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Phytochemical and Antimicrobial Activities of the Leaf Extract of *Stachytarpheta Angustifolia* (MILL) Vahl Verbenaceae

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ABSTRACT

Petroleum ether and partitioned leaf ethanolic extract of *Stahytarpheta angustifolia* were analysed, Phytochemically and evaluated for their antimicrobial activity against some pathogenic species: *Staphylococcus aureus, streptococcus pyogenes*. *Escherichia coli and salmonella typhi*. The partitioned leaf ethanolic extract reveals the presence of cardiac glycoside, Saponins, tannin, Terpeniod and steroid. The Maximum zone of inhibition of 27mmm was exhibited by the chloroform extract. The best (MIC) and (MBC) values were observed for the microorganisms sensitive to chloroform portion of the extract. Ampiclox 5mg/ml was used as the standard antibiotic (control). The greater and remarkable antimicrobial activity of the chloroform portion was recorded against *S. aureus and S. Pyogenes*. These results provide the rationalization for the traditional use of the leaf for the treatment of some infectious diseases.

Keywords: *Stachytarpheta angustifolia*, antimicrobial activity, Verbenaceae.

1. INTRODUCTION

Biologically active compounds from natural sources are of interest as possible new drugs for infectious diseases [1]. *Stachytarpheta angustifolia* commonly known as devils coach, bastard vervain or Brazilian tea is a seasonal shrub that grows to about 4 feet high. The shrub has a soft cylindrical bark which are simple or slightly branched and often rather succulent. The flowers are mostly pale blue with or without centre. The shrubly plant can easily be recognized by its opposite leaves of length (2-5cm) and (1-2mm) broad, distinctly petiole, oblong – lanceolate, acute and glabrous. They have deep spikes which are slender (6 – 9 inch) long. The calyx is nearly as the bracts with a minute tooth while the corolla is as twice and long as the calyx having a small blue limb. The inflorescence is a curious whip like of 1.5ft long covered with spiral of bracts [2]. The leaves are use for the relief of sprain by rubbing the juice on the affected part and also use as an adulterant in tea. The aerial part of the whole plant is boil and taken as a remedy against diarrhoea, Intestinal parasite and skin ulcer. [3]. This plant is reported to contain a glucosidal substance stachytarphine which is reported to be Abortificient. The decoction of the whole plant is taken as an antihelmintic agent, while the infusion of the plant mixed with patron is taken as a remedy against gonorrhoea, syphilis and other related venerable infectious diseases [4]. In this study the antimicrobial activities of the leaf extract of *Stahytarpheta angustifolia* was investigated.

2. MATERIAL AND METHOD

Plant Material

The leaf portion of *Stachytarpheta angustifolia* (Mill) verbanaceae was collected from a village outskirt of Zaria, Nigeria. The plant was authenticated at the Herbarium of the Department of Biological Sciences Ahmadu Bello University, Zaria and a voucher specimen (N0 900188) was deposited.

Extraction Procedure

The air-dried powdered material (800g) was successively defatted at room temperature with petroleum ether 60^0c – 80^0c (5 x 600ml) to exhaustion using cold maceration method. The defatted marc was exhaustively extracted with 95% ethanol [5]. The ethanolic and pet-ether extract were concentrated to afford a dark brown gummy mass and an oily mass coded as 'Es" and 'Ps" respectively. The ethanolic extract (30g) was suspended in water and successively partition with chloroform, ethyl acetate and n-butanol. The various partition portions were concentrated at room temperature while the n-butanol and Aqueous portion were obtain using rotary evaporator. These were coded Cl, Ea, n-but and Aq [6]. The partitioned leaf extracts were hence subjected to phytochemical screening using standard protocols [7], [8], [9].

Tested Microorganisms

Antimicrobial activity of *Starchytarpheta angustifolia* leaf was investigated against four bacteria isolate comprising of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Salmonella typhi* which were obtain from Department of Pharmaceutical Microbiology Ahmadu Bello University Teaching Hospital Zaria, Nigeria.

Inoculum Preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37^0C for 4 hours. The turbidity of

actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1.2×10^8 colony-forming units per milliliter (cfu/ml). The grown suspension was used for further testing [10].

Antimicrobial Bioassay

The antimicrobial activities of *Starchytarpheta angustifolia* leaf were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards [11], [12] . Muller Hinton Agar was used for the antimicrobial activity test. Under aseptic conditions in the Biosafety chamber, 15ml of Nutrient agar medium was dispensed into pre-sterilized Petri dishes to yield a uniform depth of 4 mm and inoculated by the bacterial culture. Sterile disks (Diameter 6mm) were impregnated with 20mg/ml concentration of *Starchytarpheta angustifolia* leaf dried for 10-15 minutes. The dried disks were placed on Nutrient agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. The discs were spaced far enough to avoid reflections wave from the edges of the Petri dishes and overlapping rings of inhibition. Finally, the Petri dishes were incubated for 18 to 24 hours at 37^0C . The diameter of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured and recorded [13], [14].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) was determined for each of the plant extract showing antimicrobial activity against the test isolates using broth micro dilution method [15]. The MIC values were taken as the lowest concentration of the extracts in the well of the test tube that showed no turbidity after incubation. The turbidity of the wells in the test tube was interpreted as visible growth of microorganisms. The minimum bactericidal concentration (MBC) was determined by sub culturing from each well showing no apparent growth. Least concentration of the extract showing no visible growth on sub culturing was taken as MBC [10], [16]

3. RESULTS

Table 1: Phytochemical Constituents of the Leaf Extract of *S. angustifolia.*

CONSTITUENTS			INFERENCE			
	Ps	Es	Cl	Ea	n-But	Aq
Carbohydrate	-	++	-	-	-	++
Terpenoids	++	++	++	++	+	-
Steroids	+	+++	+	++	-	-
Anthraquinones	-	-	-	-	-	-
Tannins	-	+++	-	+	++	++
Alkaloids	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Cardiac glycoside	-	++	-	+	++	-
Saponin	-	++	-	-	++	++

Key - = Absent, + = Faintly present, ++ - moderately present, +++ - highly present.
Ps = Pet – ether, Es = Ethanolic extract, Cl = Chloroform Ea = Ethyl acetate, n-But = N –Butanol and Aq = Aqueous portion.

Table 2: Antimicrobial Activities of the Solvent used against the Microbes (control)

Test Organism	Ps	Es	Cl	Ea	n-But	Aq
S. aureus	R	R	R	R	R	R
S. pyogenes	R	R	R	R	R	R
E. coli	R	R	R	R	R	R
S. typhi	R	R	R	R	R	R

Key: R = Resistance, Ps = Pet ether, Es = Ethanolic, Cl = Chloroform, Ea = Ethyl acetate n-But = N-Butanol and Aq = Aqueous extract.

Table 3: Antimicrobial Activities of the Leaf Extract

Test Organism	Ps	Es	Cl	Ea	n-But	Aq
S. aureus	S	S	S	S	S	S
S. pyogenes	R	S	S	S	S	S
E. coli	S	S	S	S	S	S
S. typhi	R	S	S	S	S	S

Key = S = Sensitive, R = Resistance

Table 4: Zones of Inhibition of the Leaf Extract

Test Organism	Ps	Ea	Cl	n-But	Aq	Amp
S. aureus	10	22	27	14	21	28
S. pyogenes	R	20	22	12	12	26
E. coli	14	19	21	17	15	23
S. typhi	00	14	17	10	14	25

Table 5: Determination of Minimum Inhibitions Concentration of the Leaf Extract against the Microorganism (MIC)

	Ps					Ea					Cl					n-But							
	20	10	5	2.5	1.25	20	10	5	2.5	1.25	20	10	5	2.5	1.25	20	10	5	2.5	1.25	20	10	
	-	-	ox	+	+	-	-	-	ox	+	-	-	-	ox	+	-	-	ox	+	++	-	-	c
	-	-	ox	+	++	-	-	-	ox	+	-	-	-	ox	+	-	-	ox	+	++	-	ox	
	-	-	-	ox	-	-	-	ox	+	++	-	-	ox	+	++	-	-	ox	+	++	-	-	c
	-	-	-	-	ox	-	-	ox	+	++	-	-	ox	+	++	-	-	ox	+	++	-	-	

Table 6: Determination of Minimum Bactericidal Concentration of the Leaf Extract (MBC)

Ps					Ea					Cl					n-But							
CON. Mg/ml																						
20	10	5	2.5	1.25	20	10	5	2.5	1.25	20	10	5	2.5	1.25	20	10	5	2.5	1.25	20	10	
-	ox	+	+	+	-	-	ox	+	++	-	-	ox	+	++	-	ox	+	++	+++	-	ox	+
-	ox	+	++	+++	-	-	ox	+	++	-	ox	+	+	+++	-	ox	+	++	+++	-	ox	-
-	-	-	ox	+	-	ox	+	++	+++	-	ox	+	++	+++	-	ox	+	+++	+++	-	ox	+
-	ox	+	++	+++	-	-	ox	+	++	-	-	ox	+	++	-	ox	+	++	+++	-	-	o

Key (-) = No growth, OX = (MIC), + = turbid ++ - Dense growth, +++ = Highly growth, Ps = Ethanolic extract, Cl = Chloroform extract, n-But = n-Butanol, Ps = Petroleum ether, Aq = Aqueous Extract, Ea = Ethyl acetate. Amp=Ampiclox

4. DISCUSSION

The result of the preliminary phytochemical Screening of the partition leaf extract portion of *S. angustifolia* reveals the presence of cardiac glycoside, Tannins, Terpenoids, steroid, Saponins and carbohydrate (Table 1). The antimicrobial activities of the leaf extract portion of *Stahytarpheta angustifolia* shows remarkable sensitivity against all the clinical isolate use in this research as represented in table 3. However, *Streptococcus pyogenes and Salmonella typhi* were resistant to pet-ether portion of the extract. Table 4 shows higher zones of inhibition ranging from 20mm to 27mm was recorded against *Staphylococcus aureus, Streptococcus pyogenes Escherichia coli* using Ea, CL and Aq extract. While moderate zone of inhibition ranging from 14mm to 17mm was recorded against *Staphylococcus aureus, Escherichia coli and Salmonella typhi* using Ps, n-butanol, Aq, CL. Weak zone of inhibition ranging from 10mm to 12mm was recorded against

Staphylococcus aureus and Salmonella typhi, using Ps and n-butanol. A higher zone of inhibition ranging from 23mm to 28mm against the tested bacteria was recorded when ampiclox (5mg/ml) was used as the standard antibiotic (control). These were compared with the antimicrobial activities of the various extracts of *Stahytarpheta angustifolia* used in this research. It was observed that the CL portion of *Stahytarpheta angustifolia* was relatively the same when compared with the standard antibiotic (ampiclox 5mg/ml). This further suggests that, this particular extract can be used to produce new drugs that will replace the existing antibiotic that are no longer effective against most of the pathogenic organisms.

The result of MIC carried out represented on table 5 revealed that, almost all the extract portion are effective against the tested isolate used in this research, even at a lower concentration ranging from 1.25mg/ml to 5.0mg/ml. The best MIC of 1.25mg/ml was recorded against *Salmonella typhi* using Ps and Aq while moderate MIC ranging from 2.5mg/ml to 5.0mg/ml was recorded against the isolates using different portions of the extracts. Finally, the MBC result represented in table 6 further proof the effective of this plant could be used in ethno medicinal use in the treatment of infection associated with the bacterial isolates used in this research. The highest zone of inhibition of 27mm (Table 4) was exhibited by the chloroform portion of the extract [13]. The ethanolic portion of the leaf extract of *S. angustifolia* inhibits the growth of the tested isolates. This shows that, the extract contains substances that can inhibit the growth of some micro-organism at different concentration [14], [15] .The zone of inhibition of the chloroform fraction was found to be more than the ethanolic portion. This observation suggest that, the active principle responsible for these actions present in the extracts are inactivated in the presence of other compounds presents in the extract [16], [17] . Therefore, the antimicrobial effects of the partitioned leaf extract may be attributed to the presence of tannins, steroids, Cardiac glycosides, Saponins and Terpenoids.

5. CONCLUSION

The observed antimicrobial properties of the partituoned leaf extract of *S. angustifolia* against the tested isolates used in this research corroborate its use in the traditional medicine for the treatment of dysentery, eye trouble, antihelmintic, Gonorrhea, syphilis and other Venereal Infectious diseases. Further studies should be carried out on other part of the plant to evaluate their phytochemical and possibly their antimicrobial activities. Finally, studies should also be carried out to understand more on the mechanism of the Antispasmodic action of *S. angustifolia* on the gastrointestinal organ and also to isolate the active principle(s) responsible for this action.

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