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Research Article
Antimicrobial Activity and Phytochemicals Analysis of *Vernonia aemulans*, *Vernonia amygdalina*, *Lantana camara* and *Markhamia lutea* Leaves as Natural Beer Preservatives

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Abstract

Background: African traditional beers are both considered as food and beverages for African people and hence preserving them using the natural additive is of utmost importance. In the present study, the antimicrobial activity of aqueous and ethanol extracts of Rwandan plants *Vernonia aemulans*, *Vernonia amygdalina*, *Lantana camara* and *Markhamia lutea* leaves were tested against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Sacharomyces cerevisiae.* **Methodology:** The antimicrobial activity was carried out by the disc diffusion method. The phytochemical screening of ethanolic extracts of these Rwandan plants was determined using standard method of analysis. **Result:** The results showed that the ethanol and aqueous extracts of *V. aemulans*, *V. amygdalina*, *L. camara* and *M. lutea* leaves have antibacterial activity against food spoilage bacteria and food-borne pathogens with inhibitory zone diameters ranging between 3-26 mm. All extracts analyzed did not possess antimicrobial activity against *S. cerevisaie*, which plays major role in African beers fermentation. The Gram-negative bacteria tested were found to be resistant only against the extracts of *M. lutea* leaves. The extracts of *V. aemulans*, *V. amygdalina* and *L. camara* possess antibacterial activities both against the Gram-positive (*B. subtilis* and *S. aureus*) and negative (*E. coli* and *S. typhimurium*) bacteria with the minimum inhibitory concentration ranging from 2-16 mg mL^{-1}. These inhibitory properties had been attributed to the presence of tannins (9.2-99 mg g^{-1}), flavonoids (62.4-87.4 mg g^{-1}), saponins (39.8-65 mg g^{-1}), phenolic compounds (22.6-42.8 mg g^{-1}) and alkaloids (32-40.7 mg g^{-1}) in these plants. **Conclusion:** The findings established that *V. aemulans*, *V. amygdalina* and *L. camara* leaves can be used as natural beer preservatives with considerable market opportunities in African brewing industry due to their strong antimicrobial activity imparting extended shelf-life with less harmful effects.

Key words: Beer preservatives, *Vernonia aemulans*, *Vernonia amygdalina*, *Lantana camara*, *Markhamia lutea*, antimicrobial activity, phytochemical analysis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In sub-Saharan Africa, traditional beers such as ikigage (Rwanda), pito (Ghana), dolo (Burkina Faso), tchoukoutou (Benin and Togo) and burukutu (Nigerian) contribute significantly to the diet of millions of African people. These beverages, prepared from sorghum, millet and unhopped are very rich in calories, B-group vitamins including thiamine, folic acid, riboflavin and nicotinic acid and are high in essential amino acids such as lysin[1,2]. However, African traditional beverages are characterized by a poor sanitary quality, variations of organoleptic quality and short shelf life. Previous studies reported the presence of *Staphylococcus aureus*, *Escherichia coli* and many food spoilage microorganisms in African traditional beers[3,4]. Thermal processing is a common method of destroying vegetative microorganisms to ensure food safety, but this technique may cause undesirable nutritional and quality effects[5]. Currently, increasing regulatory restrictions on the food preservatives, consumer negative response to chemical preservatives, the demand of food with extended shelf-life and absence of risk causing food borne infections have made that food processors focus on exploring naturally occurring preservatives. Natural preservatives have the capability of not only preserving beverages, but also being able to impart health benefits may be desirable for consumers.

The plant species have been used as beer additives for flavour and above all as food preservatives since ancient times due to their antimicrobial activities against certain pathogen microorganisms[6-10], antioxidative properties and essential oils[11,12].

In Western brewing beers, the female flowers of plant *Humulus lupulus* (generally called hops) are widely used as one of the standard ingredients of beer since the enactment of the reinheitsgebot in 1516. Hops are mainly responsible for the bitterness and some of the flavours and aromas of beer. They also contribute to the biological stability of beer. During wort boiling the bitter and aromatic hop components are transferred into the wort. It had been assumed that hops offer complete protection of beer from microbial contamination[13]. However, hop plant is a temperate crop[14] and cannot be successfully grown in tropical regions like sub-Saharan Africa. Hence, the use of hops is not appropriate in the traditional context of African brewing beers. It is imperative to find hop substitutes from local plants.

In Rwanda, during the preparation of Ikigage beer, the sorghum wort is inoculated by a traditional leaven "Umusemburo" as fermentation starter, which is prepared from malted sorghum with the leaves of certain local plants, mainly *Vernonia amygdalina*, *Vernonia aemulans*, *Markhamia lutea* and *Lantana camara*[3,15]. But, the role of these plants in the preparation of Rwandan traditional leaven is not yet well-known.

The *V. amygdalina*, commonly called bitter leaf or called umubirizi in Rwandan language is a tropical plant belonging to the Astaraceae family and is used widely as vegetable and medicinal plant. It is a shrub of about 2-5 m with a petiolate leaf of about 6 mm in diameter and elliptic shape. The leaves are green with a characteristic odour and bitter taste. It does not produce seeds and has to be distributed or propagated through cutting. It grows under a range of ecological zones in Africa and produces a lager mass of forage and it is drought tolerant, with about 200 species including *V. aemulans* (called Idoma in Rwandan language). However, extract of *V. amygdalina* had been reported to exert antibiotic action against drug resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities[16]. The *V. aemulans* is used in Rwanda against some bacterial infections especially gonorrhoea[17-19].

Markhamia lutea (known as Umusave in Kinyarwanda), native to Eastern Africa and cultivated for its large bright yellow flowers is a tree species of the plant family Bignoniaceae and is used locally to treat anaemia and diarrhoea and various microbial and parasitic diseases[20].

Lantana camara, known as umuhengeri in Rwanda is a flowering ornamental plant belonging to family Verbenaceae and is well known as medicinal plant in traditional medicinal system[21]. It contains lantadenes, pentacyclic triterpenes which is reported to possess a number of useful biological activities. Several previous reports have described antifungal, anti-proliferative and antimicrobial activities of *L. camara*[22,23].

The purpose of the present study was to determine and compare the potential of *V. aemulans*, *V. amygdalina*, *L. camara* and *M. lutea* as antimicrobial agent against the microorganisms responsible of food spoilage and food poisoning such *Bacillus subtilis*, *Sacharomyces cerevisiae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*.

MATERIALS AND METHODS

Collection of plant samples: The fresh leaves of *V. aemulans* (Idoma), *V. amygdalina* (Umubirizi), *L. camara* « Umuhengeri» and *M. lutea* «Umusave» were collected from Huye and Musanze districts of Rwanda. The plants were authenticated at the Botanic Laboratory, Department of Biology, School of Science, College of Science and Technology, University of Rwanda.

Preparation of extracts: Plant leaves collected were washed thoroughly with distilled water. The leaves were dried under oven at 45°C for 3 days. The dried leaves were well grinded into fine powder using electrical grinder and then stored in air tight containers for further use. Two hundred and fifty grams of the pulverized plant material was extracted for 3 days in absolute ethanol (Sigma-Aldrich) and sterile water[24]. The separated extracts were then filtered through Whatman's No. 1 filter paper and the filtrates were then separately condensed to dryness using rotary evaporator. The thick extracted mass was then dried at room temperature. Dried extract was collected in an air tight container and stored at 4°C for further analysis.

Antimicrobial activity essay: The antimicrobial activity of various extracts from *L. camara*, *M. lutea*, *V. amygdalina* and *V. aemulans* were screened against *Escherichia coli* (CWBI-WT), *Bacillus subtilis* (CWBI-FZB42), *Saccharomyces cerevisiae* (CWBI-F451), *Staphylococcus aureus* (CHUB) and *Salmonella typhimurium* (CHUB) obtained from the collection of Centre Wallon de Biologie Industrielle (CWBI) and Butare University Teaching Hospital (CHUB). The bacterial isolates were first sub-cultured in a nutrient broth (Sigma-Aldrich) and incubated at 37°C for 18 h, while the yeast was sub cultured on a Sabouraud broth (Sigma-Aldrich) for 48 h at 28°C.

The antimicrobial assay was carried out by the disc diffusion method as described elsewhere[25,26]. The extracts were concentrated and dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) to a final concentration of 10 mg mL^{-1} and sterilized through 0.45 µm millipore filters. One hundred microliters of suspension containing 10^3 colony forming unit (CFU mL^{-1}) of bacteria and 10^5 CFU mL^{-1} of yeast were inoculated on Mueller Hinton agar (Sigma) and Sabouraud agar (Sigma-Aldrich), respectively. Discs of 6 mm diameter were impregnated with 10 µL of extracts and placed on the inoculated agar. The plates containing bacteria were incubated at 37°C for 24 h, while those containing yeast were incubated at 28°C for 48 h. The inhibition zones were measured for determining the antimicrobial activity and the findings were tabulated.

Minimum inhibitory concentration: The determination of Minimum inhibitory concentration for bacterial and yeast strains was performed using agar dilution method[27]. Varying concentrations of each extract (0.125-20 mg mL^{-1}) were prepared in 5% DMSO and filter sterilized (0.45 µm). The extract was mixed with sterilized Mueller Hinton agar dispensed into sterile petri dish. Five microliters of standardized inocula (matching 0.5 McFarland turbidity standards) of various test isolates was seeded on Mueller Hinton agar plate. Test strains on solvent free Mueller Hinton agar and DMSO incorporated in Mueller Hinton agar served as growth and solvent controls respectively. Minimum inhibitory concentration was determined after 24-48 h incubation at 37°C.

Qualitative and quantitative analysis of phytochemicals: To detect the presence of tannins, alkaloids, flavonoids, saponins, glycosides, carbohydrates steroids, phenolics, phlobatannins and terpenoids, the phytochemical screening of the extracts was done using the method described elsewhere[28-30].

The phytochemicals which are present in the ethanol extracts of *V. aemulans*, *V. amygdalina*, *L. camara* and *M. lutea* were determined and quantified by standard procedures as described by Gracelin *et al*.[31].

Determination of total phenols: A total phenolic compound was determined spectrophometrically according to Folin-Ciocalteu colorimetric method[32]. One hundred micrograms of the extract of the sample was weighed accurately and dissolved in 100 mL of Triple Distilled Water (TDW). One milliliter of this solution was transferred to a test tube, then 0.5 mL 2 N of the Folin-Ciocalteu reagent and 1.5 mL 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 mL with TDW followed by vigorous shaking and finally allowed to stand for 2 h after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

Determination of total flavonoids: The method is based on the formation of the flavonoids aluminium complex which has an absorptivity maximum at 415 nm[33]. One hundred microliters of plant extracts in methanol (10 mg mL^{-1}) was mixed with 100 µL of 20 % aluminum trichloride in methanol and a drop of acetic acid and then diluted with methanol to 5 mL. The absorbance at 415 nm was read after 40 min. Blank samples were prepared from 100 mL of plant extracts and a drop of acetic acid and then diluted to 5 mL with methanol. The absorption of standard rutin solution (0.5 mg mL^{-1}) in methanol was measured under the same conditions. All determinations were carried out in triplicates.

Determination of total alkaloids: Five grams of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic

acid in methanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed[28].

Determination of total tannins: Sample (500 mg) was weighed into a 50 mL plastic bottle. Fifty millilitres of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtered was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min[34].

Determination of total saponins: The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty millilitres of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated[35].

Statistical analysis: The experiments were conducted in triplicate and the results were expressed as mean with standard deviation. Statistical analysis of the data was performed using SPSS package program. Statistical significance was taken at 95% confidence interval when $p<0.05$. When analysis of variance (ANOVA) revealed a significant effect ($p<0.05$), the data means were compared by the least significant difference (Duncan's multiple range test) test.

RESULTS

Antimicrobial evaluation: The results of antimicrobial activities of *M. lutea*, *V. aemulans*, *L. camara* and *V. amygdalina* leaves are presented in Table 1. These results shown that all extracts tested possess high inhibitory effects against *B. subtilis* (9.2-26.5 mm of inhibition zone diameter) and *S. aureus* (5.3-23.1 mm of inhibition zone diameter), but not possess any antimicrobial activity against *S. cerevisiae*. The extracts of *V. aemulans*, *V. amygdalina* and *L. camara* possess antibacterial activities against *E. coli* and *S. typhimurium* with inhibition zone diameter ranging from 3.0-18.6 mm. These Gram-negative bacteria were found to be resistant against the extracts of *M. lutea* leaves. However, the diameters of inhibition zones of ethanol extracts were larger than those of aqueous extracts ($p<0.05$) for all bacteria tested.

Ethanol extracts of *V. amygdalina*, *V. aemulans*, *M. lutea* and *L. camara* leaves showed varying degree of Minimum Inhibitory Concentration (MIC) (Table 2). Growth of *B. subtilis* was inhibited at a MIC of 2 mg mL^{-1} for all extracts analyzed. The MIC values of 2, 8 and 16 mg mL^{-1} were recorded for extracts of *L. camara*, *V. amygdalina* and *V. aemulans* respectively against *S. typhimurium*. The *E. coli* was inhibited at a MIC of 2 mg mL^{-1} for extracts of *V. aemulans* and *V. amygdalina* and at a MIC value of 16 mg L^{-1} for *L. camara*. It was observed also that the extracts of *M. lutea* exerted more activity on *S. aureus* with MIC value of 1.5 mg mL^{-1}.

Phytochemical analysis: Phytochemical screening of the extracts was carried out to detect the presence of tannins,

Table 1: Antimicrobial activities of extracts of some Rwandan plants (100 µ mL^{-1})

		Zone of growth inhibition (mm)				
Rwandan plants	Extract solvent	*Escherichia coli*	*Salmonella typhimurium*	*Bacillus subtilis*	*Staphylococcus aureus*	*Sacharomyces cerevisiae*
Lantana camara	Ethanol	8.3±0.4[a]	11.6±0.5[a]	19.0±0.8[b]	13.6±1.4[a]	R
	Water	3.0+0.1[b]	4.8±0.6[b]	11.2±06[a]	6.1±0.6[b]	R
Vernonia aemulans	Ethanol	18.0±0.8[c]	9.0±0.8[a]	22.8±0.4[b]	12.9±0.1[a]	R
	Water	9.5±1.2[a]	4.0±0.7[b]	10.2±0.5[a]	5.8±0.3[b]	R
Vernonia amygdalina	Ethanol	18.6±1.6[c]	9.3±1.2[a]	20.1±1.6[b]	15.0±0.3[a]	R
	Water	8.0+0.2[a]	3.8±0.5[b]	9.2±1.6[a]	5.3±0.4[b]	R
Markhamia lutea	Ethanol	R	R	26.5±0.3[c]	23.1±1.5[c]	R
	Water	R	R	10.8±1.2[b]	11.6±1.3[a]	R

R: Resistant at the concentration of 100 µ mL^{-1} of aqueous and ethanol extracts, [a-c]Means in a same column with different letters are significantly different ($p<0.05$)

Table 2: Minimum Inhibitory Concentration (MIC) of some Rwandan plants against *E. coli*, *S. aureus*, *S. typhimurium*, *B. subtilis* and *S. cerevisiae*

	Concentrations (mg mL^{-1}) of ethanol extracts			
Microorganisms	*Lantana camara*	*Vernonia amygdalina*	*Vernonia aemulans*	*Markhamia lutea*
Escherichia coli	16.0	2.0	2.0	R
Salmonella typhimurium	8.0	2.0	16.0	R
Bacillus subtilis	2.0	2.0	4.0	2.0
Staphylococcus aureus	2.0	2.0	2.0	1.5
Sacharomyces cerevisiae	R	R	R	R

R: Resistant to the concentrations from 0.125-20 mg mL^{-1} of extracts

Table 3: Phytochemical screening of ethanol extracts of *V. aemulans, V. amygdalina, L. camara* and *M. lutea* leaves

	Rwandan plants			
Phyto-constituents	*Vernonia amygalina*	*Lantana camara*	*Vernonia aemulans*	*Markhamia lutea*
Alkaloids	+	+	+	+
Glucosides	+	+	+	+
Carbohydrate	+	+	+	+
Flavonoids	+	+	+	+
Phenols	+	+	+	-
Saponins	+	+	+	+
Steroids	+	-	+	+
Phlobatannins	-	-	-	-
Tannins	+	+	+	+
Terpenoids	+	-	+	-

+: Presence, -: Absence (not detected)

alkaloids, flavonoids, saponins, glycosides, phenolics, steroids, phlobatannins and terpenoids in ethanol extract of *V. amygdalina*, *V. aemulans*, *L. camara* and *M. lutea* from Rwanda and the positive results are showed in Table 3. On a total of 9 phytochemical tests, 8 were positive in ethanol extract of *V. amygdalina*, *V. aemulans* and *L. camara* leaves. Seven tests were positive in ethanol extracts from *M. lutea* leaves.

The amount of phytochemicals which were found in ethanol extracts of *V. aemulans*, *V. amygdalina*, *L. camara* and *M. lutea* were quantitatively determined by standard procedures. Among the five components analyzed tannins content (99±2.6 mg g^{-1}) was highest in *V. amygdalina* extracts followed by flavonoids (70.2±1.5 mg g^{-1}), saponins (64±2.3 mg g^{-1}), phenolic compounds (35.5±2.2 mg g^{-1}) and alkaloids (32±0.6 mg g^{-1}) as shown in Fig. 1. While flavonoids content were high in the extracts of *V. aemulans*, *L. camara* and *M. lutea* with the concentrations of 87.4±1.7, 58.3±1.8 and 62.4±3 mg g^{-1}, respectively. The *V. aemulans* extract contained 82.6±3 mg g^{-1} of tannins, 44±0.6 mg g^{-1} of saponins, 40.7±0.7 mg g^{-1} of alkaloids and 22.6±0.3 mg g^{-1} of phenolic compounds. In *L. camara* extract 42.8±1.8 mg g^{-1} of phenolic compounds, 37.8±0.1 mg g^{-1} of alkaloids, 30.8±1.3 mg g^{-1} of saponins and 9.2±0.6 mg g^{-1} of tannins were found. In *M. lutea* extract 27±1.7 mg g^{-1} of

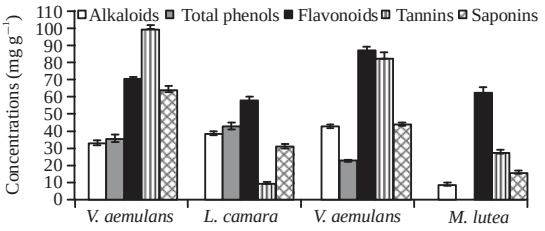

Fig. 1: Phytochemical components of ethanol extract of *V. aemulans*, *V. amygdalina*, *L. camara* and *M. lutea* leaves

tannins, 17.1±0.8 mg g^{-1} of saponins and 8±0.5 mg g^{-1} of alkaloids were observed.

DISCUSSION

Many plant extracts have been known to possess antimicrobial activities and are proposed as of food preservatives[26,36,37]. In the present study, the aqueous and ethanol extracts of *V. amygdalina*, *V. aemulans*, *L. camara* and *M. lutea* leaves possessed antibacterial activity against food spoilage bacteria and food-borne pathogens.

However, ethanol extracts of *V. amygdalina*, *L. camara* and *V. aemulans* exhibited pronounced activities against Gram-positive (*B. subtilis* and *S. aureus*) than Gram-negative bacteria (*E. coli* and *S. typhimurium*). In certain case, e.g., in extracts of *M. lutea* leaves, the results showed the total absence of antibacterial activity against the Gram-negative bacteria tested. Pelczar *et al.*[38] suggested that the difference in susceptibility of Gram-positive and Gram-negative bacteria to various antimicrobial agents probably depends on structural differences in their cell walls. Cell wall of Gram-negative bacteria is protected by an outer membrane that prevents permeation of the active molecule.

Several similar studies reported the antibacterial activities of *V. amygdlina*, *L. camara* and *M. lutea* against *E. coli* and *S. aureus*[39-43]. But, the antimicrobial activities of these plants against *S. cerevisiae* and *S. typhimurium* were not tested in their studies.

In this study, the results showed that all extracts analyzed did not possess antimicrobial activity against *S. cerevisiae,* which is reassuring for their use as hops substitute in African brewing beers due to the role played by *S. cerevisiae* in African beers fermentation. This resistance can be explained by the presence of bitter compounds in these plants as the case in hops. Studies of the antibacterial properties of hop compounds showed that they inhibit growth of Gram-positive bacteria and not Gram-negative bacteria and *S. cerevisiae*[44-46].

Unlike bacteria, yeast cells have a number of unique features that prevent cell damage from bittern compounds. These include modifying the cell wall in response to hop stress; reducing the concentration of alpha acids in the cell vacuole and actively purging alpha acids from the cell itself[46].

However, according to their microbial properties, *V. amygdalina*, *V. aemulans* and *L. camara* have an advantage over the hops because they are able to inhibit the growth of both Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-negative bacteria such us *E. coli* and *S. typhimurium*. The antibacterial inhibitory effects of these plants can be attributed to the presence of tannins, flavonoids, saponins, phenolic compounds and alkaloids in extracts of *V. amygdalina*, *V. aemulans* and *L. camara* leaves.

These phytochemical constituents were further reported to be responsible for many antimicrobial activities of different plant species[41,47,48]. Their concentration, composition, structure and functional groups serve an important role in determining antimicrobial activity. Phenolic compounds are generally the most effective due to their chemical structures[49], which may be divided into different categories including simple phenolic compounds, flavonoids, quinones, tannins and coumarins. Phenolic compounds contribute to the sensory properties when added to food and have antioxidant and antimicrobial properties[36,50], characteristics that are useful in extending the shelf-life of food. The antimicrobial effect of phenolic compounds may be due to their ability to alter microbial cell permeability, thereby permitting the loss of macromolecules from the interior. They could also interfere with membrane function (electron transport, nutrient uptake, protein, nuclein acid synthesis and enzyme activity) and interact with membrane proteins, causing deformation in structure and functionality[37]. Flavonoids have been reported to be synthesized by plants in response to microbial infections and are good antibacterial agent[51]. Tannins have been demonstrated antibacterial activities[52]. With proline-rich proteins, tannins form irreversible complexes which may be able to inhibit the cell-wall-protein synthesis of bacteria[52].

Some alkaloids from plants have also been used as antimicrobials in food[53]. Recently, saponins have been used as a preservative and/or used as a part of a preservative system to inhibit and/or reduce growth of spoilage microorganisms of beverages and foods[54].

CONCLUSION

The results of the present study indicate that all extracts of *V. aemulans*, *V. amygdalina*, *L. camara* and *M. lutea* leaves possess potent antibacterial activity against selected food spoilage bacteria and food-borne pathogens which might be due to the presence of tannins, flavonoids, saponins, glucosides and phenolic compounds in these plants. However, *M. lutea* extracts did not exhibit antibacterial activity against Gram-negative bacteria such as *E. coli* and *S. typhimurium*. All extracts analyzed did not possess antimicrobial activity against *S. cerevisiae*, which plays an important role in beer fermentation. Therefore, *V. aemulans*, *V. amygdalina* and *L. camara* leaves can be used as natural beer preservatives with considerable market opportunities in African brewing industry due to their strong antimicrobial activity imparting extended shelf-life with less harmful effects.

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