

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

ABSTRACT: The antimicrobial and Phytochemicals activities of methanol 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 and bacteria species in relations to causing diarrhoea in under five populations in Bauchi State, Nigeria. The phytochemical screening revealed the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, alkaloids, phenolics. Antimicrobial activity was determined against *Giardia lamblia* and *Salmonella* sp.; 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 produced on the bacteria were expressed as mean \pm SEM (Standard Error of Mean) and the differences between means were statistically analysed and compared. The results obtained showed that methanolic extracts of *Aloe vera* and *Hyptis suaveolens* plants singly and in combinations had inhibitory effects on *Giardia lamblia* and *Salmonella* sp. In all cases, the extraction solvents, plants, concentrations and time were determinant factors for the anti-giardial and antibacterial activity. Anti-giardial activity was best with extracts of *Aloe vera* which showed anti-giardial activity of (0.002 \pm 0.553), and activity was greater in combined *Aloe vera* and *Hyptis suaveolens* which showed anti-giardial activity of (0.002 \pm 0.679). Also, antibacterial activity of methanol extracts of these plants on *Salmonella* species, showed higher zone of inhibitions with extracts of *Aloe vera* (0.895 \pm 20.17) and the efficacy becomes higher in combined *Aloe vera* and *Hyptis suaveolens* which zone of inhibitions is (0.423 \pm 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

46 *Salmonella typhi* which cause typhoid fever in humans and the broad host range *Salmonella*
47 *typhimurium* causing gastroenteritis in humans and other mammals [14]. Giardiasis is a
48 protozoan infection principally of the upper small intestine and remains largely asymptomatic
49 bringing on acute self-limited diarrhea [3]; [4]. Its occurrence is world-wide. Children are
50 infected more frequently than adults. Prevalence is higher in area of poor sanitations in
51 institutions with overcrowded human conditions and areas of children not toilet trained [7].
52 Medicinal plants are widely used to treat different diseases in different parts of the world, as part
53 of complementary and alternative medicine, a number of phyto-medicines including those
54 obtained from African plants are in global markets [1]. Even though medicinal plants may not
55 have been used systematically in Africa as in the western and eastern countries, medicinal plants
56 remain the backbone of African healthcare system. It is therefore pertinent that African plants
57 should be investigated systematically for better use in healthcare systems. Several plant extracts
58 and phytochemicals obtained from them have shown activities against certain types of
59 microorganisms including Gram positive and Gram negative bacteria [11].

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

63 **MATERIALS AND METHODS**

64 The design was both community and hospitals-based prospective cross-sectional study. The
65 design of the study allows for the collections, extractions of both *Aloe vera* and *Hyptis suaveolens*
66 L., laboratory isolation, detections and culturing of *Giardia lamblia* and *Salmonella* sp. occurring
67 in both symptomatic and asymptomatic infections among children and the antimicrobial
68 potentials of the crude extracts of *Aloe vera* and *Hyptis suaveolens* L. against them in Bauchi
69 Metropolis. The plants were randomly collected in around densely populated areas in Jos,
70 Plateau State. The plants were authenticated by the plant curator at the Herbarium of Federal
71 College of Forestry, Jos, Plateau State, Nigeria. The air dried leaves of *Hyptis suaveolens* L. was
72 grounded into powder soaked in methanol for 72 hours, placed in Gallenkamp shaker rotating at
73 65 revolutions per minute, the contents were then homogenized and filtered using Whatman filter
74 paper no.1. The filtrate were poured into a round bottom flask and concentrated using a Buchi
75 Rotavapor R-200 to yield *Hyptis suaveolens* in required concentrates and also, the grounded
76 powder *Aloe vera* soaked in methanol in conical flasks and left to stand for 3days as reported by
77 [12]. Stool samples collected, placed in a clean disposable plastic tubes with tight fittings,
78 microscopically examined for *Giardia lamblia* cysts and trophozoites presence, positively
79 detected 50 mg of stool was inoculated immediately in an axenic medium for culture of *Giardia*
80 *lamblia* trophozoites. Also, *Salmonella* species, stool samples collected were inoculated within
81 two hours of collections onto selective and differential media: MacConkey (MAC) agar,
82 *Salmonella-Shigella* (SS) agar, and xylose lysine deoxycholate (XLD) agar, using a calibrated
83 inoculating loop in the spread plate method. The media were then incubated aerobically at 35°C
84 for 18 to 24 hours as described by [15] and [9].

85 **RESULTS AND DISCUSSION**

86 The results in table 4.1, shows the plant extracts of *Aloe vera* and *Hyptis suaveolens* were
87 qualitatively tested for the presence of phytochemicals. All the plant extracts were found to
88 contain saponins, tannins, alkaloids, flavonoids, terpenoids, alkaloids, phenolics.

89 **Table 4.1: Phytochemical Constituents of *Aloe vera* and *Hyptis suaveolens***

Name of Test	<i>Aloe vera</i>	<i>Hyptis suaveolens</i>
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Extractions	Methanol	Methanol
Saponins	+	-
Tannins	+	+
Flavonoids	+	-
Terpenoids	-	-
Steroids	-	-
Cardiac glycosides	-	-
Anthraquinones	-	-
Alkaloid (Wagner's test)	+	+
Alkaloid (Mayer's test)	+	-
Phenolics	-	+

90 **Key:** (+) present, (-) absent

91 The fecal culture sample of *Giardia lamblia* trophozoites produced after 72 hours in an estimated
 92 numbers are $0.9-1 \times 10^3$ /ml. Hence, the results as presented in table 4.2, shows the mean efficacy
 93 of treatments and time of *Aloe vera* on cultured *Giardia lamblia* trophozoite produced after 48
 94 hours was significantly ($P=0.05$) different after 48 hours reveals the highest mean value
 95 treatment with 80mg/ml and 48 hours of time resulted in higher efficacy with methanol
 96 extractions (0.002 ± 0.553) and (0.002 ± 0.550) when compared with positive control
 97 (0.002 ± 0.633).

98 **Table 4.2: Standard Error and Mean Efficacy of Treatments (*Aloe vera*) and Time on**
 99 **Cultured *Giardia lamblia* Trophozoite**

100
 101 **S.E \pm 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

102
 103 *Each value is a mean of \pm standard error of three replicates. Mean followed by the same*
 104 *superscripts in a column are not significantly different from each other.*
 105

106 Table 4.3 shows the results of mean efficacy of treatments and time of *Hyptis suaveolens* on
 107 cultured *Giardia lamblia* trophozoite produced after 48 hours, revealed that the effect of *Hyptis*

108 *suaveolens* extracts was significantly (P=0.05) and the highest mean value treatment was with
 109 80mg/ml and 48 hours of time (0.002±0.377) and (0.002±0.412) when compared with positive
 110 control (0.002±0.586).

111 **Table 4.3: Standard Error and Mean Efficacy of Treatments (*Hyptis suaveolens*) and Time**
 112 **on Cultured *Giardia lamblia* Trophozoite**

113 **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

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

116
 117 Table 4.4 as presented shows the mean efficacy of treatments and time of combined *Aloe vera*
 118 and *Hyptis suaveolens* on cultured *Giardia lamblia* trophozoite produced after 48 hours, revealed
 119 that the effect of combined *Aloe vera* and *Hyptis suaveolens* extracts was significantly (P=0.05)
 120 and the highest mean value treatment was with 80mg/ml and 48 hours of time (0.002±0.679) and
 121 (0.002±0.742) when compared with positive control (0.002±0.627).

122 **Table 4.4: Standard Error and Mean Efficacy of Treatments (*Aloe vera* and *Hyptis***
 123 ***suaveolens*) and Time on Cultured *Giardia lamblia* Trophozoite**

124
 125 **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

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

128
 129 The result in table 4.5, shows the mean efficacy of inhibitions zones of treatments with *Aloe vera*
 130 on cultured *Salmonella* species, the average zones of inhibition formed by the effect of *Aloe vera*
 131 extracts was significantly (P=0.05) different which reveals the highest zone of inhibition value
 132 treatment with 80mg/ml (0.895±20.17) compared with positive control (0.895±29.33).

133 **Table 4.5: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments**
 134 **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

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

138
 139 The result in table 4.6, shows the mean efficacy of inhibitions zones of treatments with *Hyptis*
 140 *suaveolens* on cultured *Salmonella* species, the average zones of inhibition formed by the effect
 141 of *Hyptis suaveolens* extracts was significantly (P=0.05) different which reveals the highest zone
 142 of inhibition value treatment with 80mg/ml (0.309±13.33) compared with positive control
 143 (0.309±28.67).

144 **Table 4.6: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments**
 145 **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

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

149
 150 The result in table 4.7, shows the mean efficacy of inhibitions zones of treatments with combined
 151 *Aloe vera* and *Hyptis suaveolens* on cultured *Salmonella* species, the average zones of inhibition
 152 formed by the effect of combined *Aloe vera* and *Hyptis suaveolens* extracts was significantly

153 (P=0.05) different which reveals the highest zone of inhibition value treatment with 80mg/ml
 154 (0.423±27.50) compared with positive control (0.423±29.00).

155 **Table 4.7: Standard Error and Mean Efficacy of Inhibition Zone Diameters of Treatments**
 156 **of combined *Aloe vera* and *Hyptis suaveolens* on Cultured *Salmonella* species**

157

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

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

160
 161 **Conclusion**

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

168
 169
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