In vitro antibacterial activity of ethanolic crude extracts of *Capsicum annum* against *Staphylococcus aureus* and *Escherichia coli* isolated from pus and stool samples at Ruhengeri Referral Hospital, Rwanda

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Abstract

This work aimed at determining the antibacterial activity of ethanolic crude extracts of leaves and fruits of *Capsicum annum* against *Staphylococcus aureus* and *Escherichia coli*; isolated from pus and stools samples of patients in Ruhengeri Referral hospital, Rwanda. Fruits and leaves of *C. annum* samples were collected from Sina Gerard enterprise in Rulindo District in July, 2012. Plant samples were shade dried for 10 days, and then standard method was used for extraction using 96% ethanol as a solvent. Antibacterial activities of ethanolic crude extracts of fruits and leaves of *C. annum* were determined against *E. coli* and *S. aureus* using standard disc diffusion method. Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC) were also determined. Results showed that crude extracts of leaves and fruits of *C. annum* had antibacterial efficacy against *S. aureus* and *E. coli*. The mean diameter of inhibition zones and standard deviation of fruits crude extract against *S. aureus* and *E. coli* ranged from 8.17± 0.15 to 11.0 ± 0.89 mm, and 14.0 ± 0.25 to 17.9 ± 0.35 mm; respectively, while those of leaves crude extract ranged from 9.7 ± 0.26 to 14.7± 0.25mm, and 8.7± 0.35 to 11.1 ± 0.38 mm; respectively, at increasing extract concentration from 50 to 200 mg/ ml. MIC of fruits ethanolic crude extract against *E. coli* and *S. aureus* was 25 and 12.5mg /ml; respectively, while MBC was found to be 50 and 25mg/ ml, respectively. However, MIC of leaves ethanolic crude extract against *E. coli* and *S. aureus* were 50 and 12.5mg/ ml respectively, while MBC was found to be 100 and 50 mg/ ml, respectively. We concluded from this study that, ethanolic crude extract of both fruits and leaves of *C. annum* collected from Rwanda had antibacterial potential against tested bacteria, thus could be used as sources of new drugs.

Keywords: *Capsicum annum*, bacteria, pus, stool, Antibacterial activity, Ruhengeri Referral Hospital, Rwanda

1. Introduction

Global burden of diseases caused by *S. aureus* and *E. coli* particularly the resistant strains were still high (Kirk *et al*., 2015; Boswihi and Udo, 2018). These microorganisms were one of the major causes of morbidity and mortality in both hospital and acquired community infections (Kirk *et al*., 2015; Gahamanyi *et al*., 2017). Most strains of these bacteria developed resistance to the commonly prescribed drugs in the health care centers (Gahamanyi *et al*., 2017). These make it difficult to have effective drugs treatment of diseases caused by these and other related microbes. Thus there is an urgent need to look for alternatives or new drugs to face these global challenges (WHO. 2017).

The role of plants in herbal medicine as the major source of remedy in traditional care systems has been in medical practice for thousands of years (Ajanaku *et al*., 2018). According to Adia *et al*., (2014), the majority of the world's population in developing countries still relies on herbal medicine to meet their primary health care needs. Plants have a great potential for producing new drugs of great benefits to mankind (Adia *et al*., 2014). Folk medicine and systematic screening of these products may result in the discovery of novel effective compounds (Pavithra *et al*., 2010; Sandhya and Vijayakumar, 2016). The scientific evaluation of ethno medicinally important plants has received a global attention as new current fields of research; due to the increased global burden of antimicrobial drug resistances. Janvier *et al*., (2018) reported that although a vast indigenous knowledge about the medicinal plants was available in Rwanda; however only few of them have been scientifically and experimentally validated. Several previous researches in Rwanda showed the presence of a huge number of medicinal plants (Vlietinck *et al*., 1995; Amir and Kumar, 2004; Latha *et al*., 2010; Francine *et al*., 2015).

Capsicum annum (Red pepper or Bell pepper) is a member of the Solanaceae; which is a large tropical family. The exact origin of *C. annum* is still controversial, but several literatures showed that this plant may have originated from districts of Central America (Menichini *et al*., 2009; Vieira *et al*., 2015); or may have been domesticated in Mexico (Aguilar-Melendez *et al*., 2009). *C. annum* which is usually used to add color, pungency, and aroma to dishes in Rwanda; can also be used in modern medicine for the treatment of many symptoms such as; stomach ache, diarrhoea, dysentery and spasms of muscles in spine to mitigate pain as a topical analgesic (Sen *et al*., 2016; Sandhya and Vijayakumar, 2016). In Rwanda, many researchers reported antibacterial activities of different types of plants (Cos *et al*., 2002; Francine *et al*., 2015); but there was paucity of data on antimicrobial activity of Rwandan *Chili* spp. especially *C. annum*. Therefore this research was conducted to detect the antibacterial activities of ethanolic crude extract of fruits and leaves of *C. annum* against *E. coli* and *S. aureus* isolated from pus and stool of patients at Ruhengeri Hospital Referral, Rwanda.

2. Materials and methods

2.1. Study site, sampling and identification of *C. annum* plants

This study was carried out in biomedical laboratory Science, Department of Biomedical laboratory science, Ruhengeri Institute of applied sciences, Faculty of applied fundamental sciences, Institute of Applied Sciences Ruhengeri, Rwanda. The fresh leaves and fruits of *C. annum* were collected from Sina Gerard enterprise in Rulindo District, Rwanda, on July 2012 (Fig. 1). These *C. annum* plants were authenticated by a taxonomist. They were identified using Martin the Kew database at www.theplantlist.org. After that, the plants Voucher samples were deposited in Physico-chemistry laboratory of INES-Ruhengeri. Collection of plant materials was carried out according to the method described by Gayathri *et al*., (2016).
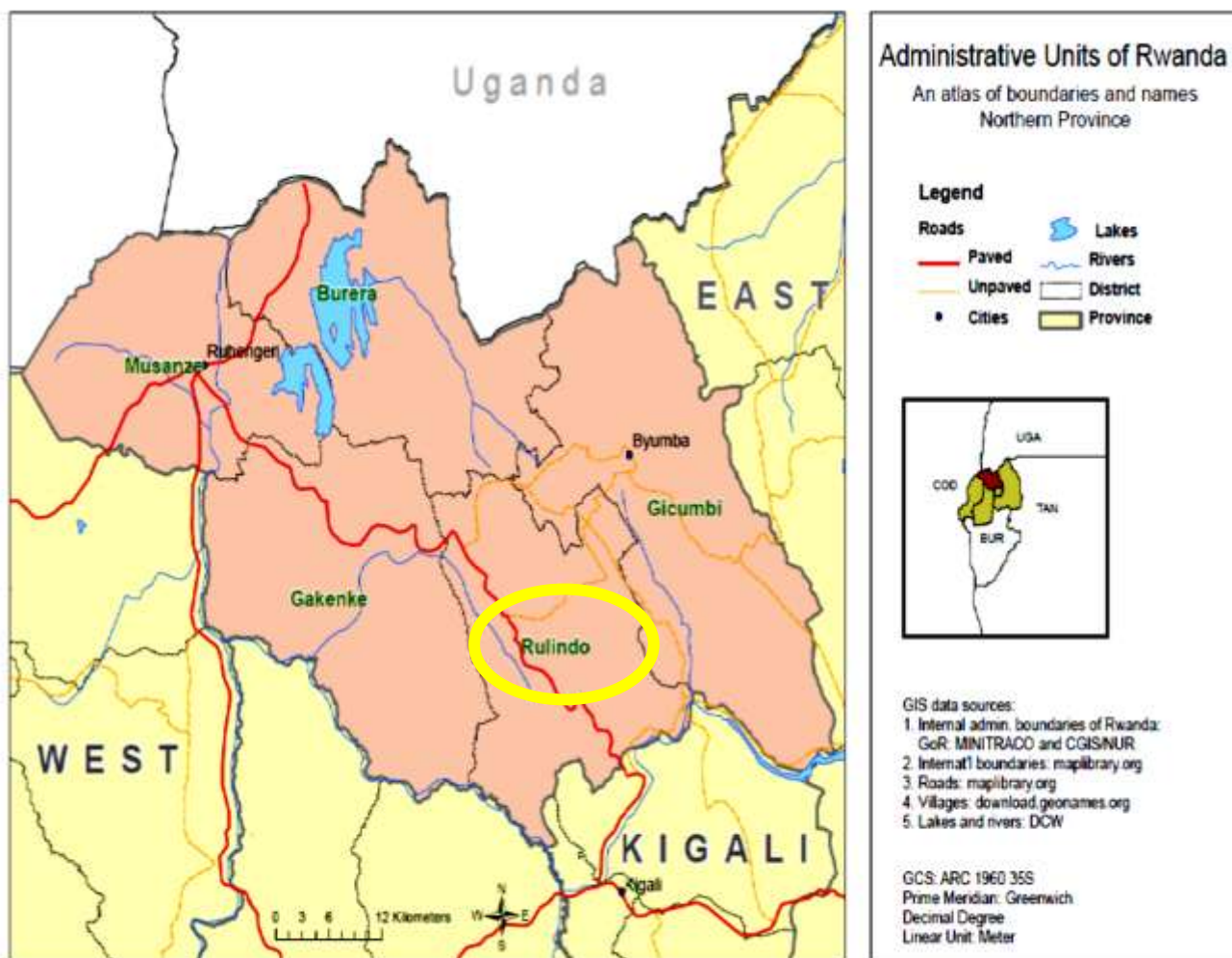
Fig. 1: Map of Rwanda showing the sampling site area circled in yellow (National Institute of Statistics, Rwanda, 2015).

2.2. Preparation of *C. annum* crude extracts

The collected plant materials were washed with sterile dist. water and then shade dried for 10 days. These dried plants were crushed using sterile mortar and pestle for leaves samples; and using mauler machine for fruits.

C. annum (i.e. leaves and fruits) ethanolic crude extracts were prepared using maceration method according to Anokwuru *et al*., (2011). The plant powder (100 g) was dissolved in 500 ml of 96% ethanol for 48 h with continuous shaking. The mixture was then filtered using Whatman no. 1 filter paper. The filtrate solution was concentrated using rotary vacuum evaporator (Büchi brand, France) at a temperature of 60°C to remove the ethanol remains. This was done to both plants samples (leaves and fruits). Dried crude extracts were stored at 4°C till further study.

2.3. Determination of percentage yield of crude extracts

The percentage yield of crude extracts from dried leaves and fruits was calculated using method described by Kumar *et al*., (2016), according the following formula:

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$$\text{Yield (\%)} = (W1 \times 100)/W2$$

Where: W1 is the weight of dry extract, and W2 is the weight of powder before drying.

2.4. Isolation and identification of test microorganisms

Pus and stool samples were collected from patients attending to Ruhengeri Referral Hospital according to the method described by (Virpari *et al*., 2013; Yakubu *et al*., 2014; Ibrahim *et al*., 2018). Mannitol Salt Agar (MSA) (Oxoid-CM0085, UK); and MacConkey agar (HiMedia-CM0115, India) were used as selective media for isolation of *S. aureus* and *E. coli*, respectively. Samples of pus and stool were inoculated by streaking on the surface of freshly prepared media; and then incubated at 37°C for 24 h. After incubation, colonies suspected to be *S. aureus* isolates (medium sized colonies that appeared golden yellow in color; and turned the MSA medium color into yellow after fermentation of mannitol sugars); and those colonies suspected to be *E. coli* (colonies that appeared bright pink/ red colored having dry and flat surfaces with bile precipitation in medium), were purified and then sub-cultured on MSA and MacConkey agar slants; respectively, to obtain pure cultures.

Identification of *S. aureus* and *E. coli* isolates were done using standard microscopical and biochemical tests such as; Grams staining, Indole production, Nitrate reduction, Methyl red, Coagulase, Oxidase, Voges proskauer, Citrate, Urease, Catalase, Motility test and Triple Sugars Iron test; Hydrogen sulphide production, fermentation of sugars (i.e. Glucose, sucrose and lactose), as described by Cheesbrough, (2006). All identified bacterial isolates were maintained on nutrient agar (NA) slants; and then stored at 4°C for further study.

2.5. Antibacterial potential of *C. annum* ethanolic crude extracts

2.5.1. Preparation of test bacteria and concentrations of crude extracts

The concentrations of the test bacteria (*E. coli* and *S. aureus*) were adjusted to turbidity standard of 0.5 McFarland standards (1-2.0×10⁸ cells/ ml) as described by Senjobi *et al*., (2017). Four different concentrations of each crude extract were prepared; 200, 150, 100 and 50 mg/ ml, using 10% Dimethyl sulphoxide (DMSO) as diluent (Sandhya and Vijayakumar, 2016). Filter paper discs (6mm each) were impregnated with 10 µl of each concentration of the extracts. Ciprofloxacin (10µl) disc and DMSO 10% were used as positive and negative controls, respectively.

2.5.2. Determination of the *in vitro* antibacterial potential of the *C. annum* crude extracts

Antibacterial activities of the crude extracts of fruits and leaves of *C. annum* were determined using standard disc diffusion method as described by CLSI. (2012). 0.1 ml (1× 10⁵ cells/ ml) of each test bacterium were spread over the surface of plates containing Mueller Hinton agar medium (HiMedia-M173, India) using sterile cotton swabs. The surfaces of the inoculated plates were left to dry for 30 min. at room temperature. This was followed by addition of impregnated discs that contained different concentrations of each extract; positive control (Ciprofloxacin, 10 µg) and negative control (10% DMSO), using sterile forceps. These plates were left at room temperature for 15 min. to allow the extract to diffuse, after which were incubated at 37°C for 24 h. The diameters of inhibition zones were measured using a ruler in millimeters (mm) and results were recorded. All treatments were conducted in triplicates, and results of inhibition zones (mm) were expressed as mean and standard deviations.

2.6. Determination of minimum inhibitory concentrations (MIC) of crude extracts

MIC of both fruits and leaves ethanolic crude extracts were determined using broth dilution method as described by (Andrews, 2002; Obiagwu *et al*., 2011; Rachuonyo *et al*., 2016). This assay was performed using Brain Heart infusion (BHI) broth medium (Oxoid-CM1135, UK). Two fold serial dilutions of both crude extracts from the stock concentration (200 mg/ ml) were carried out to obtain the following concentrations in tubes: 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/ ml. An equal volume (1ml) of each bacterial suspension at 1×10^6 cells/ ml was inoculated into each tube that contained 1ml of diluted extract concentration to form final bacterial density of 5×10^5 cells/ ml. Tubes were then incubated at 37°C for 24 h. Two controls were used; positive control tube containing BHI broth and test bacterium; while two negative control tubes were included; one tube containing BHI broth only, while the other had crude extract only. After incubation, tube that showed no visible turbidity next to the one showing turbidity (bacterial growth) was considered as MIC of the extract concerned in mg/ ml. MIC is defined as the lowest concentration of the extract in broth tube showing no visible bacterial growth.

2.7. Determination of minimum bactericidal concentrations (MBC) of crude extracts

MBC was determined by subculture of contents from BHI broth tubes which showed no visible bacterial growth on the surface of freshly prepared Mueller Hinton (MH) agar plates. Inoculated plates were then incubated at 37°C for 24 h. Absence of bacterial growth was interpreted as MBC of extract in mg/ ml. Obiagwu *et al*.,(2011) and Rachuonyo *et al*., (2016) defined MBC as the lowest concentration (mg/ ml) of the extract that kills 99.9% of the test bacteria.

2.8. Statistical analysis

Statistical analysis was carried out using PAST (3.14) software (Hammer *et al*., 2001). Data obtained was presented as mean and standard deviation. The mean diameters of inhibition zones of different concentrations of each ethanolic crude extract against each test bacteria; were compared using one way ANOVA. Statistical significance was considered at $p \leq 0.05$.

3. Results

3.1. Morphological and biochemical characterization of test bacteria

Identification of bacterial isolates obtained from pus and stools samples were confirmed using morphological and biochemical tests (Table 1). The confirmed isolates were used to determine the *in vitro* antibacterial activities of *C. annum* fruits and leaves ethanolic crude extracts.

3.2. Percentage yield of crude extracts

The percentage yield of ethanolic crude extracts of both fruits and leaves of *C. annum* is presented in Table (2). Results showed that both leaves and fruits crude extracts had almost the same extraction yields of 16.35% and 16.14%, respectively.

3.3. *In vitro* antibacterial potency of fruits and leaves ethanolic crude extracts

Results of antibacterial potential of both the leaves and fruits crude extracts of *C. annum* against the tested bacteria are shown in Table (3). The mean and standard deviations of zones of inhibition due to different concentrations of fruits ethanolic extract against *S. aureus* ranged from 8.17± 0.15 to 11.0± 0.89 mm; whereas, those due to leaves ethanolic extract ranged from 9.7± 0.26 to 14.7± 0.25 mm. On the other hand; the mean and standard deviations of zones of inhibition due to different concentrations of fruits ethanolic crude extract against *E. coli* ranged from 14.0± 0.25 to 17.9± 0.35 mm, while those due to leaves ethanolic crude extract ranged from 8.7± 0.35 to 11.1± 0.38 mm. Ciprofloxacin (10 µg) used as a positive control antibacterial agent showed mean and standard deviations of zones of inhibition which ranged from 20.3± 0.2 to 21.8±1.72 mm;

which were higher than those of the fruits and leaves ethanolic extracts. Ten percent (10%) DMSO diluent used as a negative control showed no antibacterial activity. MIC of fruits ethanolic crude extract of *C. annum* against *E. coli* and *S. aureus* were found to be 25 and 12.5 mg /ml, respectively, whereas the

MBC were observed to be 50 and 25 mg/ ml; respectively (Table 4). However, MIC of leaves ethanolic crude extract against *E. coli* and *S. aureus* were 50 and 12.5 mg/ml; respectively, while MBC were found to be 100 and 50mg/ ml, respectively (Table 5).

Table 1: Morphological and biochemical characterization of *E. coli* and *S. aureus* isolated from pus and stool samples

S/ no.	Suspected bacteria	Gram's reaction	Indole	Nitrate reduction	Methyl red	Voges proskauer	Coagulase	Oxidase	Citrate	Urease	Catalase	Motility	TSI Test Glucose	Sucrose	Lactose	H_2S	Gas production
1	*E. coli*	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	+
2	*S. aureus*	+	-	+	+	+	+	-	-	+	+	+	-	-	-	-	+

Table 2: Percentage yield of fruits and leaves ethanolic crude extracts of *C. annum*

Part of plant	Mass of powder (g)	Volume of solvent (ml)	Mass of extracts obtained (g)	Percentage yield (%)
Fruits	100	500	16.14	16.14
Leaves	100	500	16.35	16.35

Table 3: Mean and standard deviation of diameters of inhibition zones due to ethanolic crude extracts of fruits and leaves of *C. annum* against *S. aureus* and *E. coli*

Extracts concentration (mg/ ml)	Mean and standard deviation of diameters of inhibition zones (mm)			
	Fruits extracts		Leaves extracts	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
50	8.17±0.15[a]	14.0±0.25[a]	9.7±0.26[a]	8.7±0.35[a]
100	8.4±0.60[a]	16.0±0.25[a]	11.0±0.57[b]	8.57±0.45[a]
150	10.5±0.75[b]	16.2±0.30[a]	10.5±0.50[a]	10.1±0.31[a]
200	11.0±0.89[c]	17.9±0.35[b]	14.7±0.25[b]	11.1±0.38[b]
Ciprofloxacin (10μg)	20.3±0.2[d]	21.1±1.71[c]	21.1±0.67[c]	21.8±1.72[c]
DMSO 10%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Where: DMSO: Dimethyl sulphoxide. Mean and standard deviation value in the same column with different letters were significantly different at $p \leq 0.05$

Table 4: MIC and MBC of fruits ethanolic crude extract of *C. annum* against *E. coli* and *S. aureus*

S/ no.	Test organisms	Fruits crude extract concentrations (mg/ ml)							MIC (mg/ ml)	MBC (mg/ ml)
		100	50	25	12.5	6.25	3.125	1.5625		
1	*E. coli*	-	-	-	+	+	+	+	25	50
2	*S. aureus*	-	-	-	-	+	+	+	12.5	25

Where: MIC: Minimum Inhibitory concentration, MBC: Minimum bactericidal concentration. (-): Growth/ turbidity absent, (+): Growth/turbidity present

Table 5: MIC and MBC of leaves ethanolic crude extract of *C. annum* against *E. coli* and *S. aureus*

S/ no.	Test organisms	Leaves crude extract concentrations (mg/ ml)							MIC (mg /ml)	MBC (mg/ ml)
		100	50	25	12.5	6.25	3.125	1.5625		
1	*E. coli*	-	-	+	+	+	+	+	50	100
2	*S. aureus*	-	-	-	-	+	+	+	12.5	50

Where: MIC: Minimum Inhibitory concentration, MBC: Minimum bactericidal concentration,   (-): Growth/turbidity absent, (+): Growth/ turbidity present

4. Discussion

Recently, there is an increased burden of drug resistant microorganisms; especially pathogenic bacteria that caused higher morbidity and mortality rates in both hospital and community settings (WHO. 2017; Hay *et al*., 2018). This makes it difficult to provide effective health care treatments to the diseases caused by these microbes. Accordingly, there is an urgent need to search for new drugs from effective natural products such as; plants, soil microbes and other related sources to face this global challenge (Pavithra *et al*., 2010; Sandhya and Vijayakumar, 2016; WHO. 2017). This study was therefore undertaken to determine the antibacterial activities of *C. annum* fruits and leaves ethanolic extracts against pathogenic *S. aureus* and *E. coli* isolated from pus and stool samples. Identification of these isolated bacteria was confirmed using biochemical tests according to Cheesbrough, (2006).

Results of estimating percentage yield of *C. annum* fruits and leaves ethanolic crude extracts showed that the plant had good percentage of yield which was within the recommended range (10.96-20.29%); according to the Pharmacopoea British Herbal. (1996).

The current study detected the antibacterial activities of *C. annum* fruits and leaves ethanolic extracts against tested pathogenic bacteria. Both fruits and leaves extracts had almost similar means and standard deviations of zones of inhibitions against *S. aureus*; though there were statistically significant differences between the different concentrations used. Gayathri *et al*., (2016) also reported similar results (21 mm) zone of inhibition of acetone crude extract of *C. chinense* fruits, however, lower zone of inhibition (15 mm) was recorded from leaves acetone crude extract against *S. aureus*. Moreover, Sen *et al*., (2016) reported similar results from fruits of three different spp. of Chili

aqueous and methanol crude extracts against *S. auereus*, though there were differences in mean and standard deviations of zones of inhibition between the solvents used for extraction. This was in contrary to the findings of Sandhya and Vijayakumar, (2016) who reported differences in zones of inhibition of ethanolic crude extracts of leaves and fruits of eight different *Capsicum* spp. from kanyakumari district, Tamilnadu, India.

On the other hand, results of antibacterial potential of fruits and leaves ethanolic crude extracts against *E. coli*; showed that there were statistically significant differences in mean and standard deviations of zones of inhibition at lower concentrations (50-100 mg/ ml), but had almost the same deviations values at higher concentrations (150- 200 mg/ ml). This was inconsistent with the findings of Gayathri *et al*., (2016), who reported significant differences in mean and standard deviations of zones of inhibition (10 and 14 mm) between leaves and fruits acetone crude extracts of *C. chinense*, respectively. In addition, Sen *et al*., (2016) also reported differences in mean and standard deviations between the aqueous and methanol crude extracts of three different spp. of Chili against *E. coli*.

The abilities of fruits and leaves ethanolic crude extracts of *C. annum* to show biological activities against tested bacteria could be attributed to the presence of bioactive compounds such as; carbohydrates, tannins, saponins, flavonoids, phenols, alkaloids, terpenoids and volatile oils in these extracts; as reported earlier by Gayathri *et al*., (2016) from *C. chinense*. Presence of these compounds in plants have been reported earlier by Lee *et al*., (2000); Kurian and Starks, (2002); Vatsalya *et al*., (2012) to have anti-allergic, anti-inflammatory, anti-microbial, anti-mutagenicity, anticancer effects and high antioxidant activities.

Conclusion

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Current results reported that leaves and fruits ethanolic crude extracts of *C. annum* had antibacterial efficacy against the growth of *S. aureus* and *E. coli* isolated from pus and stool samples of patients at Ruhengeri Referral Hospital, Rwanda. The fruits ethanolic extracts had a higher antibacterial activity against Gram negative bacteria (*E. coli*) than Gram positive bacteria (*S. aureus*); whereas, leaves ethanolic extracts exhibited higher activity against *S. aureus* compared to *E. coli*. Accordingly, *C. annum* extracts could be used as prospective natural sources of antibacterial agents used in the treatment of ailments caused by these pathogenic bacteria. Thus these extracts represent potential alternatives to synthetic drugs; and overcome the burden of drug resistant bacteria in Rwanda. It was observed that the standard antibacterial agent i.e. Ciprofloxacin (10 µg) had the highest mean and standard deviations of zones of inhibition against the tested bacteria compared with the ethanolic crude extracts. This could be due to the purity of the standard drug compared with the crude extracts which might contain some compounds that have antagonistic effects; thus lowering extracts antibacterial activities against the tested bacteria.

Acknowledgment

Conflict of interests

The authors declare no conflict of interests.

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