Original Research Article

SCREENING FOR ANTIMICROBIAL ACTIVITIES OF METANOLLIC EXTRACTS OF *ALOE VERA* AND *HYPTIS SUAVEOLENS* AGAINST CO-INFECTIONS OF *GIARDIA LAMBLIA* AND *SALMONELLA* AMONG DIARRHOEAGENIC CHILDREN

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ABSTRACT: The antimicrobial and Phytochemicals activities of methanol extracts obtained from *Aloe vera* and *Hyptis suaveolens* plants were investigated individually and combined in an attempt to evaluate their medicinal potentials and efficacies on protozoan; *Giardia lamblia* and bacteria; *Salmonella* species as co-infections causing diarrhoea in under five populations in Bauchi State, Nigeria. The phytochemical screening revealed the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, alkaloids, phenols. Antimicrobial activity was determined against *Giardia lamblia* and *Salmonella* species; anti-giardial activity, an *in-vitro* susceptibility assays method was performed and antibacterial activity was carried out by Kirby-Bauer method. The parasites mortality was determined by counting in hemocytometer under a light microscope and the zone of inhibition diameter produced against the bacteria were determined, expressed as mean ±SEM (Standard Error of Mean) and the differences between means were statistically analyzed and compared. The results obtained showed that methanol extracts of *Aloe vera and Hyptis suaveolens* singly used and in combinations had inhibitory effects on *Giardia lamblia* and *Salmonella* species. In all cases, the extractions, plants, concentrations and time were determinant factors for the anti-giardial and antibacterial activity. Anti-giardial activity was best recorded with extracts of *Aloe vera* which showed anti-giardial activity of (0.002±0.553), and activity was greater in combined *Aloe vera* and *Hyptis suaveolens* which showed anti-giardial activity of (0.002±0.679). Also, antibacterial activity of methanol extracts of these plants on *Salmonella* species, showed higher zone of inhibitions diameter with extracts of *Aloe vera* (0.895±20.17) and the efficacy becomes higher with the combined *Aloe vera* and *Hyptis suaveolens* which zone of inhibitions is (0.423±27.50).

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

INTRODUCTION

Diarrhoea, nevertheless, remains a major cause of mortality and morbidity among children under five years of age especially in developing countries [2]; [8]. Acute diarrhoea disease has 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 leading etiologic agents [10]; [5]. Since 2000, childhood mortality due to diarrhoea has diminished by 6.5% every year, but this trend requires an acceleration to reach

the 2030 objectives. Diarrhoea infections are associated with acute gastroenteritis, one of the most common alimentary diseases; caused by the consumptions of contaminated water and food especially meat [13]. The prevalence rate in Nigeria is about 18.8%, one of the worst in sub-Saharan Africa and accounts for over about 16% of child-deaths and estimated 150,000 deaths chiefly among children less than five years of age which occurs annually due to this disease which is caused by poor sanitations and poor hygiene practices [8]. *Salmonella* is a genus of enteric pathogens consisting of two species; *Salmonella enterica* and *Salmonella bongori* which cause diseases in broad range of hosts, [6]. This sub-species includes host-restricted serovars like *Salmonella typhi* which cause typhoid fever in humans and the broad host range *Salmonella typhimurium* causing gastroenteritis in humans and other mammals [14]. Giardiasis is a protozoan infection principally of the upper small intestine and remains largely asymptomatic bringing on acute self-limited diarrhea [3]; [4]. Its occurrence is world-wide. Children are infected more frequently than adults. Prevalence is higher in area of poor sanitations in institutions with overcrowded human conditions and areas of children not toilet trained [7]. Medicinal plants are widely used to treat different diseases in different parts of the world, as part of complementary and alternative medicine, a number of phyto-medicines including those obtained from African plants are in global markets [1]. Even though medicinal plants may not have been used systematically in Africa as in the western and eastern countries, medicinal plants remain the backbone of African healthcare system. It is therefore pertinent that African plants should be investigated systematically for better use in healthcare systems. Several plant extracts and phytochemicals obtained from them have shown activities against certain types of microorganisms including Gram positive and Gram negative bacteria [11].

This study is aim at determining the antimicrobial potentials of medicinal plants; *Aloe vera* and *Hyptis suaveolens* extracts against co-infections of *Salmonella* sp. and *Giardia lamblia* and to evaluate their qualitative phytochemical compositions.

MATERIALS AND METHODS

The design was both community and hospitals-based prospective cross-sectional study. Conducted between April, 2018 and February, 2019, the design of the study allows for the collections, extractions of both *Aloe vera* and *Hyptis suavolens* L., laboratory isolation, detections and culturing of *Giardia lamblia* and *Salmonella* sp. occurring in both symptomatic and asymptomatic infections among children and the antimicrobial potentials of the crude extracts of *Aloe vera* and *Hyptis suavolens* L. against them in Bauchi Metropolis. The plants were randomly collected in around densely populated areas in Jos, Plateau State. The plants were authenticated by the plant curator at the Herbarium of Federal College of Forestry, Jos, Plateau State, Nigeria. The air dried leaves of *Hyptis suavolens* L. was grounded into powder soaked in methanol for 72 hours, placed in Gallenkamp shaker rotating at 65 revolutions per minute, the contents were then homogenized and filtered using Whatman filter paper no.1. The filtrate were poured into a round bottom flask and concentrated using a Buchi Rotavapor R-200 to yield *Hyptis suavolens* in required concentrates and also, the grounded powder *Aloe vera* soaked in methanol in conical flasks and left to stand for 3days as reported by [12]. Stool samples collected, placed in a clean disposable plastic tubes with tight fittings, microscopically examined for *Giardia lamblia* cysts and trophozoites presence, positively detected 50 mg of stool was inoculated immediately in an axenic medium for culture of *Giardia lamblia* trophozoites. Also, *Salmonella* species, stool samples collected were inoculated within two hours of collections onto selective and differential media: MacConkey (MAC) agar, *Salmonella-Shigella* (SS) agar, and xylose lysine deoxycholate (XLD) agar, using a calibrated inoculating loop in the spread plate

method. The media were then incubated aerobically at 35°C for 18 to 24 hours as described by [15] and [9].

RESULTS AND DISCUSSION

The results in table 1, shows the plant extracts of *Aloe vera and Hyptis suaveolens* were qualitatively tested for the presence of phytochemicals. All the plant extracts were found to contain saponins, tannins, alkaloids, flavonoids, terpenoids, alkaloids, phenolics.

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

Name of Test	***Aloe vera***	***Hyptis suaveolens***
Extractions	**Methanol**	**Methanol**
Saponins	+	-
Tannins	+	+
Flavonoids	+	-
Terpenoids	-	-
Steroids	-	-
Cardiac glycosides	-	-
Anthraqinones	-	-
Alkaloid (Wagner's test)	+	+
Alkaloid (Mayer's test)	+	-
Phenolics	-	+

Key: (+) present, (-) absent

The fecal culture sample of *Giardia lamblia* trophozoites produced after 72 hours in an estimated numbers are $0.9\text{-}1\times10^{3}$/ml.

Hence, the results as presented in table 2, shows the mean efficacy of treatments and time of *Aloe vera* on cultured *Giardia lamblia* trophozoite produced after 48 hours was significantly (P=0.05) different after 48 hours reveals the highest mean value treatment with 80mg/ml and 48 hours of time resulted in higher efficacy with methanol extractions (0.002±0.553) and (0.002±0.550) when compared with positive control (0.002±0.633).

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

		S.E ± Mean Effects after 48 hours
EXTRACTIONS		**Methanol**
Treatment	**$-_{ve}$ Ctrl**	0.002 ± 0.004^{g}
	$+_{ve}$ Ctrl	0.002 ± 0.633^{a}
	40mg	0.002 ± 0.067^{f}
	50mg	0.002 ± 0.294^{e}
	60mg	0.002 ± 0.407^{d}
	70mg	0.002 ± 0.470^{c}
	80mg	0.002 ± 0.553^{b}
Time (Hours)	**8**	0.002 ± 0.112^{f}
	16	0.002 ± 0.210^{e}
	24	0.002 ± 0.320^{d}

	32	0.002±0.405 [c]
	40	0.002±0.485 [b]
	48	0.002±0.550 [a]

Each value is a mean of ± standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

Table 3 shows the results of mean efficacy of treatments and time of *Hyptis suaveolens* on cultured *Giardia lamblia* trophozoite produced after 48 hours, revealed that the effect of *Hyptis suaveolens* extracts was significantly (P=0.05) and the highest mean value treatment was with 80mg/ml and 48 hours of time (0.002±0.377) and (0.002±0.412) when compared with positive control (0.002±0.586).

Table 3: Standard Error and Mean Efficacy of Treatments (*Hyptis suaveolens*) and Time on Cultured *Giardia lamblia* Trophozoite

S.E ± Mean Effects after 48 hours

EXTRACTIONS		**Methanol**
Treatment	**$-_{ve}$ Ctrl**	0.002±0.008 [g]
	$+_{ve}$ Ctrl	0.002±0.586 [a]
	40mg	0.002±0.017 [f]
	50mg	0.002±0.159 [e]
	60mg	0.002±0.224 [d]
	70mg	0.002±0.296 [c]
	80mg	0.002±0.377 [b]
Time (Hours)	**8**	0.002±0.077 [f]
	16	0.002±0.119 [e]
	24	0.002±0.220 [d]
	32	0.002±0.271 [c]
	40	0.002±0.330 [b]
	48	0.002±0.412 [a]

Each value is a mean of ± standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

Table 4 as presented shows the mean efficacy of treatments and time of combined *Aloe vera* and *Hyptis suaveolens* on cultured *Giardia lamblia* trophozoite produced after 48 hours, revealed that the effect of combined *Aloe vera* and *Hyptis suaveolens* extracts was significantly (P=0.05) and the highest mean value treatment was with 80mg/ml and 48 hours of time (0.002±0.679) and (0.002±0.742) when compared with positive control (0.002±0.627).

Table 4: 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 S.E ± Mean Effects after 48 hours

EXTRACTIONS	**Methanol**

Treatment	**$-_{ve}$ Ctrl**	0.002±0.007 [g]
	$+_{ve}$ Ctrl	0.002±0.627 [b]
	40mg	0.002±0.383 [f]
	50mg	0.002±0.458 [e]
	60mg	0.002±0.520 [d]
	70mg	0.002±0.603 [c]
	80mg	0.002±0.679 [a]
Time (Hours)	**8**	0.002±0.168 [f]
	16	0.002±0.289 [e]
	24	0.002±0.465 [d]
	32	0.002±0.525 [c]
	40	0.002±0.620 [b]
	48	0.002±0.742 [a]

Each value is a mean of ± standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

The result in table 5, shows the mean efficacy of inhibitions zones of treatments with *Aloe vera* on cultured *Salmonella* species, the average zones of inhibition formed by the effect of *Aloe vera* extracts was significantly (P=0.05) different which reveals the highest zone of inhibition value treatment with 80mg/ml (0.895±20.17) compared with positive control (0.895±29.33).

Table 5: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments of *Aloe vera* on Cultured *Salmonella* species

	S.E ± Mean Effects after 48 hours	
EXTRACTIONS		**Methanol**
Treatment	**$-_{ve}$ Ctrl**	0.895±0.333 [f]
	$+_{ve}$ Ctrl	0.895±29.33 [a]
	40mg	0.895±8.883 [e]
	50mg	0.895±11.83 [d]
	60mg	0.895±17.00 [c]
	70mg	0.895±18.67 [bc]
	80mg	0.895±20.17 [b]

Each value is a mean of ± standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

The result in table 6, shows the mean efficacy of inhibitions zones of treatments with *Hyptis suaveolens* on cultured *Salmonella* species, the average zones of inhibition formed by the effect of *Hyptis suaveolens* extracts was significantly (P=0.05) different which reveals the highest zone of inhibition value treatment with 80mg/ml (0.309±13.33) compared with positive control (0.309±28.67).

Table 6: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments of *Hyptis suaveolens* on Cultured *Salmonella* species

	S.E ± Mean Effects after 48 hours	
EXTRACTIONS		**Methanol**
Treatment	**$-_{ve}$ Ctrl**	0.309±0.000[g]
	$+_{ve}$ Ctrl	0.309±28.67[a]
	40mg	0.309±5.000[f]

	50mg	0.309±7.333[e]
	60mg	0.309±9.333[d]
	70mg	0.309±10.33[c]
	80mg	0.309±13.33[b]

Each value is a mean of ± standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

The result in table 7, shows the mean efficacy of inhibitions zones of treatments with combined *Aloe vera* and *Hyptis suaveolens* on cultured *Salmonella* species, the average zones of inhibition formed by the effect of combined *Aloe vera* and *Hyptis suaveolens* extracts was significantly (P=0.05) different which reveals the highest zone of inhibition value treatment with 80mg/ml (0.423±27.50) compared with positive control (0.423±29.00).

Table 7: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments of combined *Aloe vera* and *Hyptis suaveolens* on Cultured *Salmonella* species

S.E ± Mean Effects after 48 hours		
EXTRACTIONS		**Methanol**
Treatment	**-$_{ve}$ Ctrl**	0.423±0.667[g]
	+$_{ve}$ Ctrl	0.423±29.00[a]
	40mg	0.423±14.17[f]
	50mg	0.423±18.33[e]
	60mg	0.423±21.00[d]
	70mg	0.423±24.67[c]
	80mg	0.423±27.50[b]

Each value is a mean of ± standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

CONCLUSION

Based on the findings of this research work, methanol extracts of *Hyptis suaveolens, Aloe vera* and of combined *Aloe vera* and *Hyptis suaveolens* all exhibited good activity on *Giardia lamblia* and *Salmonella* species, hence, they possess antimicrobial potentials. There was the presence of phytochemicals in these plant extracts, it is thus concluded that these plants are promising and are very important for the future treatment of *Giardia lamblia* and *Salmonella* sp. causing diarrhoea.

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