Original Research Article

Evaluation of Antibacterial Activity of Zobo and Bay leaf Extracts on Enteropathogenic Bacteria

ABSTRACT

Aim: The antibacterial activity of Bay leaf (*Laurus nobilis*) and Zobo leaf (*Hibiscus sabdariffa*) extracts on enteropathogenic bacteria was investigated

Study design: the study utilized well in agar diffusion to investigate the antimicrobial properties of the extracts.

Place and Duration of Study: Department of Microbiology, Rivers State University and the study was carried out in August, 2018 to October, 2018.

Methodology: Faecal samples were collected from a medical laboratory and inoculated on eosin methylene blue and mannitol salt agar plates for *E. coli* and *S. aureus* using standard microbiological techniques. The bacterial isolates were subjected to biochemical and molecular (PCR) identification so as to ascertain the distinctiveness of the isolates. Hot water and absolute alcohol were used as the extracting solvents. Concentrations of the extracted solvents was tested against *E. coli* and *S. aureus* using the well in agar method.

Results: The result showed that both hot aqueous and alcoholic extracts of Bay leaf (*Laurus nobilis*) showed no sensitivity against the tested bacteria, whereas the extracts of hot dry aqueous and alcohol of Zobo leaf (*Hibiscus sabdariffa*) showed remarkable zones of inhibition against the tested bacteria. The zones of inhibition in the dry hot aqueous extract of zobo leaf with concentrations of 0.25 µg/ml, 0.125 µg/ml and 0.063 µg/ml were 31.3±0.1, 25.6±1.2 and 10.0±0.0, respectively. The minimal inhibitory concentration of the dry hot aqueous of zobo extract was observed at 0.063 µg/ml for *Escherichia coli*, while zones of inhibition of 33.3±0.0, 30.1±0.3, 17.2±1.0 and 15.0±0.1 mm were recorded from the dry alcoholic extract of zobo leaf on *Escherichia coli* given similar concentrations and the MIC was observed at the 0.031 µg/ml concentration. The result also showed that out of the four concentrations of the dry hot aqueous extract, only the 0.25 µg/ml concentration was able to show 14.2±0.0 mm inhibition on *Staphylococcus aureus,* while the concentrations of 0.25 µg/ml and 0.125 µg/ml were the only two concentrations of the dry alcohol that showed levels of sensitivity with zone diameters of 29.3±1.0 and 25.2±0.0, respectively.

Conclusion: The plant extracts of zobo leaves which displayed remarkable activity at fairly-low concentrations could be recommended for use against similar bacteria. Thus, investigation and adoption of plant extracts in modern medicine should be encouraged as this may be the break through needed to combat the ever-increasing resistance to commonly used antibiotics.

Keywords: antimicrobial properties, Laurus nobilis, *Hibiscus sabdariffa*, *enteropathogenic bacteria*

1. INTRODUCTION

For decades, plants have been the mainstay of traditional medical practice and have remained an inestimable source of natural health products for humans, particularly in the last few decades, with more thorough researches having being carried out to explore natural

therapies [1]. The use of herbs in the treatment of diseases has become widespread and is increasingly achieving popularity worldwide not only due to their continuous usage in developing countries for primary health care of the poor, but also in societies where conventional medicine is prevalent in their health care system [2]. Approximately eighty percent of the world's population practises herbal medicine, which may explain the constant rise in the annual global market value of these herbal remedies estimated at over US $60 billion currently [3]. Presently, the use of medicinal plants alongside western medicine is of great significance in the Nigerian health care system, a type of health care referred to as "herbalism" [4]. Due to the constant rise in sophistication across the world, it is essential to refer to herbal medical practice as alternative or complimentary medicine, so as to appeal to large populations of people regardless of their cultures and/or religions [5].

Medicinal plants contain certain substances which possess the healing properties known as "phytochemicals" [6]. Phytochemicals are non-nutritive, biologically active chemical compounds occurring naturally in these plants, which confer the characteristic colour, aroma and flavour to them and in some cases, constitute their natural defence mechanisms [7, 8]. Phytochemicals are chiefly categorized into two broad groups namely: primary constituents and secondary metabolites [9]. Primary constituents include proteins, amino acids, common sugars and chlorophyll, whereas, secondary constituents include glycosides, alkaloids, phenolic compounds, flavonoids, saponins, essential oils, tannins and terpenoids ([9]. At present, many countries have shown a stepwise increase in their employment of phytochemicals for pharmaceutical uses [2]. It has been reported by the World Health Organization (WHO) that medicinal plants would serve as the best source of varieties of drugs [10]. Nearly eighty percent of individuals, particularly in developed countries, engage in traditional medicine, which makes use of compounds gotten from medicinal plant parts [3]. Recently, numerous studies have been conducted in various countries to demonstrate the efficiency and significance of various crude plant extracts and phytochemicals of known antimicrobial characteristics in modern therapeutic care [11]. Hence, many plants have found usefulness in medical practice by virtue of their respective antimicrobial properties which are conferred upon them by the secondary metabolites they synthesize [11]. Due to the constantly rising incidence of new and re-emerging infectious diseases, there is a pressing need to find new antimicrobial agents with varying chemical structures and newer mechanisms of action [12]. This is also necessitated by some of the adverse side effects associated with certain antibiotics as well as the increasing development of resistance to the antibiotics currently in use [12]. As such, necessary actions must be taken to prevent excessive and unnecessary intake of antibiotics, to better comprehend the various genetic antibiotic resistance mechanisms and to enable further researches in the development of newer drugs [13]. There are various means of treating and controlling the infections caused by Multi-Drug Resistant (MDR) bacteria. One of such means is by isolating active phytochemicals in plants that can help stop the transmission of infection [2]. Thus, the aim of this study is to investigate the antibacterial activity of zobo and bay leaf extracts commonly used in Nigeria against some human enteropathogenic bacteria.

2. MATERIAL AND METHODS – SHOULD MENTION ABOUT STATISTICAL ANALYSIS FOLLOWED

2.1 Sample Collection

Bay leaf (*Laurus nobilis*), and Zobo leaf (*Hibiscus sabdariffa*) were bought from the Rumuokoro Slaughter Market which is one of the major markets in Port Harcourt City Local Government Area, Rivers State. The samples were taken to the Botany Department of the Rivers State University for identification before being taken to the Microbiology Laboratory for preparation.

2.1.1 Preparation of Samples

The plant samples were shade dried at room temperature (30-35 ^{o}C) for eight (8) days. After which, they were pulverized into fine powder using a mortar and pestle which has been sterilized using ethanol (99.9 %) and cotton wool.

2.1.2 Extraction of extract

Hot distilled water and ethanol were used for extraction. For the hot distilled water extraction, fifty grams (50g) of the powdered samples were transferred in to sterile beakers containing 200ml each of sterile distilled water (which was sterilized by autoclaving at 121 ^{o}C for 15 minutes) and labelled accordingly. While in the ethanol extraction, fifty grams (50g) of the powdered samples were transferred into sterile conical flasks containing 200ml ethanol (99.9%). The samples were swirled and allowed to stand for 72 hours. Both samples were sieved using filter paper. The filtrates obtained were evaporated to dryness using the water bath and the residues were stored in sterile containers for further use.

2.1.3 Test for Sterility of Extracts

The sterility of the extracts was determined by streaking them on MacConkey and nutrient agar plates. plates were later incubated for 24-48 hours at 37 ^{o}C. The absence of microbial growth after incubation showed that the extracts were not contaminated (i.e. were sterile) [14].

2.1.4 Preparation of Various Concentrations from the Extracts

The extracts were diluted into four (4) concentrations (0.25 µg/ml to 0.031µg/ml) using the two-fold dilution method described by Obire and Ogbonna [15]. One gram of extract was diluted into 2ml of the sterile diluent and a step-wise 2-fold dilution was carried out to achieve the required concentrations.

2.4 Microbiological Analysis

2.4.1 Isolation and Identification

Twenty stool samples were collected in sterile bottles from a medical laboratory and transferred to the Microbiology Laboratory of the Rivers State University for analysis. The stool samples were analyzed according to the methods described by Cheesbrough [16]. The stool samples were moistened in normal saline and were streaked on the surface of Eosin methylene blue (EMB) agar and Mannitol salt agar (MSA) plates and incubated at 37 ^{o}C for 24 hours. Discrete colonies on the respective plates were isolated and streaked on fresh nutrient agar plates until pure isolates were obtained and preserved in agar slants. Isolates were identified by their colonial morphology microscopy, biochemical test and molecular methods.

2.4.2 Characterization of bacterial isolates

The bacterial isolates were characterized using the methods described by Cheesbrough [16] and further confirmation of isolates was done using the Bergy's manual of determinative bacteriology. The biochemical tests adopted include catalase, motility, sugar fermentation, citrate utilization, oxidase, MRVP and Indole. Further confirmation of the isolates was carried out using molecular (genomic) characterization.

2.5 Antimicrobial Susceptibility Test of the Extracts

The Well in agar diffusion method was used. The standardized inoculum was swabbed on the surface of the Mueller-Hinton agar plates and were allowed to dry. A sterile 6mm well borer was used to bore holes on the surface of the seeded plates. The holes were bored in such a way that each hole did not get to the bottom of the agar so as to prevent leakage. The already prepared extracts at different concentrations were then transferred into the

holes, after which plates were incubated at 37 ^{o}C for 18-24 hours without inverting the plates.

3. RESULTS AND DISCUSSION

After the mega blast for the search of highly similar sequences of the already obtained 16S rRNA sequences from the NCBI data base, the 16S rDNA of the isolates showed a percentage similarity to other species at 99%. The evolutionary distances which was computed with the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16s rDNA of the isolates as presented in Fig. 1. Four bacterial isolates belonging to *Escherichia coli, Enterobacter xiangfengesis, Pseudomonas aeruginosa* and *Staphylococcus aureus* were identified. The percentage yield of the plant extract using the different solvents are presented in Table 1.

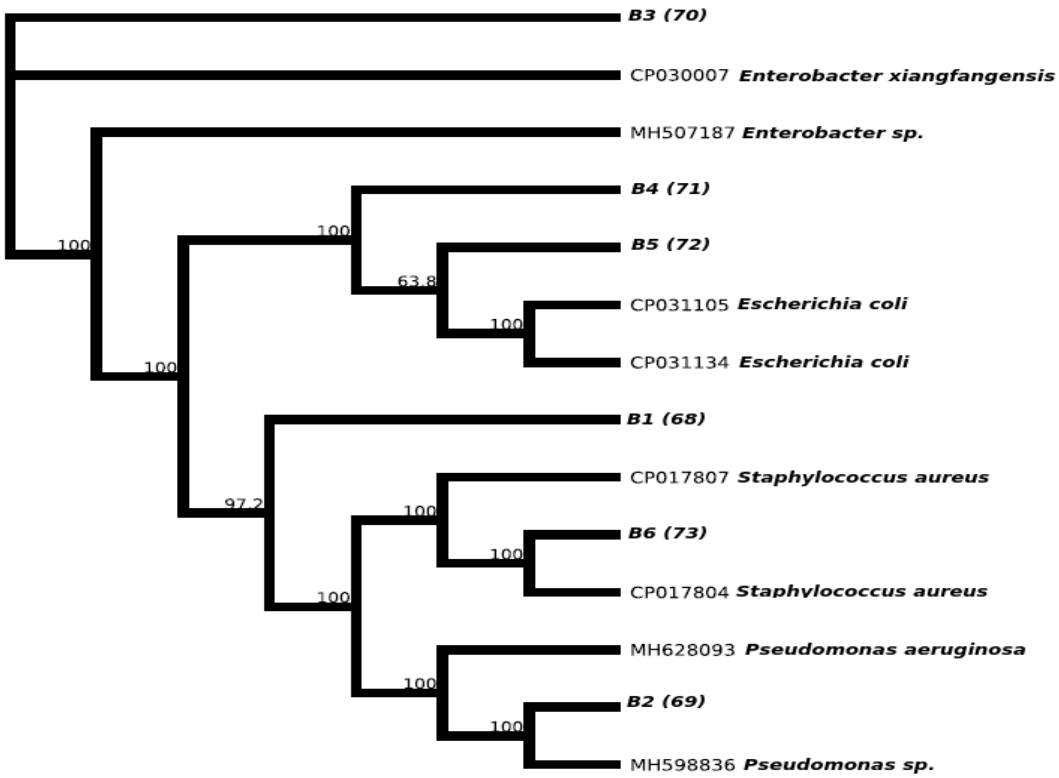

Fig 1: Phylogenetic tree showing the evolutionary distance between the bacterial isolates

Table 1: Percentage yield of crude extracts

Medicinal plant	Extracting solvent	Type of extract	Colour of extract	Weight of macerated sample used	Weight of extract	Percentage yield of extract (%)

				(g)		
Bay leaf	Hot water	DHA	Light green	50	5.2	10.4
	Alcohol	DA	Light green	50	4.91	9.82
Zobo leaf	Hot water	DHA	Red	50	5.0	10
	Alcohol	DA	Red	50	5.1	10.2

DHA: dry hot aqueous, DA: dry alcohol

Table 2: Zones of inhibition (mm) of the different extracts of Bay leaf - is this table required as all are zero you can write in result running matter ????

Bacterial isolates	Type of extract	Inhibitory zone diameters(mm) at Various concentrations of extracts				MIC (µg/ml)
		0.25 µg/ml	0.125 µg/ml	0.063 µg/ml	0.031µg/ml	
Escherichia coli	**DHA**	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0
Escherichia coli	**DA**	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0
Staphylococcus aureus.	**DHA**	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0
Staphylococcus aureus	**DA**	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0

DHA: dry hot aqueous, DA: dry alcohol

Table 3: Zones of inhibition (mm) of the different extracts of Zobo leaf

Bacterial isolates	Type of extract	Inhibitory zone diameters(mm) at Various concentrations of extracts				MIC (µg/ml)
		0.25 µg/ml	0.125 µg/ml	0.063 µg/ml	0.031 µg/ml	
Escherichia coli	**DHA**	31.3±0.1	25.6±1.2	10.0±0.0	0.0±0.0	0.063
Escherichia coli	**DA**	33.3±0.0	30.1±0.3	17.2±1.0	15.0±0.1	0.031
Staphylococcus sp.	**DHA**	14.2±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.25
Staphylococcus sp.	**DA**	29.3±1.0	25.2±0.0	0.0±0.0	0.0±0.0	0.125

DHA: dry hot aqueous, DA: dry alcohol

Susceptibility of the test organisms to *Laurus nobilis*

The susceptibility of the antimicrobial activity of Bay leaf is presented in Table 2. The result showed that both the dry hot aqueous and dry alcohol extracts of *Laurus nobilis* demonstrated no inhibitory activity on the test organisms. Thus, the findings in this study do not agree with previous studies which has demonstrated the antimicrobial property of bay leave extracts (*Laurus nobilis*) on *E. coli, Staphylococcus sp., Salmonella sp., Pseudomonas sp., Shigella sp.* and *Klebsiella* [17, 18, 19, 20, 21].

Susceptibility of the test organisms to Zobo leaf (*Hibiscus sabdariffa*)

The result of the zones of inhibition of the Zobo leaf (*Hibiscus sabdariffa*) extract is presented in Table 3. From the results, both dry hot aqueous and dry alcoholic extracts of *Hibiscus sabdariffa* both demonstrated remarkable inhibitory activity on the growth of the test bacterial isolates. For the dry hot aqueous extracts, the zones of inhibition in the extract

concentrations of 0.25 µg/ml, 0.125 µg/ml and 0.063 µg/ml were 31.3±0.1, 25.6±1.2 and 10.0±0.0 respectively for *Escherichia coli*. The least concentration which represented the MIC was noted in the 0.063 µg/ml. whereas higher zones of inhibition were recorded from the alcoholic extract on *Escherichia coli* given similar concentrations and the MIC was observed at the 0.031 µg/ml concentration. The result also showed that out of the four concentrations of the dry hot aqueous extract, only the 0.25 µg/ml concentration was able to show 14.2±0.0 mm inhibition on *Staphylococcus aureus,* while the concentrations of 0.25 µg/ml and 0.125 µg/ml were the only two concentrations of the dry alcohol that showed levels of sensitivity with zone diameters of 29.3±1.0 and 25.2±0.0, respectively. The antimicrobial activities of zobo leaf extracts have been reported by previous studies [22, 23, 24, 25, 26, 27]. In the study of Salem *et al* [22], it was shown to inhibit *S. aureus*, *K. pneumoniae* and *E. coli*, at minimum concentrations ranging from 0.30 to1.30±0.2mg/ml for the three organisms. In the study done by Higginbotham *et al* [28], *E. coli* and *S. aureus* were inhibited at concentrations of both 40 and 60mg/ml, while in the study carried out by Al-Hashimi [27], aqueous and ethanolic extracts of *H. sabdariffa* caused growth inhibition of *E. coli, S. aureus* and *P. aeruginosa,* with inhibitory zone diameters ranging within 17 and 46mm for all three organisms. Results from the study of Saeidi *et al* [23] showed that *H. sabdariffa* extracts inhibited growth of *E. coli*, *Shigella sp*. and *S. aureus* at concentrations of 1.25-20mg/ml, while the study of Nwaiwu *et al* [25] showed that it inhibited *Salmonella sp*., *Shigella sp*. and *Enterobacter sp*. each at 200mg/ml. Results similar to those obtained from this study were also seen in that of Panaitescu and Lengyel [24] in which *H. sabdariffa* extracts were found to inhibit growth in *E. coli, S. typhi, K. pneumonia* and *S. aureus* used in the study. Inhibitory concentrations were 4, 10, 20 and 100% respectively, while inhibitory zone diameters ranged within 0.1 and 5.0mm. The work of Jantrapanukorn *et al* [26] showed that it caused inhibition in *S. typhi*, *S. paratyphi A*, *S. flexneri, S. boydii, S. dysenteriae* and *S. sonnei* at a minimum concentration of 3.125mg/ml. The results of this study also agreed with those of Sekar *et al* [29], [30], [31] **– put all in one [29, 30 and 31]** in which *E. coli, P. aeruginosa, S. aureus, S. enterica* and *K. pneumoniae* were all inhibited.

4. CONCLUSION

The emergence and re-emergence of antibiotic-resistant organisms has become a serious problem in clinical practice due to the fact that some common antibiotics in use no longer demonstrate any significant effects on these organisms. This research was carried out in a bid to discover novel means of combating this public health scourge, as medicinal plants apparently offer promising solutions to this problem. Interestingly, the plant extracts of zobo leaves displayed remarkable activity at fairly-low concentrations, whereas extracts of bay leaf were completely not sensitive against the bacterial isolates. This means that in the nearest future, these common medicinal plants will have a place in modern medical practice.

COMPETING INTERESTS

No competing interest exist between authors

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