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Antibacterial and Antioxidant Activity of Herbal Extracts of *Curcuma longa* L., *Careya arborea* Roxb., *Madhuca longifolia* (Koenig) Macbr. and *Punica granatum* L.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GMPJB, TSPJ, HADR and HADR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GMPJB and HADR managed the analyses of the study. Author UNNU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Development of multiple resistances against pathogenic bacteria has become a global concern at the moment. This problem has enforced scientists to search for new antimicrobial substances from various sources including medicinal plants. Hence the study was designed to determine the effect of ethanol extracts of commonly used some herbs rhizomes of *Curcuma longa*, bark of *Careya arborea*, seed of *Madhuca longifolia* and leaves of *Punica granatum* at different levels of concentrations (125, 100, 75 and 50 mg/ml) in Sri Lanka on (*Salmonella typhimrium* (ATCC 14028)*, Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922)).

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Study Design: This is a laboratory-controlled experimental design.
Place and Duration of Study: Laboratory of Livestock Production, Faculty of Agricultural Sciences and Laboratory of Chemistry, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.
Methodology: Ethanol herbal extracts at four different concentrations (125, 100, 75 and 50 mg/ml) were screened for its antimicrobial activity by Kirby-Bauer disc diffusion assay method. *In vitro*, the antioxidant ability was determined using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay method.
Results: *Punica granatum* showed the highest inhibition against *S. typhimrium*; *15.46±0.1, 14.71±0.14, 13.11±0.06 and 11.81±0.09 mm* at 125, 100, 75 and 50 mg/ml concentrations respectively. *Careya arborea* showed the highest inhibition against *B. subtilis* with inhibitory zones of *10.34±=0.12, 9.22±0.25, 7.55±0.15 and 7.05±0.05 mm* and second highest inhibition was against *E. coli*; *12.89±0.07, 12.43±0.10, 11.34±0.19* and *10.67±0.08 mm* for all concentrations respectively. *Careya arborea* exhibited the highest inhibition against *B. subtilis* with inhibitory zones of *10.34±=0.12, 9.22±0.25, 7.55±0.15* and *7.05±0.05 mm*. When considering the effect of *Curcuma longa, B. subtilis* and *E. coli* had shown significant highest inhibition at 125 mg/ml and *S. typhimurium* had lowest inhibition. Highest antimicrobial effect on *B subtilis* (*7.49±0.31 mm*) was shown by the ethanolic extract of *M. longifolia* (125 mg/ml) compared to other two bacteria (for *S. typhimrium*, *6.53±0.18 mm* and *E. coli 6.67±0.18 mm*). *Careya arborea* exhibited the highest ascorbic acid equivalent antioxidant concentrations (*718.40±22.78* mg ascorbic/g extract) followed by *Punica granatum* (*213.43±17.82* mg ascorbic/ g extract) and *Curcuma longa* (*144.28±6.40* mg ascorbic/g extract). *Madhuca longifolia* had lowest (*4.11±0.27*) and inhibitory concentration at 50% (IC_{50}) of DPPH radical scavenging activity (*2.84±0.08, 9.62±0.76, 14.14±0.61 and 498.01±34.63* IC_{50} µg/ml) in the ethanol extracts of *Careya arborea*, *P. granatum*, *C. longa* and *Madhuca longifolia* respectively.
Conclusion: This study concluded that there was a significant antimicrobial effect in ethanol extracts of tested herbs against *S. typhimurium* (ATCC 14028), *B. subtilis* (ATCC 6633) and *E. coli* (ATCC 25922) at varying levels.

Keywords: Antibiotic resistance; antioxidant; antimicrobial; plant extracts.

1. INTRODUCTION

Infectious diseases create social and economic crisis all over the world. Among the etiologies of infectious diseases bacterial causes are playing a vital role and as the drug of choice for the treatment of infectious diseases caused by bacteria, antibiotics are used extensively. Nowadays many different antibiotics are used against bacterial diseases like penicillin, cephalosporin, sulphonamides, aminoglycosides, chloramphenicol, tetracycline, macrolides, and quinolones [1]. But these drugs leads resistance development which is an alarming issue globally and also it leads to product losses (milk and meat), environmental contaminations, extra medicines and labour costs and allergies.

Since the successive introduction of various synthetic antibiotics into therapeutics as well as in the food industries, the sensitivity of microorganisms changed a lot so that the proportion of antibiotic-resistant strains is at most concern group [2]. Centre for disease control and prevention (CDC) reported that each year in United States (US) at least 2 million people become infected with bacterial diseases that are resistant to antibiotics [3]. As a result, at least 23, 000 people die each year in the world and also in Sri Lanka antibiotic resistance is reported to be increased [4]. Food and drug administration stated that antibiotics that are used for livestock health improvement and increase the production are also reasons for the development of antibiotic resistance. This has effect on animal health and production and owing to that essential veterinary drugs may no longer be available for the treatment of animal diseases, the failure of disease control programmes, increased severity and longevity of diseases, increased mortality, reduced productivity, and increased risk of disease spread in animal populations and it ultimately leading to the animal productivity and national income as well [5]. Moreover, the zoonotic aspects of these antibiotic-resistant organisms can have a very severe effect on human health.

This has enforced scientists to search for new antimicrobial substances from various sources

including medicinal plants. The antimicrobial compounds found in plants are of interest as the antibiotic resistance development is becoming a worldwide public health concern [6]. Plants produce a multitude of organic compounds that have antimicrobial activity and antimicrobial properties of phytochemicals can be due to the presence of essential oils, phenolic compounds, isothiocyanates etc. and they have a significant role in controlling the ability to treat of biofilm infections [7] as well. Many Asian countries use herbs to treat many infectious diseases in Ayurveda medicine including Sri Lanka since hundreds of years. Pharmaceutical industry and scientists are paying much more attention to produce pharmaceuticals using plant crude extracts to overcome the antibiotic resistance issue [8]. Phytochemicals have shown the ability to combat bacterial diseases and there plant secondary metabolites can affect bacterial cell wall [9].

Hence this study was conducted to investigate the effect of an herbal extract of rhizomes of *Curcuma longa*, the bark of *Careya arborea*, the seed of *Madhuca longifolia* and leaves of *Punica granatum* on *S. typhimurium*, *B. subtilis* and *E. coli* and also to investigate the antioxidant activity of the herbal extracts.

2. METHODOLOGY

The effect of antibacterial activity of four herbal plant materials (extract of rhizomes of *Curcuma longa*, bark of *Careya arborea*, seed of *Madhuca longifolia*, leaves of *Punica granatum*) on three food born pathogenic bacteria (*S. typhimurium* (ATCC 14028), *B. subtilis* (ATCC 6633), *E. coli* (ATCC 25922)) was determined. The study was conducted at the Laboratory of Livestock Production, Faculty of Agricultural Sciences and Laboratory of Chemistry, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.

2.1 Effect of Plant Extract on Different Bacteria

Effect of ethanol extracts of plant materials on *S. typhimurium* (ATCC 14028), *B. subtilis* (ATCC 6633), and *E. coli* (ATCC 25922) was investigated using Kirby-Bauer disc diffusion method. Rhizomes of *Curcuma longa*, bark of *Careya arborea*, *Madhuca longifolia* seed and *Punica granatum* leaves were used for the study.

Fig. 1. Plant materials used in the study

2.2 Preparation of Ethanol Extracts

Plant materials were collected washed and air dried without exposing to the direct sunlight. All plant materials were powdered using grinder following drying. Ethanol extraction was done according to the method described by Tiwari et al [10]. Plant materials were immersed in 200ml of absolute ethanol (1:10) for 24 hours. The supernatant was filtered through Whatman filter paper No.1 and the filtrate was evaporated using rotary evaporator on a 40°C water bath to remove all the solvent materials. This was taken as the concentrated extract and these extracts were stored in refrigerated condition at 4°C after weighing it until used. Four different concentrations (125 mg/ml, 100 mg/ml, 75 mg/ml and 50 mg/ml) of each plant extracts were prepared using dried ethanol extract mixing with Dimethyl Sulfoxide (DMSO) and stored in the refrigerator at 4°C for subsequent antibacterial assay.

2.3 Preparation of Bacterial Cultures

Pure cultures of *S. typhimurium* (ATCC 14028) *B. subtilis* (ATCC 6633) and *E. coli* (ATCC 25922) used were kindly donated by Professor Indrani Karunasagar, the Centre for Science Education & Research, UNESCO MIRCEN for Medical and Marine Biotechnology, Nitte University, Mangalore, India. The cultures were recovered from the stock cultures and single colony was used for further study. Colonies were grown in Luria Bertani broth for the study and both *S. typhimurium* and *E. coli* were incubated at 37°C overnight and *B. subitilis* were incubated overnight and at 28°C. The bacterial cell suspension was prepared at 1.0 $\times 10^6$ colony forming units per ml (CFU/ml) following the McFarland 0.5 turbidity standard. Cells were harvested and washed by centrifugation and number of cells was determined according to the optical density (at 640 nm) by using UV spectrophotometer (Thermo Scientific, Genesys 10S UV-V1S).

2.4 Antibacterial Assay

Antibacterial assay was performed by using disc diffusion method described by Bauer et al. [11]. Sterilized paper discs with the diameter of 5.5 mm were made from Whatman filter paper and the discs were sterilized prior to use. 0.5ml of each bacterial culture (10^6 CFU/ml) was spread on Muller Hinton agar plates and prepared sterilized disc were placed on the inoculated surface. Discs were impregnated with the test extracts (10 µl) i.e. aqueous extracts and ethanol extracts of *Curcuma longa* rhizome, *Careya arborea* bark, *Madhuca longifolia* seed and *Punica granatum* leaves (at concentrations 125 mg/ml, 100 mg/ml, 75 mg/ml and 50 mg/ml for aqueous preparation and ethanol extract) and kept at room temperature for absorption followed by incubation at 37°C for *S. typhimurium* (ATCC 14028), *E. coli* (ATCC 25922) and at 28°C for *B. subtilis* (ATCC 6633) overnight.

Plate sets with ampicillin (25 µg), chloramphenicol (30 µg) and nalidixic acid (30 µg) discs (Himedia India Pvt. Ltd., Mumbai) were used as a positive control and respective solvents were taken as the negative control (DMSO, distilled water and absolute ethanol). At the end of incubation, inhibition zones formed around the disc were measured by using a vernier calliper and the tests were performed in six replicates.

2.5 DPPH Radical Scavenging Assay

DPPH Radical Scavenging activity was measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution. It was prepared by dissolving 20 mg in 100 ml of methanol. Five different concentration of each herbal samples were prepared in methanol. 20 mg of each herbal extracts were dissolved in 20 ml of methanol (1 mg/ml). Extracts were initially dissolved in DMSO. Standard sample was prepared 20 mg of L-Ascorbic acid dissolving in methanol. Then it was diluted in methanol. DPPH Radical Scavenging assay was performed according to a method described by Blois [12].

In the assay, 450 µl DPPH and 2550 µl methanol was used as a control and 750 µl from sample was mixed with 2250 µl of methanol in cuvette as blank as the extracts were having colour. Herbal extracts sample was prepared as 750 µl extract solution, 450 µl DPPH and 1800 µl methanol mixing in glass cuvette. Then the sample was darkened and incubated at room temperature 25±2°C for 10 minutes. Absorbance was recorded at 517 nm (Thermo Scientific, Genesys 10S UV-V1S).

2.6 Calculation of % Radical Scavenging Activity of Sample/Standard

$$= \left[\frac{A_{control} - A_{Sample\ test}}{control}\right] \times 100$$

(Equation for radical scavenging activity)

2.7 Calculation of IC_{50} (Concentration of Sample/Standard at 50% inhibition) of Sample/Standard

Percentage of radical scavenging activity at each concentration of the sample/standard was measured in five different concentrations with triplicates. Concentration vs. percentage of radical scavenging activity was plotted in the scatter graph. Using the equation Y=mx+c (x=sample concentration, Y=percentage of radical scavenging activity) x was calculated considering that the Y=50.

2.8 Analysis of Data

The individual analysis of variance and the means were compared by Duncan's Multiple range test at the level of 95% of confidence level by statistical software SAS™ (9.0).

3. RESULTS AND DISCUSSION

3.1 Effect of Herbal Extracts on Microorganisms

When considering the ethanolic extracts of *Punica granatum* has shown significant highest (*P=.05*) inhibition zone against *S. typhimurium* when compared to the other plant extracts. *Punica granatum* had 15.46±0.1 mm, 14.71±0.14 mm, 13.11±0.06 mm and 11.81±0.09mm of inhibition at 125 mg/ml, 100 mg/ml, 75 mg/ml, and 50 mg/ml concentration levels respectively (Table 1). Second highest inhibition against *S. typhimurium* was by ethanolic extract of *Careya arborea* and lowest inhibition was by *M. longifolia* (Table 1). The ethanolic extract of *Careya arborea* has shown significant highest (*P=.05*) inhibition zone against *B. subtilis* and it *Careya arborea* had 10.34±0.12 mm, 9.22±0.25 mm, 7.55±0.15 mm and 7.05±0.05 mm of inhibition at 125 mg/ml, 100 mg/ml, 75 mg/ml, and 50mg/ml concentration levels respectively (Table 1).

When considering the *E. coli*, ethanolic extract of *Punica granatum* has shown significant highest (p=*.05*) inhibition zone when compared to other plant extracts. *Punica granatum* had 12.89±0.07 mm, 12.43±0.10 mm, 11.34±0.19 mm and 10.67±0.08 mm of inhibition at 125 mg/ml, 100 mg/ml, 75 mg/ml, and 50 mg/ml concentration levels respectively (Table 1). Second highest inhibition against *E. coli* was by ethanolic extract of *Careya arborea.*

Commercial antibiotics used in this study had the significant effect on all the three bacterial species studied (*S. typhimurium*, *B. subtilis* and *E. coli*) in comparison with plant extracts. Among the four antibiotics used, Chloramphenicol showed the significantly highest inhibition against *S. typhimurium*, *B. subtilis* and *E. coli* with the inhibition zones of 30.85±0.34 mm, 32.83±0.62 mm and 30.04±0.49 mm respectively, whereas the second higher inhibition for *S. typhimurium* (26.08±0.19 mm) and for *E. coli* (25.47±0.32 mm) was with ampicillin and for *B. subtilis* that was nalidixc (11.96±0.10 mm) (Table 1).

Ethanolic extract of *Careya arborea* has shown significantly highest (p≤0.05) inhibition zone at 125 mg/ml level against *B. subtilis* (10.34±0.12 mm) and *E. coli* (7.64±0.12 mm) when compared to other concentrations (100 mg/ml, 75 mg/ml and 50 mg/ml) used in this study. But considering the *S. typhimurium* at 125 mg/ml, 100 mg/ml and 75 mg/ml did not show significant different (*P=.05*) effect from each other. Inhibition zone at 125 mg/ml, 100 mg/ml and 75 mg/ml were 7.61±0.27 mm, 7.37±0.31 mm and 7.07±0.21 mm respectively. *Curcuma longa* extract at 125 mg/ml has shown the highest (p≤0.05) inhibition zone against *S. typhimurium* (6.16±0.12 mm) *B. subtilis* (7.72±0.13 mm) *E. coli* (7.38±0.33 mm) and also for the *Punica granatum* highest concentration has shown the highest (*P=.05*) inhibition zone against *S. typhimurium* (15.46±0.10 mm) *B. subtilis* (7.42±0.15 mm) *E. coli* (12.89±0.07mm). None of the concentration levels of *Madhuca longifolia* did show significant inhibition zone on *S. typhimurium*. But the effect of *Madhuca longifolia* on *B. subtilis* and *E. coli* at 125 mg/ml has shown significantly high inhibition zone (7.49±0.31 mm and 6.67±0.18 mm) respectively (Table 1).

Ethanolic extract of *Careya arborea* at 125 mg/ml and 100 mg/ml level highest significant (p=*.05*) effect was shown on *B. subtilis* (10.34±0.12 mm) compared to other two bacteria. When considering the effect of *Curcuma longa B. subtilis* and *E. coli* had significant highest (p=*.05*) effect at 125 mg/ml level and *S. typhimurium* had the lowest effect. The ethanolic extract of *Madhuca longifolia* at 125 mg/ml level highest significant (p=*.05*) effect was shown on *B. subtilis* (7.49±0.31 mm) compared to other two bacteria. When considering the effect *Punica granatum* on three bacterial strains, at 125 mg/ml, 100 mg/ml, 75 mg/ml and 50 mg/ml levels showed significant (p=*.05*) effect on three bacteria. The highest significant (p=*.05*) effect was on *S. typhimurium*

(15.46±0.10 mm). The second highest effect was on *E. coli* (12.89±0.07 mm) and the lowest effect was on *B. subtilis* (7.42±0.15 mm) at 125 mg/ml level (Fig. 2).

Curcuma longa extract contained alkaloids, tannin, flavonoid, glycoside and carbohydrate. There are reports showing that alkaloids and flavonoids are the responsible compounds for the antibacterial activities in higher plants [13]. Study done by Gupta et al. (2015) found that antibacterial effect of *Curcuma longa* extract on *Staphylococcus* spp, and it is also proven by Negi [14] who reported the inhibitory effects of ethanol and hexane extract of turmeric against *S. aureus*. Tyagi et al. [15] studied the effect of curcumin which is an essential constituent of *Curcuma longa* against different organism i.e, *Staphylococci, Pseudomonas*, *E. coli* and *Enterococci* and they confirms the broad-spectrum antibacterial nature of curcumin, and its membrane damaging property which enables the antibacterial property. In *B. subtilis* it has shown that cucumin of *Curcuma longa* induce the filamentation suggesting that it inhibits bacterial cytokinesis thereby perturbation of the GTPase activity and it is lethal to bacteria and indicated that curcumin inhibits bacterial cell proliferation [16]. The observations of the present study which shows that there is an antibacterial effect of *Curcuma longa* against *E.coli*, *B. subtilis* and *S. typhimurium* are in agreement with the findings of previous studies which showed decrease in *E. coli* cell growth, inhibition of *Staph. aureus* and *P. aeruginosa*, *E. faecalis* and *B. subtilis* [17,18]. In agreement with the current study, Luer [19] also found that effect of *Curcuma longa* extracts against *E. coli*, *Staphylococcus aureus*, *Salmonella typhi* and *C. albicans* by disc diffusion method. Our results are compared with some other studies done, Chandrana et al. [20] and Kim et al. [21] who reported that *Curcuma longa* extract was effective against *E. coli, B.subtilis* and *Staphylococcus aureus* which may be due to the presence of curcuminoid, a phenolic compound. The antimicrobial activity of *Curcuma longa* is reported to be due to the presence of essential oil, curcumins, curcuminoids, turmeric oil, turmerol and valeric acid [16,22-24].

Careya arborea extract also has shown the effects of different microorganisms and It is known to have flavonoids, tannins, alkaloids and terpenoids in *Careya arborea* [25-27]. The current study revealed that there is a an antibacterial effect of *Careya arborea* against all the three microorganisms (*B. subtilis*, *E. coli* and *S. typhimurium*) tested with highest activity against the *B. subtilis* and it is in agreement with the findings of Kumar et al, [28] who found the antimicrobial activity against many microorganism such as *Pseudomonas*, *E. coli*, *Salmonella, Shigella*, *Vibrios*, *Staphylococci*, *Streptococci*, against some fungal species as well. Furthermore, they have found that there is a strong antioxidant activity; it is also comparable to this study. In line with the current study, Mali and Wadje, [29] found that there is an inhibition of *Staphylococcus aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *Aspergillus niger* and *Candida albicans*. Prabhakaran et al. [30] and Panda et al. [31] explained the effect of extracts of fruits of *Careya arborea* against some bacteria and fungus in parallel to this study.

In line with this study, Chakma [32] found an antimicrobial effect of *Madhuca longifolia* against *Staphylococcus aureus*, *B. subtilis, E. coli* and *Pseudomona*s and also the survey by Pandey and Agarwal [33] showed that this plant extract has high potency to inhibit the growth of *E.coli* and *Staphylococci* and some fungal species. Kalavani and Jegadeesan [34] concluded in their study that the *Madhuca longifolia* extracts have significant antimicrobial activities against *Staphylococcus aureus, B. subtilis, E. coli, P. aeruginosa, A. oryzae* and *A. niger*, which supports the current study. Similar studies were done by several earlier workers [35-41] who also reported the antibacterial and antioxidant activity *of Madhuca longifolia*.

3.2 DPPH Radical Scavenging Activity

All tested herbal extracts have shown DPPH radical scavenging activities. It was measured five concentrations of ethanol extracts using three replicates. Concentration levels were prepared half dilution series. The stock solution was 1 mg/ml each plant extracts. DPPH radical scavenging activity of herbal extracts was calculated as L-ascorbic acid equivalent antioxidant concentration values and IC_{50} values.

DPPH radical scavenging activity of standard antioxidant ascorbic acid was used to calculate ascorbic acid equivalent antioxidant concentration values. A standard curve is given in Fig. 3.

Table 1. Effect of ethanolic extract of rhizomes of *Curcuma longa*, bark of *Careya arborea*, Seed of *Madhuca longifolia* and leaves of *Punica granatum* on, *Salmonella typhimurium*, *Bacillus subtilis* and *Escherichia coli*

Inhibition zone diameter of *Salmonella typhimurium* (mm)							
	Careya arborea	***Curcuma longa***	***Madhuca longifolia***	***Punica granatum***	**Chloramphenicol 30 µg**	**Nalidixic acid 30 µg**	**Ampicillin 25 µg**
125 mg/ml	$7.61\pm^{e}0.27$	$6.16\pm^{fg}0.12$	$6.53\pm^{f}0.18$	$15.46\pm^{d}0.1$	$30.85\pm^{a}0.34$	$24.62\pm^{c}0.22$	$26.08\pm^{b}0.19$
100 mg/ml	$7.37\pm^{e}0.31$	$5.93\pm^{fg}0.01$	$6.66\pm^{ef}0.61$	$14.71\pm^{d}0.14$	$30.85\pm^{a}0.34$	$24.62\pm^{c}0.22$	$26.08\pm^{b}0.19$
75 mg/ml	$7.07\pm^{e}0.21$	$5.73\pm^{f}0.02$	$6.62\pm^{e}0.16$	$13.11\pm^{d}0.06$	$30.85\pm^{a}0.34$	$24.62\pm^{c}0.22$	$26.08\pm^{b}0.19$
50 mg/ml	$6.79\pm^{e}0.13$	$5.63\pm^{g}0.03$	$6.26\pm^{f}0.08$	$11.81\pm^{d}0.09$	$30.85\pm^{a}0.34$	$24.62\pm^{c}0.22$	$26.08\pm^{b}0.19$
Inhibition zone diameter of *Bacillus subtilis* (mm)							
	Careya arborea	***Curcuma longa***	***Madhuca longifolia***	***Punica granatum***	**Chloramphenicol 30 µg**	**Nalidixic acid 30 µg**	**Ampicillin 25 µg**
125 mg/ml	$10.34\pm^{c}0.12$	$7.72\pm^{de}0.13$	$7.49\pm^{de}0.31$	$7.42\pm^{de}0.15$	$32.83\pm^{a}0.62$	$11.96\pm^{b}0.10$	$7.92\pm^{d}0.01$
100 mg/ml	$9.22\pm^{c}0.25$	$6.13\pm^{e}0.37$	$6.51\pm^{e}0.20$	$6.52\pm^{e}0.18$	$32.83\pm^{a}0.62$	$11.96\pm^{b}0.10$	$7.92\pm^{d}0.01$
75 mg/ml	$7.55\pm^{c}0.15$	$5.77\pm^{d}0.15$	$6.46\pm^{d}0.20$	$6.36\pm^{d}0.19$	$32.83\pm^{a}0.62$	$11.96\pm^{b}0.10$	$7.92\pm^{c}0.01$
50 mg/ml	$7.05\pm^{d}0.05$	$5.76\pm^{e}0.34$	$6.46\pm^{e}0.20$	$6.36\pm^{e}0.19$	$32.83\pm^{a}0.62$	$11.96\pm^{b}0.10$	7.92±c0.01
Inhibition zone diameter of *E. coli* (mm)							
	Careya arborea	***Curcuma longa***	***Madhuca longifolia***	***Punica granatum***	**Chloramphenicol 30 µg**	**Nalidixic acid 30 µg**	**Ampicillin 25 µg**
125 mg/ml	$7.64\pm^{e}0.12$	$7.38\pm^{ef}0.33$	$6.67\pm^{f}0.18$	$12.89\pm^{d}0.07$	$30.04\pm^{a}0.49$	$24.54\pm^{c}0.39$	$25.47\pm^{b}0.32$
100 mg/ml	$7.08\pm^{e}0.20$	$6.59\pm^{e}0.16$	$5.70\pm^{f}0.11$	$12.43\pm^{d}0.10$	$30.04\pm^{a}0.49$	$24.54\pm^{c}0.39$	$25.47\pm^{b}0.32$
75 mg/ml	$7.04\pm^{e}0.17$	$6.18\pm^{f}0.16$	$5.52\pm^{f}0.02$	$11.34\pm^{d}0.19$	$30.04\pm^{a}0.49$	$24.54\pm^{c}0.39$	$25.47\pm^{b}0.32$
50 mg/ml	$6.45\pm^{e}0.12$	$5.83\pm^{e}0.18$	$0.00\pm^{f}0.00$	$10.67\pm^{d}0.08$	$30.04\pm^{a}0.49$	$24.54\pm^{c}0.39$	$25.47\pm^{b}0.32$

**Data were presented as the mean ± standard error. Means with different superscripts in the same row are significant differences at (p≤0.05)*

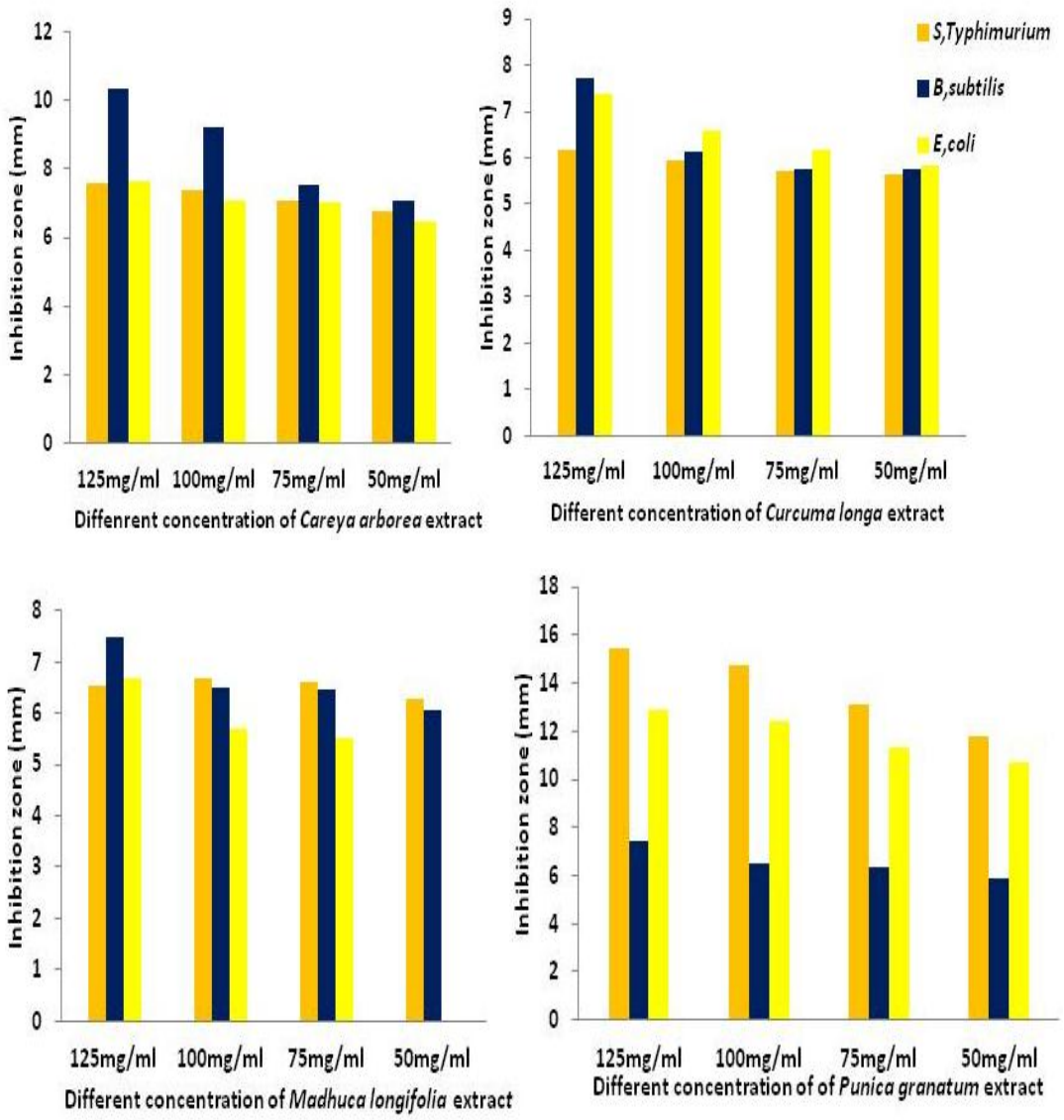

Fig. 2. Effect of herbal extracts (Rhizomes of *Curcuma longa*, bark of *Careya arborea*, *Madhuca longifolia* seed, *Punica granatum* leaves) on *Salmonella typhimurium*, *Bacillus subtilis* and *E. coli*

Table 2. Ascorbic acid equivalent antioxidant concentration (mg ascorbic/ g extract) and IC_{50} µg/ml

Extracts	Ascorbic acid equivalent Antioxidant concentration (mg ascorbic/ g extract)	IC_{50} µg/ml
Careya arborea	$718.40\pm^{a}22.78$	2.84±0.09
Punica granatum	$213.43\pm^{b}17.82$	9.62±0.76
Curcuma longa	$144.28\pm^{c}6.40$	14.14±0.61
Madhuca longifolia	$4.11\pm^{d}0.27$	498.01±34.63

**Data were presented as the mean ± standard error. Means with different superscripts in the same column are significant differences at (p=.05)*

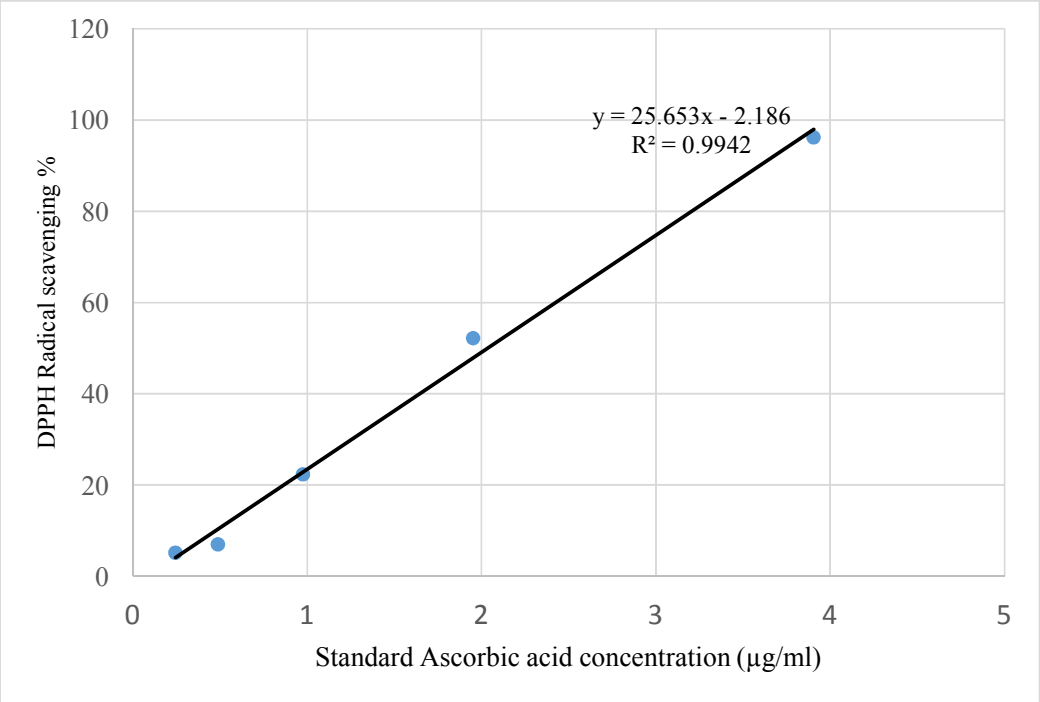

Fig. 3. Standard curve of Ascorbic acid

Table 2 has shown the highest DPPH values were showed by *Careya arborea* bark extract and the lowest value was showed by *Madhuca longifolia*. There was a significant difference within all plant extracts (p=*.05*).

DPPH radical scavenging activity was estimated as inhibitory concentration. The 50% (IC_{50}) was shown significantly highest (p=*.05*) value in *Madhuca longifolia* seed extracts and lowest value in *Careya arborea* bark extracts. Inhibitory concentrating at 50% (IC_{50}) of DPPH radical scavenging activity of herbal extracts has been shown in Table 2.

In accordance with the current study Mostafa et al. [42] found that ethanolic extracts of *Punica granatum* has inhibitory effect on strains of Gram-positive (*Staphylococcus aureus* and *B. cereus*) and three strains of Gram-negative (*E. coli, Salmonella typhi, and P. aeruginosa*). Verma et al. [43] have shown that among the three herbal extracts (*Citrus limon*, *Allium sativum* and *Punica granatum*) they have tested, *Punica granatum* as the extract with versatile antimicrobial activity inhibiting the gram positive and gram negative organisms effectively and the present study also revealed that the *Punica granatum* as the most effective extract against *Salmonella* and *E. coli*. In parallel to the current study some other studies also have demonstrated that antibacterial activity and antioxidant properties of *Punica granatum*, these studies include Al- Zorkey [44] who found that this extract as the potent inhibitor for *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli* and *Yersinia enterocolitica,* Choi et al. [45] demonstrated significant effect of *Punica granatum* extract against *S. typhimurium* in a *in vivo* study with mice. Further, the studies by Shiwaikar, Agrawal, Dahake, and Tuba [46-49] exhibited the antioxidant activity of plant extract.

4. CONCLUSION

This study concluded that there was a significant antimicrobial effect in ethanol extracts of tested herbs against *S. typhimurium*, *B. subtilis* and *E. coli* at varied levels and also it has shown that these herbs possess different antioxidant activities while some herbs have high antioxidant activities. Findings of this study could provide an impetus for further research on active constituents of these plant extracts regarding its antibiotic potential against rapidly emerging bacterial pathogens.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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