a previous study on these 22 plant extracts (Abd’Quadri-Abojukoro 2021) and LC₅₀ obtained as: \( SI = \frac{LC_{50}^{\text{drug}}}{MIC} \).

Data entry and statistical analysis

Phytochemical analysis was determined from the standard curve plotted using Microsoft Excel. The phytochemical concentration in the plant extract equivalent to the standard used were determined from the regression equation of the graph. The data were analysed using general linear model (GLM) procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) and statistical significance was set at \( P < 0.05 \).

The LC₅₀ result was then analysed using one-way ANOVA, and the means were separated using Student Newman Keuls (SNK) test \( (P < 0.05) \). All analysis was done using SAS (version 9.4). The relationship of the LC₅₀ with the phytochemicals determined was established by Pearson correlation, which was followed by multiple regression with LC₅₀ as the dependent variable.

Results

Extract yield and quantitative phytochemical analysis

The ethanolic extract yield of all the 22 plant extracts was presented as the percentage of dry plant material extracted (Table 2). The extract yield varied from 21.3% (Ananas comosus) to 63.5% (Carica papaya). The results obtained from the quantitative phytochemical analysis of the selected plants in this study (Table 3) indicated that Psidium guajava leaf extract had the highest concentration of alkaloids 219.06 ± 11.50 mg/g extract, while C. papaya had the lowest concentration, 28.91 ± 2.69 mg/g extract. Carya illinoiensis, Tulbaghia violacea, Cichorium intybus, Moringa oleifera, Vernonia amygdalina and Morus nigra had considerably higher concentrations of alkaloids of 186.88, 182.66, 176.30, 171.11, 169.98 and...
Table 3. Quantitative phytochemical content of the crude medicinal plant extracts (mean ± SD in mg/g extract).

| Plant species          | Alkaloids | Flavonoids | Steroids | Tannins | Saponin | Crude fat (mg/g) |
|------------------------|-----------|------------|----------|---------|---------|------------------|
| Acacia nilotica (leaves) | 132.36 ± 2.46 | 191.60 ± 9.07 | 44.89 ± 0.35 | 3.11 ± 0.38 | 31.68 ± 2.75 | 19.94 ± 5.57 |
| Acacia nilotica (pod)   | 95.51 ± 1.29  | 138.63 ± 1.88  | 25.84 ± 1.44  | 2.82 ± 1.16  | 29.01 ± 1.35  | 0.37 ± 0.02  |
| Acacia sieberiana       | 120.81 ± 2.67  | 40.88 ± 4.12  | 41.24 ± 1.57  | 0.45 ± 0.11  | 13.78 ± 1.23  | 624.22 ± 4.00 |
| Allium cepa             | 59.19 ± 3.00  | 37.93 ± 2.86  | 42.54 ± 1.11  | 1.22 ± 0.23  | 19.69 ± 3.80  | 2.00 ± 0.01  |
| Allium sativum          | 65.36 ± 1.22  | 31.95 ± 1.75  | 31.26 ± 1.73  | 0.65 ± 0.10  | 15.48 ± 1.28  | 149.75 ± 9.99 |
| Aloe ferox              | 72.82 ± 3.77  | 117.58 ± 4.08  | 46.91 ± 0.78  | 4.08 ± 0.22  | 28.19 ± 0.77  | 273.5 ± 15.43 |
| Anonas comosus          | 71.10 ± 1.27  | 58.17 ± 3.32  | 40.23 ± 1.13  | 0.26 ± 0.05  | 12.04 ± 1.14  | 153.30 ± 7.32 |
| Camellia japonica       | 136.01 ± 17.53 | 105.54 ± 9.67 | 44.36 ± 0.82 | 11.70 ± 1.74 | 22.08 ± 1.56 | 233.45 ± 22.8 |
| Carica papaya           | 28.91 ± 2.69  | 35.76 ± 1.05  | 28.69 ± 1.31  | 1.53 ± 0.51  | 15.70 ± 3.63  | 640.9 ± 14.21 |
| Carya illinoinensis     | 186.88 ± 4.77  | 59.94 ± 3.92  | 48.83 ± 1.31  | 21.72 ± 0.84 | 30.18 ± 3.59 | 129.57 ± 2.76 |
| Cichorium intybus       | 176.30 ± 1.05  | 80.76 ± 1.99  | 47.91 ± 2.34  | 1.70 ± 0.45  | 25.97 ± 3.58  | 293.3 ± 2.12 |
| Citrus limon            | 52.12 ± 4.08  | 57.14 ± 2.22  | 52.30 ± 3.30  | 0.66 ± 0.17  | 24.10 ± 0.21  | 183.3 ± 13.86 |
| Coffea arabica          | 79.46 ± 1.51  | 190.51 ± 1.80 | 47.68 ± 1.20  | 3.60 ± 0.39  | 32.57 ± 4.27  | 388.67 ± 4.07 |
| Ficus benjamina         | 103.61 ± 4.40 | 182.18 ± 3.61 | 36.44 ± 1.98  | 11.85 ± 2.40 | 27.54 ± 1.56  | 215.37 ± 6.49 |
| Ficus natalensis        | 52.59 ± 2.28  | 70.80 ± 1.17  | 57.39 ± 1.97  | 0.95 ± 0.23  | 28.71 ± 2.14  | 573.55 ± 4.44 |
| Moringa oleifera        | 171.11 ± 3.36 | 152.24 ± 1.32 | 70.45 ± 3.87  | 1.22 ± 0.23  | 19.69 ± 3.80  | 2.00 ± 0.01  |
| Morus nigra             | 156.96 ± 7.29 | 83.66 ± 3.36  | 68.00 ± 2.35  | 1.34 ± 0.20  | 20.46 ± 1.53  | 377.1 ± 17.43 |
| Persea americana        | 112.10 ± 10.68 | 138.62 ± 0.95 | 39.25 ± 1.01  | 2.09 ± 0.14  | 28.22 ± 1.00  | 277.68 ± 8.91 |
| Psidium guajava         | 219.06 ± 11.50 | 156.26 ± 1.75 | 29.29 ± 1.50  | 5.67 ± 0.91  | 30.13 ± 2.86  | 342.90 ± 7.96 |
| Tungbha violacea        | 182.66 ± 6.29 | 53.68 ± 3.08  | 59.23 ± 0.63  | 1.89 ± 0.04  | 29.94 ± 0.95  | 42.98 ± 5.73 |
| Vernonia amygdalina     | 169.98 ± 23.74 | 133.70 ± 1.89 | 79.84 ± 1.35  | 3.33 ± 0.79  | 30.55 ± 2.06  | 188.0 ± 38.99 |
| Zingiber officinale     | 77.03 ± 2.22  | 47.51 ± 4.07  | 48.39 ± 2.75  | 0.39 ± 0.10  | 21.46 ± 2.40  | 183.7 ± 16.34 |

P-value                  | <0.0001     | <0.0001   | <0.0001     | <0.0001  | <0.0001  | <0.0001    |
RMSE                     | 7.949       | 3.287     | 1.849       | 0.776    | 2.265    | 12.248     |

RMSE – Root mean square error.
Table 4. Cytotoxicity of crude plant extracts on monkey kidney cells LC50 (mean ± SD mg/ml).

| Plant Species | LC50  |
|---------------|-------|
| Acacia nilotica (leaves) | 0.0494 ± 0.011<sup>ad</sup> |
| Acacia nilotica (pod) | 0.0101 ± 0.006<sup>bc</sup> |
| Acacia sieberiana | 0.0856 ± 0.018<sup>b</sup> |
| Allium cepa | 0.5182 ± 0.408<sup>a</sup> |
| Allium sativum | 0.3266 ± 0.175<sup>b</sup> |
| Aloe vera | 0.4173 ± 0.219<sup>bc</sup> |
| Ananas comosus | 0.1952 ± 0.050<sup>b</sup> |
| Camellia japonica | 0.0151 ± 0.005<sup>d</sup> |
| Carica papaya | 0.2707 ± 0.071<sup>b</sup> |
| Carya illinoinensis | 0.1400 ± 0.009<sup>b</sup> |
| Chichorium intybus | 0.2294 ± 0.157<sup>b</sup> |
| Citrus limon | 0.1214 ± 0.080<sup>b</sup> |
| Coffea arabica | 0.1478 ± 0.009<sup>b</sup> |
| Ficus benjamina | 0.1406 ± 0.032<sup>bc</sup> |
| Ficus natalensis | 0.3616 ± 0.130<sup>b</sup> |
| Moringa oleifera | 0.2442 ± 0.073<sup>b</sup> |
| Morus nigra | 0.0664 ± 0.015<sup>d</sup> |
| Persea americana | 0.1572 ± 0.074<sup>b</sup> |
| Psidium guajava | 0.0481 ± 0.006<sup>d</sup> |
| Tullibahia violacea | 0.4909 ± 0.034<sup>d</sup> |
| Vernonia amygdalina | 0.0505 ± 0.009<sup>d</sup> |
| Zingiber officinalis | 0.0985 ± 0.024<sup>b</sup> |
| Dovexrubin (µM) | 2.0019 ± 0.001 |

Means with different superscripts differ significantly (P < 0.01), RMSE – Root mean square error.

R² = 0.1804. Selectivity index value (Table 6) obtained from the ratio of LC50 and MIC range from 1.325 (A. cepa against S. typhimurium) to 0.005 (C. japonica against S. faecalis). A. cepa and A. ferox has higher SI values for S. typhimurium (1.325) and S. aureus (1.061), respectively.

Discussion

Medicinal plants are characterized based on their ability to synthesize a wide range of bioactive chemical substances or their precursors that can perform specific physiological functions (Sofowora et al. 2013). Selected plants were extracted using 80% ethanol for safety reasons since further study on the extract requires living cells and animals.

All the plant extracts studied had considerable quantities of all phytochemicals except for tannins and this can be explained from the method employed which basically analysed the condensed tannins (proanthocyanidin fraction) proportion, the solvent used for extracting and the extraction process, among others. Haslam (1989) noted that not all plant species produce condensed tannins and even among those that produce these compounds, their concentration and chemical characteristics are highly variable.

The same author also indicated that condensed tannins are of more importance when it comes to research related to animal-tannin interaction. However, their chemical structure and proportion determines will have impact on their bioactivity. One of the major challenges with this area of study is comparing results with previous work due to large variations in procedure from plant material preparation to extraction and phytochemical analysis. Greathead (2003) stated that specific plant species produce phytochemicals, and their quantity and quality are varied highly within plant species and even within individual plants (plant parts). There are many factors responsible for the variations. Among these factors are geographical location, season of harvest and plant part (Marriott 2000), method of extraction (Azwanida 2014), solvent use for extraction (Obeidat et al. 2012), physiological stage and herbivory exposure of each plant (Lindroth 1989). All of these are key influencers of the phytochemical content of plant species (Marriott 2000).

Saponins have been acknowledged for their ability to improve rumen efficiency. Studies have shown that saponins and saponin-containing plant extracts inhibit methanogenesis, causes defaunation (Agarwal et al. 2006; Goel et al. 2008), favour microbial protein yield, lead to increase feed efficiency (Jayanegara et al. 2010), enhance propionate proportion of total VFA and eventually reduce methane production (Patra and Saxena 2010).

Plant tannins are well researched and their ability to decrease methane production in vitro and in vivo has been reported. Patra and Saxena (2010), stated that dietary tannins can reduce methane production directly and indirectly by inhibiting the activity of methanogenic organisms (archaea and protozoa) and reducing fibre degradation, respectively. Like saponins, tannins were able to enhance propionate production and decrease protozoa populations (Animit et al. 2008). Similarly, flavonoids have been well established to have strong antioxidant and anti-inflammatory effects in animal cells (Kim et al. 2004) thus improving the performance of farm animals (Olagaray and Bradford 2019). The inclusion of flavonoids in the diet of ruminants decreased the incidence of acidosis, lowers the acetate: propionate ratio (Balcells et al. 2012), increases milk yield (Tedesco et al. 2004) and improves organic matter digestibility (Ma et al. 2017).

Alkaloids are a group of structurally diverse phytochemicals which are responsible for the beneficial effect of most medicinal plants but also with potential harmful effects of poisoning (Cushnie et al. 2014). It has inspired the development of many drugs such as quinolones, metronidazole, linezolid and the likes (Cushnie et al. 2014). Alkaloids have also been reported for their rumen fermentation modulation effect; de Jesus Pereira et al. (2017) reported that alkaloids extracted from mesquite pod (Prosopis juliflora) was able to decrease methane production and acetate propionate ratio. Plant derived alkaloids enhance propionate concentration and increased crude protein degradation relative to the untreated group in vitro (Mickdam et al. 2016). Plant steroids, like other steroids, possess therapeutic activities such as anti-inflammatory, immune modulation, anti-bacterial, hepatoprotective, anthelmintic, growth-promoting among others (Patel and Savjani 2015). Inclusion of plant

Table 5. Pearson correlation of LC50 with the phytochemicals.

| Alkaloid | Flavonoid | Steroid | Tannin | Saponin | Crude fat |
|---------|-----------|---------|--------|---------|-----------|
| LC50    | 0.30011   | 0.42478 | 0.07899| 0.25117 | 0.1118    | 0.07188  |
| P-value | 0.1748    | 0.0488  | 0.2595 | 0.6204  | 0.7506    |          |
steroids in the diets of ruminants has been reported to have some positive results on their production parameters such as improved milk yield in dairy cows (Jin 2010). Reduced rumen ammonia nitrogen and lactate concentration with subsequent increase in microbial biomass yield and VFA production in *vitro* with the addition of plant steroids to the substrate have been observed in another study (Xi et al. 2014). Steroid metabolism linked with fat, vegetable fat and oils, has been reported to cause defaunation in the rumen environment without affecting bacteria count, improves dry matter degradation, and reduces methane production *in vitro* (Szymacher-Strabel et al. 2004). Nur Atikah et al. (2018) reported that addition of dietary vegetable oil in goat diets causes an increase in the concentration of total VFA and apparent digestibility of crude protein while reducing ammonia concentration. Beauchemin et al. (2017) also reported that inclusion of dietary lipids in the diet of ruminants reduced enteric methane emission.

Apart from the effect of these phytochemicals on rumen metabolism, other beneficial effects reported in animals include improved immunity (Seal et al. 2013), milk yield (Jin 2010), growth and overall animal performance (Ramírez-Restrepo et al. 2005). Generally, plants rich in phytochemicals have the potential of improving rumen metabolism and animal performance.

Cellular cytotoxicity is an alteration of the basic cellular function which leads to cell damage that can be observed at a microscopic level (Cureño et al. 2017). Twenty-two plant extracts were evaluated for their cytotoxic activity on mammalian *Vero* cells. The result revealed that 20 out of the 22 plant extracts were non-cytotoxic and exhibited LC_{50} values above the cut-off point of 0.03 mg/ml. According to the National Cancer Institute (NCI, USA) plant screening programme, crude plant extracts are generally considered to be cytotoxic on normal cells *in vitro*, if the LC_{50} value after incubation for 48–72 h is < 0.03 mg/ml (Fadey et al. 2013). The extract of *Acacia nilotica* pod was ranked as the most cytotoxic according to the result of this study with an LC_{50} value of 0.0101 mg/ml. Hussain and Hussain (2010) reported the strong cytotoxic activity of *A. nilotica* root extract against brine shrimp. In another study, the aqueous extract of *A. nilotica* bark was also reported to exhibit cytotoxicity on Vero cells, with a LC_{50} value of 0.0278 mg/ml (Sserunkuma et al. 2017). *A. nilotica* leaf extract used in this study had a LC_{50} value of 0.049 mg/ml which was above the cut-off point for cytotoxic crude extracts. This agreed with the findings of Sserunkuma et al. (2017) where LC_{50} values for water and acetone extract of *A. nilotica* leaf were 0.0688 and 0.2187 mg/ml, respectively. It can be deduced that only the leaf crude extract is deemed safe in this preliminary study, so other plant parts of *A. nilotica* should be employed with caution.

*Camellia japonica* leaf extract was next to *A. nilotica* pod extract in cytotoxicity ranking with a LC_{50} 0.0151 mg/ml and it is also below the cut-off point. Kuete et al. (2013) had a similar observation with the fruit extract of *C. japonica* having an LC_{50} value of 0.01 mg/ml (Sserunkuma et al. 2017). Triterpenoids isolated from the stem bark extract of *C. japonica* also displayed cytotoxicity against different cell lines (Thao et al. 2010). Unlike *C. japonica* used in this study, *Camellia sinensis*, which is in the same genus, was reported to be inactive as an antiproliferative agent towards a panel of human cell lines (Lombardi et al. 2017). It was non-cytotoxic against normal lymphocytes (Varalakshmi et al. 2011) and normal mouse fibroblast cell lines in another study (Esghaei et al. 2018).

It's worldwide acceptance indicates the level of safety of *Allium cepa* as a spice in food, and no adverse reaction to its use has been reported (WHO 1999). The result of the present study also confirms the safety of its extract on mammalian cells. *A. cepa* bulb extract was ranked as the safest of all the medicinal plant evaluated in this study with LC_{50} value of 0.5182 ± 0.408 mg/ml. Its safety was also acknowledged by Shrivastava and Ganesh (2010), who reported that *A. cepa* exhibited a significant activity as cytoprotective agent on normal lymphocyte cells whereas it shows better tumour

### Table 6. Selectivity index value of the plant extracts.

| Plants          | *S. aureus* | *S. faecalis* | *E. coli* | *S. typhimurium* |
|-----------------|-------------|---------------|-----------|------------------|
| *Acacia nilotica* (leaves) | *(1.563)0.032* | *(1.563)0.032* | *(1.563)0.032* | *(0.391)0.126* |
| *Acacia nilotica* (pod) | *(0.781)0.013* | *(0.781)0.011* | *na* | *(0.781)0.021* |
| *Acacia sieberiana* | *(1.563)0.032* | *(1.563)0.031* | *na* | *(0.781)0.027* |
| *Allium cepa* | *(3.125)0.021* | *(3.125)0.021* | *na* | *(3.125)0.101* |
| *Allium sativum* | *na* | *na* | *(1.563)0.156* | *(1.563)0.156* |
| *Aloe ferox* | *(0.391)0.126* | *(0.391)0.126* | *(0.391)0.126* | *(0.391)0.126* |
| *Ananas comosus* | *(1.563)0.032* | *(1.563)0.032* | *na* | *(0.781)0.101* |
| *Carica papaya* | *na* | *na* | *(1.563)0.179* | *(1.563)0.179* |
| *Carya illinoinensis* | *(0.781)0.150* | *(0.781)0.150* | *na* | *(0.781)0.150* |
| *Cichorium intybus* | *(0.781)0.110* | *(0.781)0.110* | *na* | *(0.781)0.110* |
| *Citrus limon* | *(3.125)0.039* | *(3.125)0.039* | *na* | *(3.125)0.039* |
| *Coffee arabica* | *na* | *na* | *(1.563)0.101* | *(1.563)0.101* |
| *Ficus benjamina* | *(3.125)0.045* | *(3.125)0.045* | *na* | *(1.563)0.090* |
| *Ficus natalensis* | *na* | *na* | *(1.563)0.116* | *(1.563)0.116* |
| *Moringa oleifera* | *(3.125)0.032* | *(3.125)0.032* | *na* | *(0.781)0.021* |
| *Morus nigra* | *(1.563)0.032* | *(1.563)0.032* | *na* | *(0.781)0.021* |
| *Persea americana* | *(3.125)0.050* | *(3.125)0.050* | *na* | *(1.563)0.010* |
| *Psidium guajava* | *(0.781)0.063* | *(0.781)0.063* | *na* | *(0.781)0.062* |
| *Tulbaghia violacea* | *(3.125)0.157* | *(3.125)0.157* | *na* | *(3.125)0.157* |
| *Vernonia amygdalina* | *(1.563)0.032* | *(1.563)0.032* | *na* | *(1.563)0.032* |
| *Zingiber officinale* | *(3.125)0.031* | *(3.125)0.031* | *na* | *(3.125)0.031* |

na – not applicable (MIC > 3.125), * – values in bracket are the MIC value used in calculating the selectivity index.
inhibition on melanoma tumour cells. *Tulbaghia violacea* followed *A. cepa* on the safety side with LC$_{50}$ very well above the cut-off point (0.4909 ± 0.034 mg/ml). There is contradiction between the findings of this study and that of Olorunnisola et al. (2011), who reported a significant lethality of the extract of *T. violacea* rhizomes on brine shrimp, but this is most likely a result of different toxicity expressed against a crustacean model and a mammalian cell line. All the other plant extracts had LC$_{50}$ values above the cytotoxic cut-off point but in vivo testing in animal models is necessary to confirm their lack of toxicity, conclusively.

The reduction in cell vigour caused by an increase in concentration of plant extract is an indication that with overdose, most of the plant extracts can become cytotoxic. Tamokou and Kuete (2014) also observed that over 40% of the medicinal plants examined in their study were identified to be potentially toxic. So many factors are responsible for the toxicity of plant compounds and they include concentration used, duration of use and route of exposure among others (McGaw et al. 2014).

Using the LC$_{50}$ cut-off point alone to determine the cellular toxicity of plant extracts may not fully depict their true cytotoxicity. Almost all the plant extracts studied had SI values below one against all the bacteria species except *A. cepa* against *S. typhimurium* and *A. ferox* against *S. aureus*, which is an indication that they generally lack selectivity. This lack of selectivity implies that these plant extracts are more cytotoxic than they are antibacterial, which can lead to the destruction of healthy cells and tissue. There are reports that some plant extracts contain phytochemicals that are cytotoxic or even genotoxic, which often target cellular processes, for example, methylazoxymethanol, which is an alkald from cycad seed (Kisby et al. 2011).

The best selectivity index (SI) values of 1.325 and 1.061 were obtained with *A. cepa* against *S. typhimurium* and *A. ferox* against *S. aureus*, respectively. It is well established that over 80% of bacteria in the rumen are Gram-negative, and the methane-producing archaea are Gram-positive (Nagaraja 2016). Therefore, plant extracts with better SI values on Gram-positive bacteria are expected to be good potential candidates for rumen fermentation improvement. Hence, for this study, the most promising plant extract is *A. ferox* with SI value of 1.061, obtained against *S. aureus*, a Gram-positive bacterium.

The LC$_{50}$ result were related to the phytochemicals determined. All the phytochemicals determined had the same trend of being negatively correlated to the LC$_{50}$. This relationship was not significant ($p >0.05$) with all of them except for flavonoid ($p <0.05$) content. The negative correlation implies that an increase in the concentration of flavonoids causes a decrease in LC$_{50}$, and the lower the LC$_{50}$, the more toxic the test compound (plant extract). This result was in resonance with the findings of Matsuo et al. (2005) in their study on the cytotoxicity of different flavonoids on normal human cells where they concluded that some flavonoids are cytotoxic towards cultured human cell at higher concentrations. The outcome of the multiple regression procedure indicated that the flavonoid content could explain only 18.04% of the variance in the LC$_{50}$ data.

### Conclusion

All the plant extracts evaluated in this study had considerable quantities of phytochemicals evaluated, and therefore, they might serve as a good source of phytochemicals for improving rumen fermentation efficiency. Most of the plant extracts tested had LC$_{50}$ values above the cut-off point of toxicity (0.03 mg/ml), and this supports their inclusion in many catalogues of folkloric medicinal plants, but unfortunately, they lack selectivity. It is important to note that in vitro cytotoxicity results serve as a preliminary screening tool to identify potentially safe or toxic plant extracts or compounds and does not ultimately address general toxicity. Hence, caution must be applied when administering even the least toxic plant extracts to the animal models with close monitoring of the animal performance.

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