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Screening of some medicinal plant extracts for antibacterial effects: A step towards natural feed additive formulation

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Abstract

Because microorganisms became resistant to the majority of antimicrobial agents, microbial infections have become potentially fatal. As a result, concerns about antibiotic resistance are being raised even in the production of animals, where the use of sub-therapeutic doses of antibiotics in animal feeds plays a significant role. Ionophores, a group of antibiotics used extensively in ruminant production to increase productivity, are prohibited in animal production in the European Union. There is a constant research for antibiotic alternatives by animal scientists in animal production. Therefore, this study aimed to assess the antimicrobial activity of a few chosen medicinal plant species against *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, and *Salmonella typhimurium*. Using the disc diffusion and microdilution procedures, the growth inhibition ability of ethanol extracts of 22 plant materials was assayed against these bacteria. All the plant extracts exhibited resistance against at least one of the bacterial strains. The MIC varied from 0.391- 3.125 mg/ml for the diffusion assay, while the inhibition zones ranged from 10.00±0.00 to 21.33±1.50 mm. *Escherichia coli* was the bacterial strain that was least affected. In comparison to the agar disc diffusion assay, plant extracts demonstrated higher antibacterial activity in the microdilution assay. This shows that for evaluating the susceptibility of bacteria to plant extracts, both the microdilution assay and the disc diffusion method should be used. Majority of the selected plants exhibited strong antibacterial properties against gram-positive bacteria. Therefore, more research is necessary to ascertain how to utilise these antibacterial properties to control rumen microorganisms for effective rumen fermentation.

1. Introduction

It has become endemic for some pathogenic microorganisms to develop resistant against available antibiotics (Guschin et al 2015). Due to repeated exposure to sub-lethal concentrations over time, the efficiency of antibiotics against bacteria has been subdued and their effectiveness has been compromised (Burt and Reinder 2003). As a result, microbial infections have emerged as a global health problem that poses a risk to life (WHO 2018), necessitating more proactive and practical efforts to offer effective countermeasures to this threat from bacteria. There are the medicinal plants whose extracts have the potential to be antibacterial agents, or their parts have components that can be used to make drugs or treat diseases (Sofowora et al 2013). The additional alternatives being researched include microbial extracts (Berdy 2005), honey (Oses et al. 2016), and marine microorganisms (Proksch et al 2003).

Considerable clinical obstacles in the management of infectious diseases are among the more worrying aspects (Lammie and Hughes 2016). These include the indiscriminate use of antibiotics as animal growth promoters, the misdiagnosis of infections (Davies 1994), and the use of sub-therapeutic levels of antibiotics in animal feeds which has flared up the antibiotic resistant strains of *Salmonella* and *Escherichia coli* species (Feinman and Matheson 1978), However, this is not the case with medicinal plants. Despite the fact that in veterinary medicine there has been the successful use of medicinal plants as insecticidal agents and for the prevention and treatment of a number of infections (Escosteguy 2014), the safety and efficiency of these plants are

not supported by any evidence (Cravotto et al 2010). Therefore, among veterinarians, animal scientists, and others associated with animal health, the acceptance of phytotherapy has been hampered by this lack of scientific validation (Phondani et al 2010).

The significance of microbes in the rumen of herbivores cannot be overstated; they help in utilisation of plant fibre in ruminants for body growth and other metabolic processes which set them apart from other animals (Hart et al 2008). However, in this process of microbial fermentation of fibre a sizeable part of nutrients get transformed to the end products such as methane, ammonia, and hydrogen, which are of no direct use to the animal (Callaway et al 2003). Methane generation and emission result in economic losses; the process can result in energy losses to the tune of 10% of gross energy intake or 14% of digestible energy intake (Cottle et al 2011). With a potential to cause 28 times as much global warming as carbon dioxide, this emission has a significant effect on the climate as well (IPCC 2014).

Several strategies have been put up to reduce methane generation and enhance fermentation process of ruminal microorganisms (Leng 1991). In ruminant production the ionophore antibiotics were most frequently used with great success. For instance, antibiotics have improved feed efficiency and decreased methane output (Callaway et al. 2003; Mbanzamihigo et al 1996; Neto et al 2009).

The use of ionophore antibiotics as rumen manipulators in ruminant production has raised a number of concerns despite its success, the most significant of which is antibiotic resistance, which is extremely concerning for the health of humans (who consume animal products) (Lammie and Hughes 2016). Therefore, this study aimed to assess the antibacterial effects of a few chosen medicinal plants against the *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, and *Salmonella typhimurium*. The medicinal plant extracts tested in this study were hypothesised to exhibit antibacterial activity against gram-positive as well as gram-negative bacteria.

2. Materials and Methods

2.1 Collection of plant materials

In this study (Table 1) the plants were selected based on the prior reports of their bioactivity in ruminants by several researchers (Fomum 2018; Ahmed et al 2012); in addition, the unpublished literature and documentation of medicinal plants by WHO were considered as supporting evidence (WHO monographs of medicinal plants). The plant materials were considered from two locations: Ukulinga research farm of the University of KwaZulu-Natal (UKZN) in Pietermaritzburg, South Africa, having an altitude of 700 m with annual rainfall of 735 mm; and the UKZN botanical garden in Pietermaritzburg, South Africa, with geographical coordinates 29°37'S and 30°24'E at an altitude of 659 m with mean annual rainfall of 735 mm. It is pertinent to highlight that every plant specimen was handled carefully and irrigated during the dry season. While *Persea americana* Mill., *Vernonia amygdalina* Delile, *Carya illinoinensis* (Wangenh) K. Koch shell, and *Psidium guajava* L. leaves were obtained from private residences around UKZN, Pietermaritzburg, South Africa, the samples of *Allium sativum* L., *Zingiber officinale* Roscoe, and *Allium cepa* L. were procured commercially from a local supermarket At the Department of Botany of the UKZN, Pietermaritzburg, South Africa,, all the plants utilised were accurately identified and verified.

2.2 Preparation of plant extracts

Based on the moisture level of the specific plant material, they were promptly cleaned with tap water after collection, chopped into tiny pieces, and oven-dried at 40 °C for 5-7 days (LABCON oven EFDO, Chamdor, South Africa). Utilising an electric blender (RETSCH, GmbH & co. ZM 200, Haan, Germany) having a sieve of 1 mm diameter, oven-dried samples were ground into a fine powder. Powdered samples were stored in sealed, well-labeled plastic containers at room temperature and away from light. In a soxhlet device 100 ml ethanol (80%) as solvent for extraction was added to which 10 g of the powdered material added and boiled for 24 h. The extract of the samples were then concentrated to dryness inside a beaker placed in a water bath set to 60 °C. Dried extracts were preserved in sealed glass vials with clear labels and stored at room temperature until they were used for screening. To get the desired concentration for an assay, ethanol (80%) was used to reconstitute the dried plant extract.

2.3 Disc diffusion *in vitro* antibacterial screening of plant extracts

2.3.1 Preparation of test microbial inoculum

Two gram-positive (*S. aureus* and *S. faecalis*) and two gram-negative (*S. typhimurium* and *E. coli*) bacteria were procured from the Microbiology department of University of KwaZulu-Natal, Pietermaritzburg, and maintained on a nutrient agar. After culturing of each bacteria separately again for 24 hours on Mueller-Hinton Agar at 37 °C two to four colonies from each were taken and transferred into a test tube containing sterile distilled water. Using a spectrophotometer set to 625 nm, the turbidity of the bacterial culture was adjusted to 0.1±0.01, which is comparable to 0.5 McFarland (1 x 10^6 cfu/ml) of a Nanodrop (ND-1000 UV-Vis Spectrophotometer, Thermo Fisher Scientific, Wilmington, USA).

2.3.2 Agar disc diffusion test

The agar disc diffusion protocol (Heartley 1944), subsequently known as the Bauer Kirby protocol of antibiotic susceptibility testing as reported by Balouiri et al. (2016), was used for examining antimicrobial properties of plant extracts prepared on the test organisms. The nutrient agar was made in

Table 1 List of plant species evaluated for their antibacterial activity

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	Liliaceae	Bulbs
Allium sativum L.	Garlic	Liliaceae	Bulbs
Aloe ferox Mill.	Aloe	Asphodelaceae	Leaves
Ananas comosus (L)Merr.	Pineapple	Bromeliaceae	Leaves
Camellia japonica L.	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.	Drum stick	Moringaceae	Leaves
Morus nigra L.	Mulberry	Moraceae	Leaves
Persea americana Mill.	Avocado	Lauraceae	Leaves
Psidium guajava L.	Guava	Myrtaceae	Leaves
Tulbaghia violacea Harv.	Society garlic	Alliaceae	Whole plant
Vernonia amygdalina Delile	Bitter leaf	Asteraceae	Leaves
Zingiber officinale Roscoe	Ginger	Zingiberceae	Rhizomes

accordance with the manufacturer's instructions (Biolab, Modderfontein, South Africa). The dried plant extract samples were reconstituted to make a final concentration of 200 mg/ml. The 9 mm diameter plain sterile filter paper discs were taken on a plain petri dish and impregnated with 50 µl of the reconstituted solution such that the concentration of 10 mg/disc is achieved. A total of 22 plant extracts were treatments. As a positive control, a common antibiotic called Neomycin stock (Sigma-Aldrich, Saint Louis, USA) was made with a concentration of 1 mg/ml and the concentration of 25 g/disc was achieved. The discs were impregnated with 80% ethanol at 50 µl/disc to serve as negative control and the impregnated discs were left to air-dry.

The inoculum suspension of 200 µl was spread on a 20 ml solidified nutrient agar uniformly in a petri plate and kept for drying for a period 10 minutes. Four impregnated 9 mm diameter discs were put on the inoculated agar plates and incubated at 37 ^{o}C for 24 hours. This antimicrobial assay was done twice with triplicate set for each extract and after 24 hours the resultant inhibition zones were measured in millimetres (mm).

2.4 Micro-dilution assay for plant extracts' antibacterial activity

2.4.1 Assessment of Minimum inhibitory concentration (MIC)

Both negative and positive controls and all the plant extracts were tested against the selected test microbes to determine their minimum inhibitory concentrations (MIC). The microtitre bioassay method was used to arrive at the MIC values as per Eloff 1998a with suitable modifications (Chukwujekwu and Van Staden 2016). A dilution series was prepared for negative and positive controls and all the plant extracts; and 100 µl from all the dilutions were placed into individual wells of a 96-well micro titration plate. The test bacterial strains of this study were cultured in Mueller Hinton broth (MHB) overnight at 37 °C and the final cell density was adjusted to 10^{6} cfu/ml in sterile MHB. Then, 100 µl of microbial inoculum was added to all the wells. The contents were mixed well and incubated for

24 hours at 37 °C. After that, each well received 40 µl of the p-iodonitro tetrazolium violet (0.2 mg/ml) (Sigma-Aldrich, Sigma Chemical Co., Steinheim, Germany), an effective growth indicator dye. The contents in the titration plate were again incubated for 30 minutes at 37 °C. The development of a reddish colour in the wells indicated bacterial growth, while clear wells after incubation were taken into account as evidence of inhibition of bacterial growth. The MIC was regarded as the minimum concentration of the substance at which growth was completely prevented (clear wells). The MIC assay was carried out quadruple times.

2.5 Experimental design and statistical analysis

Utilising the diffusion technique, all 22 plant extracts were examined for their capability to suppress the growth of four test bacterial strains. The antibacterial assays were conducted in triplicate for two separate runs. Following Moyo et al. (2011) and Eloff (2001), the MIC results were provided as mg/ml and values were converted to antibacterial activity per unit plant material. The following equation determined the precise antibacterial activity of the raw, dry plant materials because the MIC values obtained do not truly represent the antibacterial activity of the plant material:

$$\textit{Antibacterial activity (ml/g)} = \frac{\textit{Extract yield of plant material (mg/g)}}{\textit{MIC value (mg/ml)}}$$

The general linear model (GLM) procedure of SAS (2014) was used to analyse the results (diameter of inhibition zones) of diffusion method. Results are displayed as means and standard deviations (SD) of inhibition zones.

3. Results

3.1 Plant extract yield

The plant extracts yields, which varied in physical characteristics, ranged from 21.3% to 63.5% with *Ananas comosus* having the lowest yield and *Carica papaya* the highest (Table 2).

Table 2 The ethanolic extract yield and physical nature of different plant species

Plant species	Extract yield (%)	Extract yield (mg g^{-1})	Physical nature
Acacia nilotica	31.3	313	Granular
Acacia nilotica pod	38.0	380	Granular
Acacia sieberiana	41.2	412	Viscous
Allium cepa	62.5	625	Viscous
Allium sativum	24.3	243	Viscous
Aloe ferox	22.2	222	Viscous
Ananas comosus	21.3	213	Viscous
Camellia japonica	30.0	300	Granular
Carica papaya	63.5	635	Viscous
Carya illinoinensis	49.5	495	Granular
Cichorium intybus	27.6	276	Granular
Citrus limon	46.0	460	Viscous
Coffea arabica	47.4	474	Viscous
Ficus benjamina	40.5	405	Semi-solid
Ficus natalensis	25.5	255	Viscous
Moringa oleifera	32.7	327	Granular
Morus nigra	32.0	320	Viscous
Persea Americana	44.6	446	Semi-solid
Psidium guajava	29.8	298	Granular
Tulbaghia violacea	50.5	505	Viscous
Vernonia amygdalina	26.7	267	Granular
Zingiber officinale	29.7	297	Viscous

3.2 Antibacterial activity of plant extracts

3.2.1 Diameter of inhibition zones in diffusion method

The agar disc diffusion assay demonstrated that the most sensitive bacteria towards the plants extracts tested was *S. aureus* followed by *S. faecalis*. However, *E. coli* turned out to be the least sensitive bacteria (Table 3). One or more of the bacterial strains have shown susceptibility to sixteen (16) plant extracts. With inhibitory zones of more than 17 mm in diameter, the plant extracts of *Acacia nilotica*, *Psidium guajava*, *Vernonia amygdalina*, and *Camellia japonica* all demonstrated exceptional antibacterial activity. The antibacterial activity of the remaining 12 plant extracts was mild to moderate. Neomycin and *Acacia nilotica* leaf extract had inhibitory zones of 15.33±1.03 mm and 14.50±1.37 mm for *S. faecalis*, respectively. Whereas, Neomycin and *Acacia nilotica* pod extract had inhibitory zones of 9.83±1.60 mm and 18.33±2.16 mm, respectively.

3.2.2 Minimum inhibitory concentration (MIC) with dilution method

The MIC values displayed by different extracts against the microorganisms tested is shown in Table 4. The plant extracts displayed antibacterial action against at least one bacterial strains. In contrast to the disc diffusion approach, *S. typhimurium* was the most susceptible strain of bacteria, with an MIC range from 0.39 to 2.125 mg/ml, while *E. coli* was the least susceptible strain. *E. coli* displayed resistance to all plant extracts in the diffusion technique as well, except the extracts of leaves and pods of the *A. nilotica* plant, which were still able to prevent its growth. The plant extracts of *M. oleifera*, *A. cepa*, *A. sativum*, *C. papaya*, and *T. violacea* were completely

Table 3 Antibacterial activity of different plant extracts against the bacterial strains *via.* diffusion method

Plant species	Diameter of inhibition zones (mm)			
	S. faecalis	***S. aureus***	***S. typhimurium***	***E. coli***
Acacia nilotica	14.50±1.37	18.83±1.16	15.33±2.33	11.50±0.83
Acacia nilotica (pod)	12.83±0.98	21.33±1.50	18.33±2.16	11.83±0.75
Acacia sieberiana	0	12.50±1.22	0	0
Allium cepa	0	0	0	0
Allium sativum	0	0	0	0
Aloe ferox	0	11.00±1.54	0	0
Ananas comosus	0	11.00±1.54	0	0
Camellia japonica	0	18.66±1.03	0	0
Carica papaya	0	0	0	0
Carya illinoinensis	10.33±0.51	14.33±1.21	0	0
Cichorium intybus	0	11.66±0.81	10.50±0.54	0
Citrus limon	0	11.00±0.89	0	0
Coffea arabica	0	11.00±0.89	0	0
Ficus benjamina	11.83±0.98	13.16±1.72	0	0
Ficus natalensis	0	12.33±1.86	0	0
Moringa oleifera	0	0	0	0
Morus nigra	0	11.00±1.54	0	0
Persea Americana	10.33±0.51	10.16±0.40	0	0
Psidium guajava	12.33±1.21	19.00±1.26	0	0
Tulbaghia violacea	0	0	0	0
Vernonia amygdalina	0	17.33±1.50	0	0
Zingiber officinale	0	10.00±0.00	0	0
Neomycin	15.33±1.03	24.83±0.40	19.83±1.60	20.16±0.75

Inhibition zone diameters are expressed as Mean±SD

ineffective against the tested bacterial strains in the disc diffusion approach, however, they were effective against at least one or more strains tested in the microdilution assay. *A. cepa* and *M. oleifera*, with MIC values of 0.391, 1.563, and 3.125 mg/ml; and 1.563, 3.125, and 1.563 mg/ml, respectively, inhibited the growth of *S. typhimurium*, *S. aureus*, and *S. faecalis*. The *T. violacea* was effective against *S. typhimurium* and *S. aureus* with MIC value of 3.125 for both microbes. With an MIC value of 1.563 mg/ml, *A. sativum* and *C. papaya* were effective only against *S. typhimurium*.

3.3 Actual antibacterial activity of plant extracts

The actual antibacterial activity of the plant extracts against *S. aureus*, *S. faecalis*, and *S. typhimurium* are depicted in Fig. 1-3, respectively. *S. aureus* was most sensitive to the extract of *C. illinoinensis* followed by *A. ferox*, *A. nilotica* (pod), *A. cepa*, and *P. guajava*. However, *Ficus natalensis*, *Citrus limon*, *Coffea arabica*, *Carica papaya*, and *A. sativum* failed to inhibit *S. aureus* and the other plant extracts displayed intermediate antibacterial activities. Against *S. faecalis*, the antimicrobial activity of the extracts was in the following order: *Acacia sieberiana* (527.52 ml/g) > *C. illinoinensis* > *A. nilotica* (pod) > *M. oleifera* > *A. nilotica* > *A. cepa*.

The activity of other plant extracts ranged from 200.25 ml/g (*A. nilotica*) to 71.04 ml/g (*A. ferox*), whereas *S. faecalis* was not sensitive to *C. papaya*, *T. violacea*, *P. Americana*, *C. arabica*, *A. comosus*, *Z. officinale*, or *A. sativum*. *E. Coli* displayed resistance against all plant extracts tested except for the leaf and pod extract of *A. nilotica*, whose activities were 200.25 ml/g and 243.12 ml/g, respectively. Against *S. typhimurium* all extracts had activity below 1000 ml/g, but *A.*

Table 4 Minimum inhibitory concentration (mg/ml-) of plant extracts on bacterial strains

Plant species	Sa	Sf	Ec	St
Acacia nilotica	1.563	1.563	1.563	0.391
Acacia nilotica pod	0.781	1.563	1.563	0.391
Acacia sieberiana	3.125	0.781	-	3.125
Allium cepa	1.563	3.125	-	0.391
Allium sativum	-	-	-	1.563
Aloe ferox	0.391	3.125	-	1.563
Ananas comosus	3.125	-	-	1.563
Camellia japonica	1.563	3.125	-	0.781
Carica papaya	-	-	-	1.563
Carya illinoinensis	0.391	1.563	-	0.781
Cichorium intybus	3.125	3.125	-	0.781
Citrus limon	-	3.125	-	3.125
Coffea arabica	-	-	-	3.125
Ficus benjamina	3.125	3.125	-	1.563
Ficus natalensis	-	3.125	-	3.125
Moringa oleifera	3.125	1.563	-	1.563
Morus nigra	3.125	3.125	-	3.125
Persea Americana	3.125	-	-	1.563
Psidium guajava	0.781	1.563	-	0.781
Tulbaghia violacea	3.125	6.25	-	3.125
Vernonia amygdalina	1.563	1.563	-	1.563
Zingiber officinale	3.125	-	-	3.125
Neomycin	0.0016	0.0063	0.025	0.0016

Sa- *Staphylococcus aureus*, Sf- *Streptococcus faecalis*, Ec- *Escherichia coli*, St- *Salmonella typhimurium*

cepa displayed an exceptional activity of 1598.46 ml/g. However, *C. papaya* was ineffective against the other three bacterial strains, but it exhibited efficacy as high as 406.26 ml/g against *S. typhimurium*.

4. Discussion

The results of this study revealed that the antibacterial property of the plant extracts is species dependent. The bacterial species tested in this study depicted variable degree of sensitivity to the plant extracts and it is interesting to note that bacterial strain

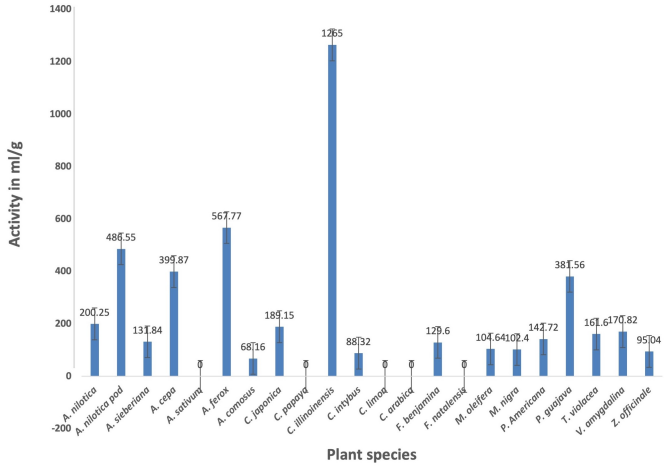

Fig. 1 Activity of plant extracts against S. aureus

tested also affects the antibacterial efficiency in addition to the plant species. This is in line with Obeidat et al. (2012), who found that test microorganisms are crucial to the antibacterial effectiveness of plant extracts. According to Chukwujekwu and Van Staden (2016), different bacteria strains have different effects on the antimicrobial activity of plant extracts. *E. coli* demonstrated the highest level of resistance across all bacterial strains in both the runs, except against *A. nilotica* leaf and pod extracts. All tested bacterial strains were suppressed by *Acacia nilotica* leaf and pod extracts at different levels. The extracts of *C. arabica, A. sativum,* and *C. papaya* suppressed the growth of only *S. typhimurium*, whereas the extracts of other plants inhibited at least two strains.

A number of studies have reported the relative resistance of gram-negative as well as gram positive bacteria against plant extracts (Vlietinck et al 1995; Rabe and Van Staden, 1997; Nostro et al 2000). In this study the selection of bacteria for antibacterial assay of plants extracts was done based on the

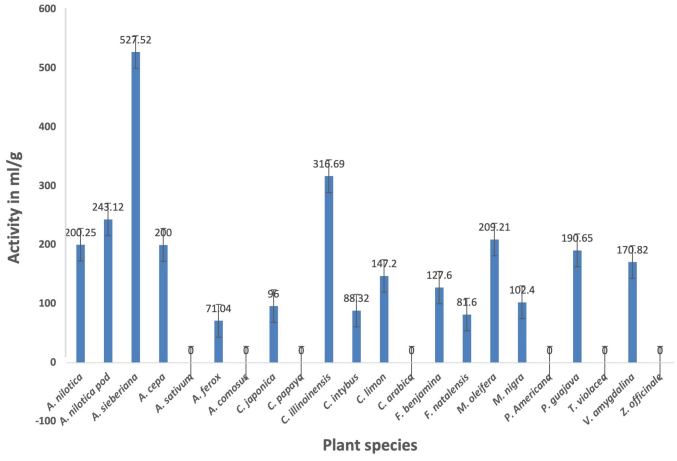

Fig. 2 Activity of plant extracts against S. faecalis

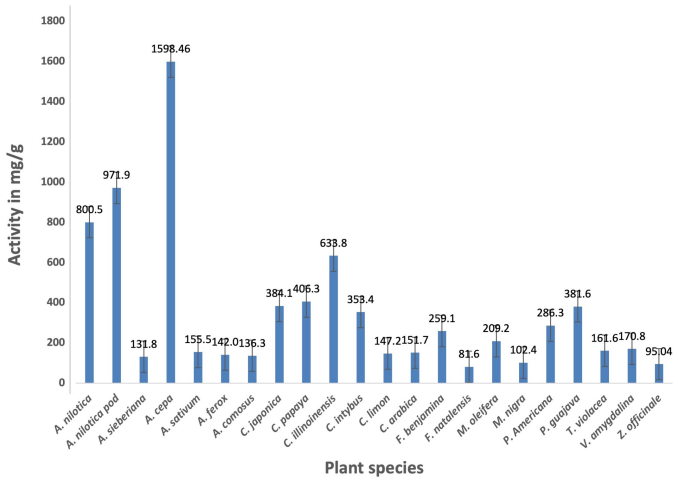

Fig. 3 Activity of plant extracts against S. typhimurium

nature of rumen bacteria gram. The outer membrane of gram-negative bacteria is made up of phospholipid and lipopolysaccharide bilayer (Worthington and Melander 2013) that restricts the influx of many antibacterial drugs into the cell and thus prevents them from reaching their intercellular targets. This is in contrast to the porous nature of peptidoglycan layer of gram-positive bacteria, which are more susceptible to influx of antibacterial plant extracts (Burt 2004). However, growth of *S. typhimurium,* a gram-negative bacteria, was inhibited by all plant extracts tested in current study.

The reports of Shekar et al (2015) and Sserunkuma et al (2017) that *A. nilotica* extract suppressed the growth of all the bacterial strains examined in their experiments is consistent with the antibacterial activity of *A. nilotica* plant parts observed in this study. Strong antibacterial activity was demonstrated by *A. nilotica* against *E. coli*, *S. typhi*, and . *typhi* (Kalaivani and Mathew 2010). There have been reports of bioactive phytochemicals, tannins, and alkaloids in *A. nilotica* plant extract, which may explain its strong activity against bacterial strains tested (Okoro et al 2014). All these bioactive substances were reported to prevent growth of bacteria (Payne et al 2013).

In the diffusion assay *Psidium guajava* demonstrated strong antibacterial activity against gram-positive bacteria and the antibacterial activity was on higher side against all bacteria except *E. coli*. On similar lines, there has been the reports of growth suppression of gram positive bacteria in earlier studies as well (Biswas et al. 2013; Nascimento et al. 2000). In another study, strong antibacterial activities of *A. cepa*, *A. sativum*, and *Z. officinale* extracts were observed against all the bacteria tested (Yousufi 2012). Furthermore, the exposure to the extract of *P. guajava* inhibited both gram-positive and gram-negative bacteria (Chanda and Kaneria 2011)). The differences in the results compared to the current study might be because of the variations in the extraction techniques and concentration levels. The inactivation of antimicrobial substances on heat treatment of fresh plant extracts of *A. cepa* and *A. sativum* was observed (Amin and Kapadnis 2005), which suggests that the bioactive compounds exerting antimicrobial effects are not heat stable.

Various earlier studies reported broad-spectrum antibacterial action of the leaf extract of *Carica papaya* (Anibijuwon and Udeze 2009; Alabi et al. 2012; Ocloo et al. 2012; Aruljothi et al. 2014), but interestingly, in the diffusion assay of present study it was ineffective against all the tested bacterial strains. But, it would be interesting to note that compared to the concentration of 10 mg/disc utilised in this study, the antibacterial activity for *Carica papaya* extract was observed at a higher concentration (Alabi et al. 2012; Aruljothi et al. 2014). For all of the bacterial strains tested, no antibacterial activity was observed by them at 50 mg/ml and 25 mg/ml levels, respectively, but at higher doses antibacterial activity was observed. This suggests that *C. papaya* extract may demonstrate action against the tested bacteria at higher doses. The present study revealed antibacterial activity only against the gram-negative *S. typhimurium*. The higher susceptibility gram-negative *S. typhimurium* to the *C. papaya* leaf was corroborated by earlier studies as well (Nirosha and Mangalanayaki 2013; Aruljothi et al. 2014).

The *Morus nigra* L. extract has shown significant antibacterial activity against gram-positive as well as gram-negative bacteria, including the highly resistant *E. coli* strains (Cestic et al. 2016). The MIC the aqueous extract ranged from 0.039 to 0.1563 mg/ml. All the bacteria tested in the present study were susceptible to the ethanolic extract of *M. nigra* at higher concentration except for the *E. coli*. Since, the efficacy of antibacterial activity of plant based extracts is dependent on the solvent used for extraction (Eloff 1998b; Obeidat et al 2012) the potential variance in these results might be caused by variations in the extraction solvents. In the present study, the extract of pecan nutshell (*C. illinoinensis*) suppressed the growth of the two gram-positive bacteria as well as the gram-negative *S. typhimurium*. On similar lines, an aqueous extract of this plant has demonstrated antibacterial activity against various gram-negative and gram-positive bacteria, including *Salmonella enteritidis* and *Pseudomonas aeruginosa*, but no action was reported against *E. coli* (Caxambu et al. 2016). The extract of pecan nutshell inhibited the growth of gram-positive bacteria, while gram-negative bacteria turned out to be resistant (do Prado et al. 2014). The diverse origins of the plant material and the extraction technique may be the cause of this difference. Similarly, a strong antibacterial activity was exerted by the *Cichorium intybus* leaf extract against *S. typhimurium* and the growth of other tested bacteria was inhibited as well, with the exception of the resistant *E. coli*. However, they were susceptible to the methanolic and acetone extracts of *C. intybus* leaf.

More number of plant extracts demonstrated the

antibacterial activity in micro dilution assay compared to agar disc diffusion test. However, similar activity was demonstrated by both assays against *E. coli*. In the diffusion assay, only three plant extracts demonstrated activity against *S. typhimurium*, whereas in dilution assay at different concentrations all plant extracts suppressed its growth. In the dilution assay, *S. typhimurium* turned out to be the most susceptible of all the microbial strains tested. The extent of diffusibility of the plant extracts through the cells can be the reason for difference in susceptibility of these bacterial strains. The disc diffusion approach may not be appropriate for determining the susceptibility of microbes because of the unique physico-chemical properties of the antimicrobial drug molecules (Wanger 2009). It has been reported that large molecular weight bioactive compounds diffuse relatively slowly in agar, which creates concentration gradients around the impregnated disc (Oses et al 2016). This restriction in diffusion may result in no inhibition zone or a smaller inhibition zone, which causes the microbe to exhibit false resistance (Kwakman and Zaat 2012).

The lower sensitivity of disc diffusion test has also been ascribed to the nature of filter paper (Burgess et al 1999; Valgas et al 2007). The filter paper discs, carrying the antimicrobial drug, is made of cellulose which has inter-linked glucose monomers. The disc's surface becomes hydrophilic due to the presence of free hydroxyl groups on the glucose monomers (Braithwaite and Smith 1990). Plant extracts contain bioactive compounds which can be either cationic or anionic (Bart 2011; Saini et al. 2016) and thus, influence their diffusion in the filter paper discs. If they are cationic in nature, they would adsorb on the disc's surface rather than disperse into the agar. Hence, the antimicrobial effects of such cationic compounds would be weakened in the disc diffusion technique, whereas the apolar compounds won't be impacted by the hydroxyl groups and will disperse freely (Valgas et al 2007). Therefore, the agar disc diffusion technique can potentially compromise the antibacterial activity of the compound and give a false impression of resistance.

The extracts of *A. cepa*, *A. sativum*, *C. papaya*, *M. oleifera*, and *T. violacea* did not affect all the tested bacterial strains in the disc diffusion assay, whereas in dilution assay all of them inhibited at least one bacteria. However, in the dilution assay, *A. cepa* exhibited extremely potent efficacy against *S. typhimurium*. It has been reported that *M. oleifera* extract contains cationic protein molecules which interact with anionic lipid membrane of microbes to aggressively inhibit bacterium cells (Saini et al 2016). This may be the reason of no efficacy of *M. oleifera* extract against any bacteria tested in the disc diffusion technique and inhibition of three bacteria out of the four tested in dilution technique. Therefore, the dilution approach need to used for testing plant extracts containing compounds of polar nature because the diffusion method is inefficient when dealing with polar extracts (Rios and Recio 2005). Earlier, Rios et al (1988) came to the conclusion that the best approach for determining the true potency of a pure substance is the liquid dilution method.

The actual antimicrobial activity of the plant materials was assessed against each of the bacterial strains tested. According to Eloff (2000), this is the highest volume to which one gram of biologically active plant material may be diluted without losing its ability to inhibit the growth of a given bacteria. Not withstanding the knowledge of authors, Eloff was the only one to report on the action of plant material on a bacteria strain (Eloff 2000, 2001). The extract yield and bacteria with the lowest MIC values were used to examine the overall antibacterial activity of plant material on microorganisms (Moyo et al. 2011). These numbers enable a useful comparison of the effectiveness of various plant components against the examined microorganisms (Fig. 1-3). For example, this study suggests that even after being diluted to 1265 ml, the bioactive substances found in one g *C. illinoinensis* still prevents the growth of *S. aureus* (Fig. 1).

The majority of research on the antibacterial effects of plant extracts do not report the extract yield Eloff (2000). In an earlier study the leaves of *A. nilotica* and *P. guajava* had extract yield of 18.96 and 25.14%, respectively (Shekar et al. 2015) which was relatively lower than the yields obtained in this study. The harvesting season, the maturity stage of the plant at harvesting, and the location of the plant contribute to variations in the extract yield. The ability of *E. coli* to synthesise tannase (TanBFnp), an enzyme that degrades tannin, a characteristic feature of fungi and certain bacteria as an adaptation mechanism to phenolic stress, may generally be used to explain the resistance of *E. coli* against most plant extracts tested (Tomas-Cortazar et al 2018). It has also been reported earlier that several bacteria, including *E. coli*, can exhibit growth when tannins and their monomers are present by utilising them as a carbon source (Scalbert 1991).

5. Conclusion

A remarkable antibacterial effect was demonstrated by the majority of the selected plants for gram-positive bacteria. It was clear that the extract of *A. nilotica* plant may be employed therapeutically against pathogens of animals as a natural broad-spectrum antibacterial. To effectively use the antibacterial capabilities to control rumen microorganisms toward an effective rumen fermentation, more research is needed.

Declarations

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