Original Research Article

ANTIDIARRHOEAGENIC POTENTIALS OF SYNERGISTIC ACTIVITIES OF WATER EXTRACTS OF *ALOE VERA* AND *HYPTIS SUAVEOLENS* AGAINST *GIARDIA LAMBLIA* AND *SALMONELLA* SPECIES INFECTIONS AMONG CHILDREN 0-5 YEARS IN BAUCHI STATE, NIGERIA.

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ABSTRACT

Background: The epidemiological significance of *Salmonella* species and *Giardia lamblia* co-infections of childhood diarrhoea differs substantially between regions depending on socio-economic conditions in the prevalence of different categories of diarrhoeagenic disease associated with diarrhoea among under 5 populations, medicinal plants extracts has increasingly been advocated in productions of antimicrobial agents as resistance with synthetic drugs increases.

Methodology: The phytochemicals constituents obtained from *Aloe vera* and *Hyptis suaveolens* were investigated to evaluate their medicinal potentials, the screening revealed the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, alkaloids, phenols. The study was conducted between June, 2018 and February, 2019 in an attempt to ascertain the efficacies of *Aloe vera* and *Hyptis suaveolens* individually and in combined against diarrhoea infections caused by *Giardia lamblia* and *Salmonella* species in under five children in Bauchi State, Nigeria. Anti-giardial activity was carried out in an *in-vitro* susceptibility assays method on *Giardia lamblia* and antibacterial activity was carried out by Kirby-Bauer method on *Salmonella* species. Parasites mortality was determined by counting in hemocytometer under a light microscope and zone of inhibitions produced against the bacteria were determined and subjected to descriptive statistics and inferential statistical analysis to determine their significance at 5% level using SPSS version 23. Means were separated using DMRT ($P \leq 0.05$).

Results: The results obtained showed that water extracts of *Aloe vera and Hyptis suaveolens* plants singly and in combinations had inhibitory effects on *Giardia lamblia* and *Salmonella* species. Shows that *Aloe vera* extracts on *Salmonella* species exhibited good zone of inhibitions 0.302±18.00, *Hyptis suaveolens* extracts 0.315±19.67 and the combined extracts 0.413±30.00. On *Giardia lamblia*, shows better activities with *Aloe vera* extracts 0.002±0.505, *Hyptis suaveolens* extracts 0.002±0.478 and the combined extracts 0.002±0.643. In all the cases, plants, concentrations and time were determinants factors for the anti-giardial and antibacterial activity.

Conclusion: Hence, *Hyptis suaveolens* extracts have better activities than extracts of *Aloe vera* while the combined extracts shows more better activities of antibacterial, *Aloe vera* have better activities than *Hyptis suaveolens* extracts while the combined extracts shows more better activities of antigiardial. Therefore, these plants possess antimicrobial potentials.

Keywords: *Aloe vera, Hyptis suaveolens, Giardia lamblia, Salmonella species, Children and Diarrhoea.*

INTRODUCTION

There is scarcity of information on the etiological agents causing diarrhoea disease in most African countries including Nigeria [11], [3]. Diarrhoea, nevertheless, remains a major cause of mortality and morbidity among children under five years of age especially in developing countries [2], [11], [3]. Acute diarrhoea disease has a significant impact on public health globally with pathogenic agents such as bacteria (*Salmonella, Shigella*, *Escherichia coli*, *Vibrio cholerae* and *Campylobacter*), parasites (*Cryptosporidium*, *Giardia lamblia* and *Entamoeba histolytica*) and viruses (Rotavirus, adenovirus, norovirus and astrovirus) recognized as the leading etiological agents [13], [8], [1], [20]. Giardiasis is a protozoan infections caused by *Giardia lamblia*; a flagellate protozoan, an infections principally of the upper small intestine and remains largely asymptomatic bringing on acute self-limited diarrhoea [5], [7]. Its occurrence is worldwide. Children are frequently more infected than adults. Prevalence is higher in area of poor sanitations, in institutions with overcrowded human conditions and in areas of children not toilet trained [10]. *Salmonella* is a genus of enteric pathogens that consists of two species, *Salmonella enterica* and *Salmonella bongori* [15], [9]. Broad host range *Salmonella typhimurium* causes gastroenteritis in humans and other mammals [19]. Plants remains one of the potential sources of effective agents against microbes including the deadly infections like HIV/AIDS, tuberculosis (*Mycobacterium tuberculosis*), syphilis (*Treponema pallidum*), gonorrhoea (*Neisseria gonorrhoea*), skin and wound infections (*Staphylococcus aureus*), diarrhoea (*Escherichia coli*), typhoid fever (*Salmonella typhus*) and *Pseudomonas aeruginosa* which directly infects the urinary tract, pulmonary tract, wounds, burns and also causes other blood infections [14]. The genus *Aloe* belongs to *Aloeaceae* (Liliaceae) family which has about 360 to 400 different species [16]. *Aloe vera* leaf components have been credited for it antibacterial, antifungal, antiviral and anti-helmintic properties [16]. *Hyptis suaveolens* commonly known as bush tea belongs to Lamiaceae family which is a potent medicinal herb [4]. Pharmacological property of *Hyptis suaveolens* includes antioxidant, antimicrobial, anticancer and anti-inflammatory [14]. This study is aim at evaluating the antimicrobial potentials of *Aloe vera* and *Hyptis suaveolens* singly and in combinations against infections caused by *Salmonella* species and *Giardia lamblia* in under five children.

MATERIALS AND METHODS

Aloe vera and *Hyptis suavolens* were randomly collected in Jos, Plateau State, authenticated by the plant curator of Federal College of Forestry, Jos, Nigeria. The design of the study is community and hospital based which allows for the collections, laboratory isolations, identifications and culturing of *Giardia lamblia* and *Salmonella* species occurring in both symptomatic and asymptomatic infections among children 0-5 years and the antimicrobial potentials of crude extracts of *Aloe vera* and *Hyptis suavolens* against them in Bauchi Metropolis. The air dried leaves of *Hyptis suavolens* was grounded into powdered, soaked for 72 hours, placed in Gallenkamp shaker rotated at about 65 revolutions per minute, the contents were then homogenized and filtered using Whatman filter paper no.1. The filtrate was poured into a round bottom flask and concentrated using a Buchi Rotavapor R-200 to yield *Hyptis suavolens* in required concentrations. The powdered *Aloe vera* was soaked and left to stand for 3days as reported by [17]. Stool samples were collected, placed in a clean disposable plastic tubes with tight fittings, microscopically examined for *Giardia lamblia* cysts/trophozoites presence. Positively detected 50 mg of stool were inoculated immediately into an axenic medium for culturing of *Giardia lamblia* trophozoites. Also, *Salmonella* species, stool samples collected were inoculated within two hours of collections onto selective and differential media:

MacConkey agar, *Salmonella-Shigella* agar and xylose lysine deoxycholate agar using a calibrated inoculated loop in spread plate method. The media were then incubated aerobically at 35°C for 18 to 24 hours as described by [21] and [12]. Biochemical test was carried out according to the methods described by [6] and [18]. The combined extracts were determined by using same solvent extractions making a combination in the ratio of 1:1 in each case. Parasites mortality was determined by counting in hemocytometer under a light microscope and zone of inhibitions produced against the bacteria were determined in triplicates, subjected to descriptive statistics and inferential statistical analysis to determine their significance at 5% level using SPSS version 23, means were separated using DMRT ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

The results in Table 1, shows the phytochemical constituents of water extracts of *Aloe vera* and *Hyptis suaveolens*, the following were found to be present and absent: saponins, tannins, alkaloids, flavonoids, terpenoids, alkaloids, phenols.

Table 1: Phytochemical Constituents of *Aloe vera* and *Hyptis suaveolens*

Name of Test	***Aloe vera* Extractions**	***Hyptis suaveolens***
Extractions	**Water (Extraction medium)**	**Water (Extraction medium)**
Saponins	+	+
Tannins	+	+
Flavonoids	-	-
Terpenoids	-	-
Steroids	-	-
Cardiac glycosides	-	-
Anthraqinones	-	-
Alkaloid	-	-
Phenolics	+	+

Key: (+) present, (-) absent

The results as presented in Table 2, shows the mean efficacy of treatments and time of *Aloe vera* and *Hyptis suaveolens* on cultured *Giardia lamblia* trophozoite produced after 48 hours which was significantly (P=0.05) different after 48 hours revealed the highest mean value treatments and time, efficacy was found with 80mg/ml of *Aloe vera* extracts (0.002±0.505) and 48hours of time (0.002±0.507) when compared with positive control (0.002±0.641). *Hyptis suaveolens*, activity was found with highest concentrations of 80mg/ml with activity (0.002±0.478) and 48hours recorded for time with activity (0.002±0.563) when compared with positive control (0.002±0.563). The result in each case equally showed that the significant value of treatments (0.000) on *Giardia lamblia* was less than the alpha value of 0.05 at 5% probability level of significant. This therefore reveals that the effect of treatments was significantly different, while the significant value of time (0.000) and the treatment/time combination (0.000) was also less than the 0.05 alpha values at 5% probability level.

Table 2: Standard Error and Mean Efficacy of Treatments (*Aloe vera*) and (*Hyptis suaveolens*) and Time on Cultured *Giardia lamblia* Trophozoite

		S.E ± Mean Effects after 48 hours	
		Aloe vera	***Hyptis suaveolens***
EXTRACTIONS		**Water**	**Water**
Treatment	**80mg**	0.002±0.505 [b]	0.002±0.478 [b]
	70mg	0.002±0.430 [c]	0.002±0.445 [c]
	60mg	0.002±0.357 [d]	0.002±0.386 [d]

	50mg	0.002±0.260 [e]	0.002±0.263 [e]
	40mg	0.002±0.041 [f]	0.002±0.058 [f]
	-$_{ve}$ Ctrl	0.002±0.004 [g]	0.002±0.009 [g]
	+$_{ve}$ Ctrl	0.002±0.641 [a]	0.002±0.638 [a]
Time (Hours)	**48**	0.002±0.507 [a]	0.002±0.563 [a]
	40	0.002±0.456 [b]	0.002±0.452 [b]
	32	0.002±0.378 [c]	0.002±0.360 [c]
	24	0.002±0.314 [d]	0.002±0.300 [d]
	16	0.002±0.169 [e]	0.002±0.177 [e]
	8	0.002±0.093 [f]	0.002±0.101 [f]

Each value is a mean of ± standard error of three replicates. Means followed by same superscripts in the column are significantly different from each other.

The results as presented in Table 3, shows the mean efficacy of treatments and time of the combined extracts of *Aloe vera* and *Hyptis suaveolens* on cultured *Giardia lamblia* trophozoites produced after 48 hours which was significantly (P=0.05) different after 48 hours revealed the highest mean value treatments and time, efficacy was found with 80mg/ml of the combined extracts with treatment activity (0.002±0.643) and 48hours of time with activity (0.002±0.719) when compared with the positive control (0.002±0.621).The result equally showed in that the significant value of treatments (0.000) on *Giardia lamblia* was less than the alpha value of 0.05 at 5% probability level of significant. This therefore reveals that the effect of treatments was significantly different, while the significant value of time (0.000) and the treatment/time combination (0.000) was also less than the 0.05 alpha values at 5% probability level.

Table 3: Standard Error and Mean Efficacy of Treatments (Combined *Aloe vera* and *Hyptis suaveolens* Extracts) and Time on Cultured *Giardia lamblia* Trophozoites

S.E ± Mean Effects after 48 hours

EXTRACTIONS		**Water**
Treatment	**80mg**	0.002±0.643 [a]
	70mg	0.002±0.582 [c]
	60mg	0.002±0.497 [d]
	50mg	0.002±0.444 [e]
	40mg	0.002±0.309 [f]
	-$_{ve}$ Ctrl	0.002±0.011 [g]
	+$_{ve}$ Ctrl	0.002±0.621 [b]
Time (Hours)	**48**	0.002±0.719 [a]
	40	0.002±0.607 [b]
	32	0.002±0.495 [c]
	24	0.002±0.420 [d]
	16	0.002±0.272 [e]
	8	0.002±0.150 [f]

Each value is a mean of ± standard error of three replicates. Means followed by same superscripts in the column are significantly different from each other.

The results as tabulated in Table 4, shows the mean efficacy of inhibition zones of treatments of *Aloe vera* and *Hyptis suaveolens* on cultured *Salmonella* species, was significantly (P=0.05) different which shows zones of inhibitions of water extractions of *Aloe vera* (0.302±18.00mm) when compared to control (0.302±29.50mm) and *Hyptis suaveolens* (0.315±19.67mm) when compared with positive control (0.315±29.50mm). The results equally reveals that the significant value treatment (0.000) on *Salmonella* species was less than the alpha value of 0.05 at 5% probability level of significant. This therefore reveals that the effect of treatments was significantly different in each case.

Table 4: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments of *Aloe vera* and *Hyptis suaveolens* on Cultured *Salmonella* species

S.E ± Mean Effects after 48 hours, measured in millimeters

		Aloe vera	***Hyptis suaveolens***
EXTRACTIONS		**Water**	**Water**
Treatment	**80mg**	0.302 ± 18.00^{b}	0.315 ± 19.67^{c}
	70mg	0.302 ± 15.00^{c}	0.315 ± 17.33^{c}
	60mg	0.302 ± 12.67^{d}	0.315 ± 14.83^{d}
	50mg	0.302 ± 9.167^{e}	0.315 ± 12.00^{e}
	40mg	0.302 ± 7.333^{f}	0.315 ± 9.167^{f}
	$-_{ve}$ Ctrl	0.302 ± 0.667^{g}	0.315 ± 0.000^{g}
	$+_{ve}$ Ctrl	0.302 ± 29.50^{a}	0.315 ± 29.50^{a}

Each value is a mean of ± standard error of three replicates. Means followed by same superscripts in the column are significantly different from each other.

Table 5 presented the minimum inhibitory concentrations and minimum bactericidal concentrations of *Hyptis suaveolens* and *Aloe vera* extracts, shows that, the extracts of *Hyptis suaveolens* has a better inhibitory effects (12.0µg/ml) on *Salmonella* species when compared with the positive control (8.0µg/ml)

Table 5: Minimum Inhibitory Concentration in Micrograms/Milliliter of *Hyptis suaveolens* Extracts Against *Salmonella* Species

Extractions	***Aloe vera***	***Hyptis suaveolens***
MIC	13.0	12.0
Positive Control	8.0	8.0
MBC	14.0	13.0

The results tabulated in Table 6, shows the mean efficacy of inhibition zones of treatments of the combined *Aloe vera* and *Hyptis suaveolens* on cultured *Salmonella* species, was significantly (P=0.05) different which shows zones of inhibitions of water extractions of the combined *Aloe vera* and *Hyptis suaveolens* (0.413±30.00mm) when compared to control (0.413±30.00mm). The results equally reveals that the significant value treatment (0.000) on *Salmonella* species was less than the alpha value of 0.05 at 5% probability level of significant.

Table 6: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments of the Combined *Aloe vera* and *Hyptis suaveolens* on Cultured *Salmonella* Species

S.E ± Mean Effects after 48 hours, measured in millimeters

EXTRACTIONS		Water
Treatment	**80mg**	0.413±30.00[a]
	70mg	0.413±28.00[b]
	60mg	0.413±24.67[c]
	50mg	0.413±22.50[d]
	40mg	0.413±18.67[e]
	$-_{ve}$ Ctrl	0.413±0.667[f]
	$+_{ve}$ Ctrl	0.413±30.00[a]

Each value is a mean of ± standard error of three replicates. Means followed by same superscripts in the column are significantly different from each other.

The results as tabulated in Table 7, shows the minimum inhibitory concentration and minimum bactericidal concentration in micrograms/milliliter of the combined *Aloe vera* and *Hyptis suaveolens* extracts against *Salmonella* species, it shows that water extracts of the combined *Aloe vera* and *Hyptis suaveolens* was more active (8.0µg/ml) against *Salmonella* species when compared with the control (8.0µg/ml).

Table 7: Minimum Inhibitory Concentration in Micrograms/Milliliter of the Combined *Aloe vera* and *Hyptis suaveolens* Extracts Against *Salmonella* Species

Extractions	Combined *Aloe vera* and *Hyptis suaveolens*
MIC	8.0
Positive Control	8.0
MBC	8.2

CONCLUSION

Based on the findings of this research work, water extracts of *Hyptis suaveolens*, *Aloe vera* and the combined, have better activities on *Giardia lamblia* and *Salmonella* species. Hence, *Hyptis suaveolens* extracts have better activities than extracts of *Aloe vera* while the combined extracts shows more better activities of antibacterial, *Aloe vera* have better activities than *Hyptis suaveolens* extracts while the combined extracts shows more better activities of antigiardial. There was the presence of phytochemicals in these plant extracts. Therefore, these plants possess antimicrobial potentials, it is thus concluded that these plants are promising and are very important antidiarrhoeagenic agents.

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