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Determination of Antimicrobial Activity of Sorrel (*Hibiscus sabdariffa*) on *Escherichia coli* O157:H7 Isolated from Food, Veterinary, and Clinical Samples

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ABSTRACT The use of medicinal plants as natural antimicrobial agents is gaining popularity. Sorrel (*Hibiscus sabdariffa*) is widely used for the treatment of diseases. The objective of this study was to investigate the antimicrobial activity of sorrel on *Escherichia coli* O157:H7 isolates from food, veterinary, and clinical samples. Phenolics of the calyces were extracted from 10 g of ground, freeze-dried samples using 100 mL of 80% aqueous methanol. Concentrations of 10%, 5%, and 2.5% methanol extract of sorrel were investigated for its antimicrobial activity. Inhibition zones were indicated by a lack of microbial growth due to inhibitory concentrations of sorrel diffused into semisolid culture medium beneath the sorrel-impregnated disk. The results of this experiment showed that the most potent sorrel concentration was 10%, then 5%, and finally 2.5%. The overall mean zone of inhibition for the sorrel extract was 12.66 mm for 10%, 10.75 mm for 5%, and 8.9 mm for 2.5%. The highest inhibition zones (11.16 mm) were observed in veterinary samples, and the lowest (10.57 mm) in the food samples. There were significant ($P < .05$) differences among mean zones of inhibition found in the food, veterinary, and clinical sources. Based on the source of samples and concentration of sorrel extract, the lowest mean inhibition was 7.00 ± 0.04 mm from clinical samples, and the highest was 15.37 ± 0.61 mm from a food source. These findings indicated that sorrel was effective at all levels in inhibiting *E. coli* O157:H7; thus it possesses antimicrobial activity and hold great promise as an antimicrobial agent.

KEY WORDS: • *antimicrobial activity* • Escherichia coli • *phenolic* • *sorrel* • *zone of inhibition*

INTRODUCTION

EMERGING FOODBORNE PATHOGENS have become a major public health concern. Many naturally occurring compounds found in medicinal plants, herbs, and spices have been shown to possess antimicrobial activities against many foodborne pathogens. The enterohemorrhagic *Escherichia coli* strain O157:H7 is a major bacterial foodborne pathogen that causes illness from mild watery diarrhea to bloody diarrhea. In children and the elderly the enteritis could lead to complications such as hemolytic uremic syndrome.[1] *E. coli* O157:H7 has been recognized as a significant cause of foodborne and waterborne illness in the industrialized world.[2] Cattle are considered to be the major reservoir for *E. coli* O157:H7, and approximately 80% of herds (beef and dairy) may be affected. This has led to varying numbers of ground beef recalls annually, where contaminated ground beef was the initial source for transmission to humans.[3] The U.S. government (2008) ordered the largest beef recall in U.S. history (143.4 million pounds) due to contamination of *E. coli* and other bacteria.[4] More recently, outbreaks have been associated with drinking contaminated water or consumption of contaminated produce.[1,5]

Synthetic antibiotics provide the main basis for the therapy of bacterial infections. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug-resistant strains of several groups of microorganisms. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand for plant-based therapeutics is increasing in both developing and developed countries because of growing recognition that they are natural products, non-narcotic, and easily biodegradable, producing minimum environmental hazards, having no adverse side effects, and being easily available at affordable prices.

Thus, in light of the evidence of rapid global spread of resistant isolates, the need to find new antimicrobial agents is of paramount importance. Researchers are increasingly

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turning their attention to herbal products, looking for new leads to develop better drugs against resistant strains.[6] Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties.[7] There have been several reports on the antimicrobial activity of different herbal extracts.[8–11] Survival and growth of both susceptible and antibiotic-resistant *Campylobacter* strains have been inhibited effectively on agar plates and in contaminated ground beef by application of roselle (*Hibiscus sabdariffa* L.).[12] In a study on blanched spinach and minced cooked beef, using clove and tea tree essential oils at three and four times the minimum inhibitory concentration in *in vitro* studies was needed to restrict *E. coli* O157:H7 populations in the food materials.[13] Various antimicrobial effects on *E. coli* have been shown by anzer tea essential oils,[14] guarana extract,[15] hydrodistilled leaf oil of *Cinnamomum chemungianum*,[16] essential oils from leaves, stems, and flowers of *Salvia reuterana* (Family Lamiaceae),[17] and linalool vapor of bergamot and linalool oils.[18] Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases, and cutaneous infections.[19] According to the World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs.[20]

Sorrel, *H. sabdariffa* L. (Family Malvaceae), a medicinal herb commonly uses to make drink and pickle, is used in folk medicine in the treatment of hypertension, liver diseases, and fever.[21–24] Okasha *et al*.[25] reported that a decoction of the seeds is given to augment or induce lactation in poor letdown and maternal mortality. *Hibiscus* anthocyanins, a group of phenolic natural pigment present in the dried flower of *H. sabdariffa* and *Hibiscus rosa-sinensis*, have been found to have cardioprotective,[26] hypocholesterolemic,[27] antioxidative, and hepatoprotective[22] effects in animals. An aqueous extract of *H. sabdariffa* enhances cardiac Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase activities.[28] Anthocyanin pigments and other phenolic compounds (*Hibiscus* protocatechuic acid) also isolated from dried flowers of *H. sabdariffa* demonstrated protective effects against *tert*-butyl hydroperoxide–induced oxidative damage and hepatotoxicity both *in vitro* and *in vivo*.[22,29] The aqueous extract was found to be effective against *Ascaris galliavium* in poultry. Also, the coloring matter of the calyces is said to be lethal to *Mycobacterium tuberculosis*.[30] In India, a decoction of the seeds was given to relieve dysuria and many cases of dyspepsia and debility. *H. sabdariffa* has been reported to be antiseptic, aphrodisiac, astringent, diuretic, emollient, purgative, sedative, stomachic, and tonic. It is also a folk remedy for abscesses, bilious conditions, cancer, cough, dysuria, and scurvy[31] and was also found to be anticarcinogenic.[32]

These findings contribute to support and qualify the importance of screening natural products. Conclusive information is, however, critical with regard to its role as an antimicrobial. The efficacy of sorrel at 2.5%, 5%, and 10% has not been tested on *E. coli* O157:H7 isolated from food, veterinary, and clinical sources. Therefore the objective of this study was to evaluate the antimicrobial activities of sorrel (*H. sabdariffa*) on *E. coli* O157:H7 isolated from food, veterinary, and clinical sources using the disk diffusion method.

MATERIALS AND METHODS

Plant material

Dried sorrel calyces were purchased from a local grocery store and freeze-dried. The freeze-dried calyces were grounded to powder and then stored at −20°C until use.

Extraction of phenolics

The phenolics in powdered freeze-dried calyces were extracted by the ultrasound-assisted method.[33] Phenolics of the calyces were extracted from 10 g of ground, freeze-dried samples using 100 mL of 80% aqueous methanol. The mixture of freeze-dried powder and 80% aqueous methanol was sonicated for 20 minutes with continual nitrogen gas purging. The mixture was filtered through Whatman (Maidstone, United Kingdom) #2 filter paper using a Buchner funnel and rinsed with 50 mL of 100% methanol. Extraction of the residue was repeated using similar conditions. The two filtrates were combined and transferred into a 1-L evaporating flask with an additional 50 mL of 80% aqueous methanol. The solvent was evaporated using a rotary evaporator at 40°C. The remaining phenolic concentrate was dissolved in 50 mL of 100% methanol and diluted to a final volume of 100 mL using distilled deionized water obtained with a NANOpure™ water system (Barnstead, Dubuque, IA, USA). The mixture was centrifuged at refrigerated temperatures, using a Sorvall (DuPont, Wilmington, DE, USA) RC-5B refrigerated superspeed centrifuge, at 10,000 *g* for 20 minutes and then stored at −4°C for future use.

Antimicrobial activities of the sorrel disk diffusion method

Food samples (ground beef, spinach, and vegetable packs) were purchased from randomly selected area grocery stores (Huntsville, AL, USA). Clinical samples (human fecal matter) were obtained from Huntsville Hospital (Huntsville). Veterinary samples (rabbit carcasses) were acquired from the Alabama A&M University rabbit farm (Normal, AL, USA). Bacterial isolates were grown in Mueller–Hinton broth for 24 hours, swabbed evenly onto the surface of Mueller–Hinton agar using sterile cotton swabs, and allowed to dry. Filter paper disks (Difco, Detroit, MI, USA) impregnated in triplicates with control or 2.5%, 5%, and 10% sorrel were placed on the surface of each plate for each isolate and then incubated at 37°C for 16 hours. Inhibition zones were then measured to the nearest millimeter. Inhibition zones were indicated by a lack of microbial growth due to inhibitory concentrations of sorrel diffused into semisolid culture media (agar) beneath the sorrel-impregnated disc.

Statistical analysis

The data were analyzed by SAS version 9.1 software (SAS Institute, Cary, NC, USA) using a completely

randomized design, and means separation was done using Tukey's Studentized range test at a significance level of $P < .05$.

RESULTS

Antimicrobial activities of various concentrations (2.5%, 5%, and 10%) of sorrel extract against *E. coli* O157:H7 isolates from food, veterinary, and clinical samples are presented in Table 1. Based on source of samples and concentration of sorrel extract, the lowest mean zone of inhibition was 7.00 ± 0.04 mm from a clinical source, whereas the highest was 15.37 ± 0.61 mm from a food source.

Results showed that within each isolate from food source, the mean zone of inhibition produced at the 2.5% level of sorrel concentration ranged from a low of 7.13 ± 0.0 mm to a high of 10.02 ± 0.04 mm. At 5% sorrel concentration, mean inhibition zones varied from 8.83 ± 0.1 mm to 13.42 ± 0.11 mm. In the case of 10% sorrel concentration, variation was between a low of 10.56 ± 0.16 mm and a high of 15.37 ± 0.61 mm (Table 1).

The highest mean inhibition against the veterinary samples at the 2.5% sorrel concentration was 11.33 ± 0.06 mm, whereas the lowest was 7.59 ± 0.23 mm (Table 2). At the 5% sorrel concentration mean inhibition varied from 9.71 ± 0.11 mm to 12.57 ± 0.11 mm. The mean inhibitions produced at the 10% sorrel concentration were between 11.42 ± 0.12 mm and 14.62 ± 0.3 mm.

Antimicrobial activities evidenced by the effect of sorrel on clinical samples produced an overall mean zone of inhibition ranging from 7.00 ± 0.04 mm to 14.98 ± 0.00 mm (Table 3). At the lowest sorrel concentration (2.5%), the mean zone of inhibition ranged from a low of 7.00 ± 0.04 mm to a high of 10.15 ± 0.15 mm. At the highest level of sorrel concentration (10%), mean inhibition zones produced were between 7.97 ± 0.07 mm and

Table 1. Antimicrobial Activity of Sorrel on *E. coli* O157:H7 from Food samples

E. coli O157:H7 *isolates*	*Source*	*Zones of inhibition (mm)*		
		2.5% sorrel	*5% sorrel*	*10% sorrel*
MF-AAMU-1	Ground beef	7.59 ± 0.2[e]	9.33 ± 0.1[e]	11.51 ± 0.4[e]
MF-AAMU-2	Ground beef	8.33 ± 0.1[d]	10.46 ± 0.1[d]	12.52 ± 0.0[d]
MF-AAMU-3	Ground beef	7.40 ± 0.1[e]	11.59 ± 0.0[c]	12.41 ± 0.1[d]
MF-AAMU-4	Ground beef	8.42 ± 0.1[d]	10.07 ± 0.4[d]	11.54 ± 0.3[e]
MF-AAMU-5	Ground beef	7.26 ± 0.1[e]	8.83 ± 0.1[d]	10.56 ± 0.1[d]
MF-AAMU-7	Ground beef	8.64 ± 0.0[d]	10.37 ± 0.0[d]	11.78 ± 0.0[e]
MF-AAMU-119	Vegetable pack	8.62 ± 0.1[d]	10.63 ± 0.0[d]	12.13 ± 0.1[d]
MF-AAMU-10	Ground beef	7.76 ± 0.0[e]	9.7 ± 0.07[e]	12.67 ± 0.0[d]
MF-AAMU-11	Ground beef	10.02 ± 0.0[b]	11.25 ± 0.1[c]	15.37 ± 0.6[a]
MF-AAMU-12	Ground beef	10.02 ± 0.0[b]	12.2 ± 0.0[b]	14.59 ± 0.1[b]
MF-AAMU-13	Ground beef	9.84 ± 0.1[c]	11.9 ± 0.01[c]	12.83 ± 0.2[d]
MF-AAMU-14	Ground beef	7.52 ± 0.1[e]	10.2 ± 0.1[d]	12.42 ± 0.1[d]
MF-AAMU-15	Ground beef	8.31 ± 0.0 [d]	9.83 ± 0.0[e]	11.78 ± 0.0[e]
MF-AAMU-120	Vegetable pack	7.45 ± 0.1[e]	9.65 ± 0.0[e]	11.55 ± 0.1[e]
MF-AAMU-17	Ground beef	9.75 ± 0.1[c]	11.3 ± 0.1[c]	13.5 ± 0.0[c]
MF-AAMU-121	Vegetable pack	10.29 ± 0.0[b]	11.42 ± 0.0[c]	12.44 ± 0.0[d]
MF-AAMU-22	Ground beef	9.3 ± 0.0[c]	10.43 ± 0.0[d]	12.36 ± 0.1[d]
MF-AAMU-23	Ground beef	7.47 ± 0.2[e]	11.2 ± 0.0[c]	13.93 ± 0.0 [c]
MF-AAMU-24	Ground beef	7.13 ± 0.0[e]	9.02 ± 0.0[e]	11.49 ± 0.1[e]
MF-AAMU-26	Ground beef	10.35 ± 0.1[b]	11.23 ± 0.1[c]	12.49 ± 0.1[d]
MF-AAMU-31	Ground beef	9.59 ± 0.2[c]	11.13 ± 0.0[c]	13.05 ± 0.2[c]
MF-AAMU-34	Ground beef	8.68 ± 0.2[d]	13.42 ± 0.1[c]	14.4 ± 0.1[b]
MF-AAMU-35	Ground beef	9.63 ± 0.1[c]	10.78 ± 0.0[d]	11.35 ± 0.2[e]
MF-AAMU-40	Ground beef	7.98 ± 0.0[e]	9.50 ± 0.2[e]	12.21 ± 0.1[d]
MF-AAMU-46	Ground beef	8.56 ± 0.1[d]	10.22 ± 0.1[d]	12.63 ± 0.1[d]
MF-AAMU-47	Ground beef	7.98 ± 0.0[e]	9.50 ± 0.2[e]	12.21 ± 0.1[d]
MF-AAMU-52	Ground beef	9.43 ± 0.2[c]	12.4 ± 0.1[b]	14.65 ± 0.0[b]
MF-AAMU-63	Ground beef	9.76 ± 0.0[c]	10.46 ± 0.0[d]	11.36 ± 0.0[e]
MF-AAMU-66	Ground beef	8.78 ± 0.0[d]	9.52 ± 0.1[e]	11.04 ± 0.0[e]
MF-AAMU-67	Ground beef	9.80 ± 0.0[c]	11.72 ± 0.1[c]	12.45 ± 0.0[d]
MF-AAMU-72	Ground beef	7.97 ± 0.0[e]	9.91 ± 0.0[e]	11.02 ± 0.0[e]
MF-AAMU-73	Ground beef	7.78 ± 0.0[e]	9.12 ± 0.0[e]	11.89 ± 0.0[e]
MF-AAMU-79	Ground beef	8.26 ± 0.0[d]	9.23 ± 0.0[e]	12.14 ± 0.0[d]
MF-AAMU-84	Spinach	9.74 ± 0.1[c]	11.44 ± 0.1[c]	13.64 ± 0.0[c]

Food samples included ground beef, spinach, and vegetable packs containing lettuce, carrot, and cabbage obtained from local grocery stores. Data are mean ± SEM values.

[abcde]Different letters denote differences in zones of inhibition for 2.5%, 5%, and 10% Sorrel.

Table 2. Antimicrobial Activity of Sorrel on *E. coli* O157:H7 on Veterinary Samples

E. coli O157:H7 *isolates*	*Source*	*Zones of inhibition (mm)*		
		2.5% sorrel	*5% sorrel*	*10% sorrel*
MF-AAMU-89	Veterinary	10.86 ± 0.0^{b}	11.17 ± 0.1^{c}	14.62 ± 0.3^{b}
MF-AAMU-101	Veterinary	9.27 ± 0.1^{c}	10.51 ± 0.0^{d}	12.89 ± 0.1^{d}
MF-AAMU-90	Veterinary	8.38 ± 0.1^{d}	11.17 ± 0.1^{c}	14.17 ± 0.0^{b}
MF-AAMU-92	Veterinary	9.31 ± 0.1^{c}	12.57 ± 0.1^{b}	13.11 ± 0.1^{c}
MF-AAMU-93	Veterinary	10.56 ± 0.0^{b}	11.06 ± 0.1^{c}	12.72 ± 0.3^{d}
MF-AAMU-94	Veterinary	10.72 ± 0.1^{b}	12.29 ± 0.0^{b}	13.52 ± 0.0^{c}
MF-AAMU-99	Veterinary	10.44 ± 0.2^{b}	11.41 ± 0.2^{c}	12.67 ± 0.1^{d}
MF-AAMU-100	Veterinary	10.67 ± 0.2^{b}	11.47 ± 0.1^{c}	13.61 ± 0.1^{c}
MF-AAMU-102	Veterinary	7.59 ± 0.2^{e}	9.86 ± 0.05^{e}	11.42 ± 0.1^{e}
MF-AAMU-103	Veterinary	8.76 ± 0.1^{d}	10.31 ± 0.0^{d}	12.4 ± 0.22^{d}
MF-AAMU-104	Veterinary	9.57 ± 0.1^{c}	10.47 ± 0.1^{d}	12.36 ± 0.0^{d}
MF-AAMU-105	Veterinary	8.55 ± 0.2^{d}	10.51 ± 0.1^{d}	13.61 ± 0.1^{c}
MF-AAMU-107	Veterinary	9.23 ± 0.0^{c}	12.46 ± 0.2^{b}	12.51 ± 0.0^{d}
MF-AAMU-108	Veterinary	9.16 ± 0.0^{c}	11.23 ± 0.1^{c}	12.61 ± 0.1^{d}
MF-AAMU-109	Veterinary	8.67 ± 0.1^{d}	9.71 ± 0.1^{e}	12.62 ± 0.1^{d}
MF-AAMU-110	Veterinary	8.7 ± 0.0^{d}	10.67 ± 0.1^{d}	12.54 ± 0.0^{d}
MF-AAMU-111	Veterinary	8.51 ± 0.2^{d}	11.46 ± 0.1^{c}	12.41 ± 0.0^{d}
MF-AAMU-112	Veterinary	9.32 ± 0.2^{c}	11.66 ± 0.1^{c}	12.42 ± 0.1^{d}
MF-AAMU-113	Veterinary	9.99 ± 0.0^{c}	12.30 ± 0.1^{b}	12.47 ± 0.2^{d}
MF-AAMU-114	Veterinary	8.75 ± 0.0^{d}	10.69 ± 0.1^{d}	13.65 ± 0.1^{c}
MF-AAMU-117	Veterinary	11.33 ± 0.0^{c}	12.37 ± 0.0^{b}	13.33 ± 0.3^{c}

Veterinary samples were from rabbit carcasses. Data are mean ± SEM values.
[abcde]Different letters denote differences in zones of inhibition for 2.5%, 5%, and 10% sorrel.

14.98 ± 0.00 mm. At the 5% sorrel concentration, mean inhibition zones varied from 7.6 ± 0.06 mm to 13.37 ± 0.11 mm (Table 3).

Results of this experiment showed that the extract of *H. sabdariffa* exhibited antibacterial activity against the *E. coli* bacterial strains. The results indicated that the most potent sorrel concentration was 10%, followed by 5%, whereas the least potent was 2.5% (Fig. 1). There were significant ($P < .05$) differences observed in mean zone inhibition among the 10%, 5%, and 2.5% concentrations of sorrel extract (Fig. 1). The overall mean zone of inhibition for the 10% concentration of sorrel was 12.66 mm, whereas for the

Table 3. Antimicrobial Activity of Sorrel on *E. coli* O157:H7 from Clinical Samples

E. coli O157:H7 *isolates*	*Source*	*Zones of inhibition (mm)*		
		2.5% sorrel	*5% sorrel*	*10% sorrel*
MF-AAMU-25	Clinical	9.49 ± 0.1^{c}	10.83 ± 0.0^{d}	13.26 ± 0.1^{c}
MF-AAMU-27	Clinical	7.66 ± 0.2^{e}	11.61 ± 0.1^{c}	14.53 ± 0.1^{b}
MF-AAMU-28	Clinical	7.75 ± 0.0^{e}	9.52 ± 0.2^{e}	13.59 ± 0.1^{c}
MF-AAMU-29	Clinical	8.81 ± 0.0^{d}	9.56 ± 0.0^{e}	14.84 ± 0.1^{b}
MF-AAMU-30	Clinical	9.43 ± 0.2^{c}	12.4 ± 0.1^{b}	14.65 ± 0.0^{b}
MF-AAMU-32	Clinical	7.40 ± 0.2^{e}	12.82 ± 0.4^{b}	13.44 ± 0.2^{c}
MF-AAMU-33	Clinical	9.26 ± 0.1^{c}	10.56 ± 0.1^{d}	12.90 ± 0.2^{d}
MF-AAMU-36	Clinical	9.21 ± 0.0^{c}	10.63 ± 0.2^{d}	12.37 ± 0.5^{d}
MF-AAMU-37	Clinical	9.87 ± 0.2^{c}	10.26 ± 0.1^{d}	11.47 ± 0.1^{e}
MF-AAMU-38	Clinical	9.41 ± 0.1^{c}	11.01 ± 0.1^{c}	13.99 ± 0.0^{c}
MF-AAMU-44	Clinical	7.44 ± 0.2^{e}	9.25 ± 0.7^{e}	12.62 ± 0.3^{d}
MF-AAMU-45	Clinical	8.68 ± 0.1^{d}	13.37 ± 0.1^{c}	14.69 ± 0.0^{b}
MF-AAMU-42	Clinical	10.15 ± 0.1^{b}	12.99 ± 0.1^{b}	14.98 ± 0.0^{b}
MF-AAMU-54	Clinical	8.78 ± 0.0^{d}	9.52 ± 0.1^{e}	11.04 ± 0.0^{e}
MF-AAMU-56	Clinical	7.00 ± 0.0^{e}	7.6 ± 0.0^{e}	7.97 ± 0.0^{e}
MF-AAMU-59	Clinical	8.81 ± 0.2^{d}	11.0 ± 0.3^{c}	14.77 ± 0.0^{b}
MF-AAMU-60	Clinical	7.02 ± 0.1^{e}	11.2 ± 0.3^{c}	13.22 ± 0.3^{c}
MF-AAMU-62	Clinical	8.91 ± 0.0^{d}	10.32 ± 0.0^{d}	12.34 ± 0.3^{d}
MF-AAMU-65	Clinical	7.81 ± 0.0^{e}	8.90 ± 0.0^{e}	11.77 ± 0.0^{e}

Clinical samples were human fecal specimens obtained from a hospital. Data are mean ± SEM values.
[abcde]Different letters denote differences in zones of inhibition for 2.5%, 5%, and 10% sorrel.

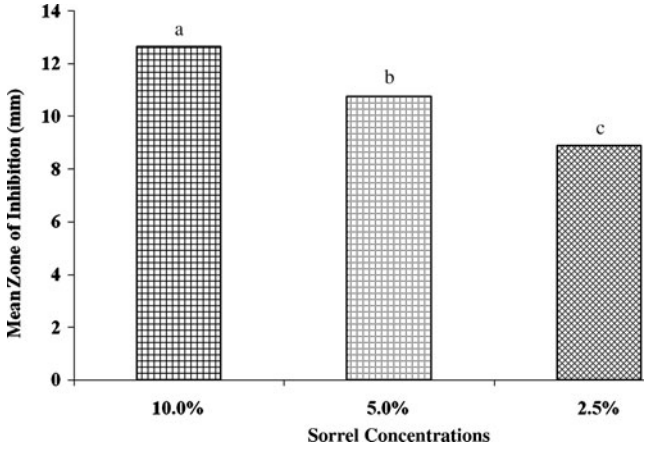

FIG. 1. Antimicrobial activities of 2.5%, 5%, and 10% sorrel concentration on zones of inhibition. Data are mean ± SEM values. [abc]Different letters denote significantly ($P < .05$) differences in zones of inhibition for 2.5%, 5%, and 10% sorrel.

2.5% concentration it was 8.90 mm; at the 5% level of concentration the mean zone of inhibition was 10.75 mm (Fig. 1).

There were significant ($P < .05$) differences between mean zones of inhibition displayed in the food, veterinary, and clinical sources (Fig. 2). The highest inhibition zones were observed in veterinary isolates, whereas the least were observed in the food isolates. The overall mean zone of inhibition observed in the veterinary isolates was 11.16 mm, whereas in the food isolates it was 10.57 mm; the overall mean zone of inhibition among the clinical isolates was 10.69 mm.

DISCUSSION

The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic use. The results of the present study support the use of *H. sabdariffa* for human and animal disease therapy and reinforce the importance of the ethnobotanical approach as a potential source of bioactive substances. This may be attributed to two reasons: first, the nature and potentiality of biologically active components (alkaloids, flavonoids, phenolics, and biterpenoids); and second, the stronger extraction capacity of methanol could have produced higher numbers and amounts of active constituents responsible for antibacterial activity.[9,34]

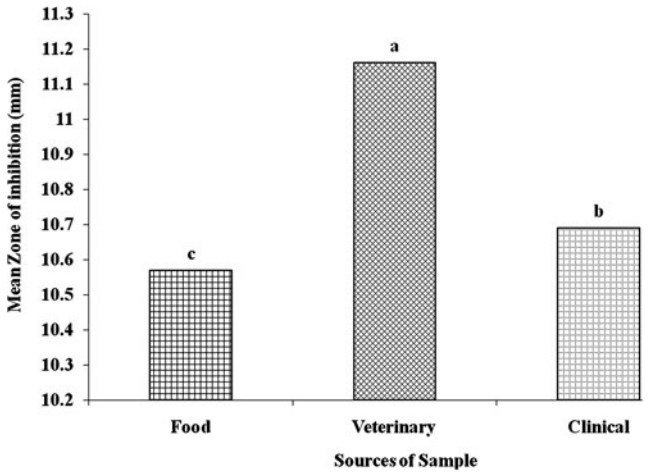

FIG. 2. Mean zones of inhibition produced among food, veterinary, and clinical sources. Data are mean ± SEM values. [abc]Different letters denote significantly ($P < .05$) differences in zones of inhibition in food, veterinary, and clinical samples.

H. sabdariffa produced zones of inhibition against *E. coli* O157:H7 isolates from food, veterinary, and clinical sources (Tables 1–3, respectively). Our study showed that the most effective sorrel concentration was 10%, whereas the least effective was the 2.5% concentration. There were significant ($P < .05$) differences in the effects of 10%, 5%, and 2.5% sorrel concentration against *E. coli* O157:H7 strains (Fig. 1). The antimicrobial activity may be the result of phenolic compounds, including flavonoids. This present study was comparable with other studies done using plant extracts on foodborne microorganisms. Krasaekoopt and Kongkarnchanatip[35] used phenolic compounds, including flavonoids, and reported that the highest zones of inhibition were displayed by *Staphylococcus aureus*, a Gram-positive organism, compared with Gram-negative bacteria (*E. coli*) with the plant extracts studied. This study was also supported by the previous studies of Nair and Chanda[20] and Rabe and van Staden.[36] Siriponputikorn *et al.*[37] reported similar results on the antimicrobial effect of Thai seasoning on common foodborne pathogens. In addition, Krasaekoopt and Kongkarnchanatip[35] found the antimicrobial activity of *Senna* and *Sesbania* flowers were higher than that of *Talisman* extract on the bacterial strains; the percentages of flavonoid found in *Sesbania* flower, *Senna* flower, and *Talisman* were 8.4%, 8.6%, and 3.4%, respectively.

The antimicrobial activity due to flavonoids may be because of their structure, as they have the ability to form a combined complex with bacterial cell walls.[38] Also, with the number of hydroxyl groups present on the phenolic ring there is increased hydroxylation, and with increased hydroxylation there will be increased antimicrobial activity. Cowan[38] reported that the site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. Flavonoids are also hydroxylated phenolic substances but occur as a C_6–C_3 unit linked to an aromatic ring. Because they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms.

E. coli strains are Gram-negative microorganisms, and the effect of *H. sabdariffa* may vary depending on Gram-negative or -positive microorganisms. In our study *H. sabdariffa* at different concentration was bactericidal against *E. coli* O157:H7. Nair and Chanda[20] also found similar effects, and they reported that standard ATCC strains of Gram-positive bacteria were more sensitive than Gram-negative

ones toward the plant extracts. The mechanism of action may be by inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the bacterial cells.[39] These processes include the inhibition of electron transport, protein translocation, phosphorylation steps, and other enzyme-dependent reactions. Nair and Chanda[20] showed inhibition at concentrations as low as 9.75 μg/mL. Antibacterial activities (minimum inhibitory concentrations of 0.30 ± 0.2–1.30 ± 0.2 mg/mL) were exhibited against *S. aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia marcescens*, *Clostridium sporogenes*, *E. coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, and *Pseudomonas fluorescence*. Cowan[38] suggested the antimicrobial action may be attributed to the intrinsic properties that are related to the permeability of their cell surface to the extracts.

Plants have an almost limitless ability to synthesize aromatic substances. Most of them are secondary metabolites, of which at least 12,000 have been isolated. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores.[40] The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant, such as phenols,[41] essential oils,[42] terpenoids,[43,44] alkaloids,[24] and flavonoids.[45]

CONCLUSIONS

Our study confirmed that *H. sabdariffa* was effective at all levels in inhibiting *E. coli* O157:H7 isolates from food, veterinary, and clinical sources. This shows that plant extracts possess antimicrobial activity and hold great promise as antimicrobial agents against foodborne pathogens, including *E. coli* O157:H7. In addition, disk diffusion provided data that reliably predicted the effectiveness of antimicrobial activity of the extracts. Plant extracts may be used as possible sources to obtain new and effective herbal medicines to treat foodborne infections, as they may be an excellent alternative to combat the further spread of multidrug-resistant microorganisms. It is important, however, to determine toxicity of the active constituents, their side effects, and pharmacokinetic properties. Future studies need to address these phenolic compounds from various plant extracts, which must be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including, in particular, toxicity studies as well as an examination of their effects on beneficial normal microbiota. Therefore, the use of natural antimicrobials such as sorrel (*H. sabdariffa*) in combination with other food preservation techniques such as low temperature, high pressure processing, and pulsed ultraviolet-light technology to create a synergistic effect would be a novel alternative.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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