

Original Research Article

Preliminary phytochemical screening and gastrointestinal study on the leaf extract of *Stachytarpheta angustifolia* Mill Vahl (Verbenaceae) in Rabbit Jejunum

Abstract:

S. angustifolia (Verbenaceae) is mostly prescribed by the folkloric healers for various gastrointestinal disorders. This study was carried out to ascertain the gastrointestinal effect of the ethanol leaf extract and other various fractions (CHCl₃, EtOAc, n- BuOH and residual aqueous) on rabbit Jejunum. The ethanol, n-butanol and residual aqueous of the extract exhibited dose concentration at (0.1, 0.2, 0.4 and 0.8mg/ml) dependent contraction of the rabbit Jejunum which was blocked by atropine suggesting that the observed pharmacological actions was mediated through the muscarinic receptors. In contrast, chloroform and ethylacetate fraction of the leaf extract exhibit dose concentration dependent relaxation of the rabbit jejunum. Intreperitoneal LD₅₀ of the extract in mice was found to be 295.8mg/kg. Preliminary phytochemical screening of the leaf extract revealed the presence of carbohydrates, tannins, saponins, cardiac glycoside, sterols and terpenoids. The result indicated that, the plant extract possesses some pharmacological activity, hence justifying its use traditionally in alleviating gastrointestinal disorder.

Keywords: *Stachytarpheta angustifolia*, Phytochemistry, Gastrointestinal study, Jejunum

INTRODUCTION

Despite the immense technological advancement in modern medicine, a lot of the Africans (approximately 80% of the population) still rely on traditional healing practices and medicinal plants for their daily health care needs (1). The floral biodiversity of Africa provides the African traditional medical practitioner with an impressive ‘natural pharmacy’ from which plants are selected as remedies or as ingredients to prepare herbal medicine (phytomedicines) for various human ailments (2). The traditional preparations comprise of medicinal plants, minerals and organic matter. The ayurvedic medicine is essentially primitive but are also preventive in therapeutic approach (3).

Stachytarpheta angustifolia is a medicinal plant that belongs to the family (Verbenaceae). It is a shrub of about 4ft high, with a soft and cylindrical bark. They are mostly simple, slightly branch and often succulent. The flowers are mostly pale blue with or without Centre (4/5). The plant is commonly known as the Devils coach whip while the Hausa’s called it Wutsiyarkadangare and the yoruba’s called it Irualangba (6/7) in Nigeria. In Brazil the triturated fresh leaf of the plant is applied locally for the treatment of ulcer and also a good

37 remedy against rheumatism. This plant is reported to contain a glucosidal substance
38 'stachytarphine' which is reputed to be an abortifacient agent (8). The cold infusion of the
39 plant is taken as a remedy against gonorrhoea and other forms of venerable infectious diseases.
40 It is also taken as a vermifuge or purging vehicle for other vermifuge. The leaf from the plant
41 is boiled and taken as a remedy against diabetes in the northern part of Nigeria (4,7). The
42 alcohol extract of the leaf has been reported to show some antimicrobial activities against
43 *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Escherichia coli*, but give a
44 negative result in antimalarial test (8).

45 The effect of this widely used plant in northern Nigeria for the treatment of gastrointestinal
46 ailments is yet to be ascertained scientifically. The present study was undertaken to ascertain
47 the preliminary phytochemistry of the plant and also to evaluate the pharmacological effect
48 of the various extracts of *S. angustifolia* on smooth muscles.

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50 MATERIALS AND METHODS

51 Plant Material

52 The whole plant material *Stachytarpheta angustifolia* (mill) vahl Verbenaceae was
53 collected from a farm land in Basawa village outskirts of Zaria, Kaduna state. The plant was
54 identified and authenticated at the herbarium Biological sciences Department, Ahmadu Bello
55 University Zaria, Nigeria. Herbarium sample was made and voucher deposited with (No. DC
56 90188).

57

58 Animals

59 Four adult's rabbits weighing 3.0-3.8kg were obtained from the animal house Department of
60 Pharmacology, Faculty of pharmaceutical science Ahmadu Bello University, Zaria. They
61 were given access to standard animal feed and water *ad libitum*. The principles of laboratory
62 animal care (NIH publication No. 85-23, revised 1985) were strictly followed, as well as
63 specific national laws were applicable. Animals were approved for use by the Animal Facility
64 Centre (AFC) committee after reviewing the protocol. All experiments were carried out in
65 accordance with the National Institute of Health Guide for the Care and Use of Laboratory
66 Animals (NIH Publications No. 80-23) revised 1996. All experiments have been examined
67 and approved by the ethics committee.

68 Drugs.

69 Acetylcholine was freshly prepared in various concentrations using distilled water just before
70 used. The extracts were also freshly prepared using distilled water.

71 Phytochemical Screening

72 The air-dried powdered material of the whole shrub and the leaf separately (360g, 470g)
73 were subjected to exhaustive extraction with petroleum ether 60°C – 80°C and subsequently

74 with 95% ethanol using cold maceration techniques. The pet ether and ethanol extract were
75 concentrated using rotary evaporator to affords 25.45g, 47.34g for the whole plant while
76 24.80g, 42.74g for the leaf extract (9).

77 The ethanol leaf extract portion (30g) was suspended in water (500ml) and partition
78 exhaustively with solvent of increasing polarity chloroform, ethyl acetate and n-butanol
79 respectively. The various partition portions of the extracts were concentrated in *vacuo* (10,
80 11). The partition portion of the extracts were subjected to phytochemical screening using
81 standard protocols (12/13).

82 **Toxicity Studies on *S. angustifolia* (LD₅₀)**

83 A total of 13 mice were used for the experiment. In the first phase, three doses of the extract
84 were administered to three groups each containing three mice. In the second phase, more
85 specific doses were administered to each group containing one mouse. The median lethal
86 dose (LD₅₀) value was determined as the geometric mean of the highest non-lethal dose and
87 the lowest lethal dose of which there is 1/1 and 0/1 survival (13)

88

89 **Pharmacological Studies on Isolated Rabbit Jejunum**

90 The method described by (14) and modified by (20) was adopted. The four adult rabbits
91 obtained were starved overnight prior to the experiment. The animals were sacrificed by a
92 blow on their head, exsanguinated and their abdomen cut open. Segments of their jejunum
93 3.0cm long were cut and placed separately in to 25ml organ baths containing Tyrode's
94 solution of 136.8mMNaCl, 2.7mMKCl, 1.3mM CaCl₂, 12mMNaHCO₃, 0.5mMMgCl₂,
95 0.14mMNa₂HPO₄, 5.5mMglucose well aerated and maintained at 37°C. An initial tension of
96 1.0g was applied to the tissue and a 60min period of stabilization was observed. During this
97 period, the physiological solution was changed every 15min after which the effect of
98 acetylcholine at final bath concentration of (6.4x10⁻³M) was evaluated and the tissue was
99 equilibrated for 60mins before use. Dose response curve for acetylcholine (4.0x10⁻³-6.4x10⁻³)
100 bath concentrations was obtained. The contractile responses of the spasmogen were recorded
101 on the kymograph paper by means of a frontal writing lever in Ugobasile unirecorder
102 7050(GMBH, German). The tissue was washed three times with physiological solution and
103 allowed to rest before the addition of the subsequent spasmogen. The direct effect of different
104 portion of the extracts (4.0x10⁻³-6.4x10⁻³) bath concentrations were investigated after
105 allowing the tissue to rest for 30 sec. Similarly, the effect of the other portion of the extracts
106 were also investigated on submaximal dose of acetylcholine (Fig.1), so as to study the effect
107 of the extracts on these spasmogen.

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113 **Results**114 Table 1: Preliminary phytochemical screening of the Leaf extract of *S. angustifolia*

CONSTITUENTS	TEST	OBSERVATION	PORTIONS OF EXTRACTS					
			Ps	Es	Cl	Eta	n-But	Aq
Carbohydrate			Ps	Es	Cl	Eta	n-But	Aq
General Test	Molisch	Red colouring	-	+	-	-	-	++
Sugar Test	Aniline	Red colour	-	-	-	-	-	+++
Sugar (Monosaccharide)	Barfoed's	Red ppt	-	+	-	-	-	++
Red. Sugar	Fehling's	Red ppt	-	+	-	-	-	++
Tannins	Lead Ethanoate	White ppt	-	++	-	+	++	++
	Iron (III) Chloride	Blue – Black	-	+	-	+	++	+
	Ethanoic acid Methanol's	White ppt Red ppt	-	+	-	+	-	-
Saponins	Frothing	Persist frothing	-	++	-	+	++	-
Sterols	Liebermann B.	Blue or green	++	++	-	+	++	+
Saponin Glycoside	Fehling's Solution	Red ppt		++	-	+	++	-
	Tetraoxosulphate(iv) acid	Brick red	-	++	-	+	++	-
Phlobatannins	Hydrochloric Acid	Red ppt	-	++	-	-	+	-
Carotenoids	Carr price's	Blue to red colour	-	++	-	-	-	++
Emodol	Borntrager's	Red colour	-	-	-	-	-	++
Flavones aglycones	Shibata's	Red to Orange	-	-	-	-	-	-
Terpenoids	Liebermann B.	Pink to Red colour	++	++	+	+	+	-
	Dragendoff's	Orange red ppt	-	-	-	-	-	-
Alkaloids	Mayer's	Buff ppt	-	-	-	-	-	-
	Wagner's	Dark brown ppt	-	-	-	-	-	-
Flavonoids	Shinoda	Dee red	-	-	-	-	-	-
	Tetraoxosulphate (vi) acid	Deep Yellow	-	-	-	-	-	-
Cardiac glycoside	Legal's	Deep red colour	-	++	+	++	++	+
	Kedd's	Violet colour	-	+	+	+	+	-
	Keller – killiani	Reddish brown	-	++	+	++	++	+
	Baljet	Orange to Deep red	-	+	+	+	++	-

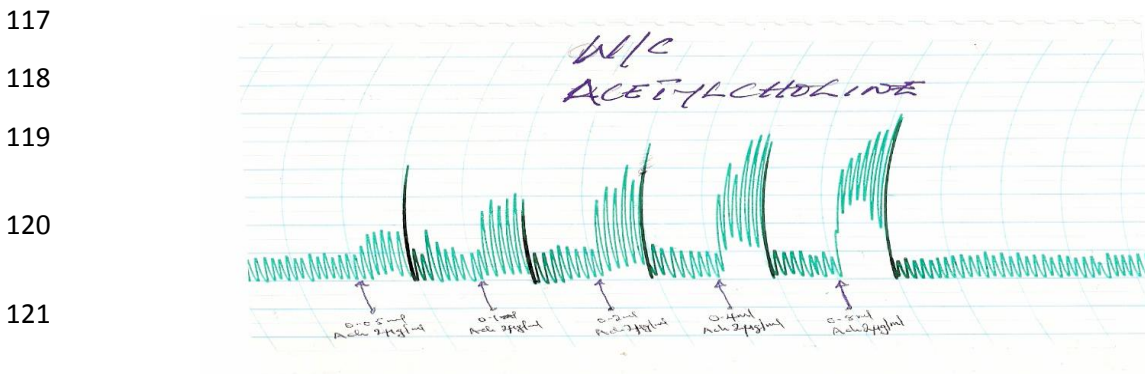
Lieberman

Bluish green

- ++ + + ++ -

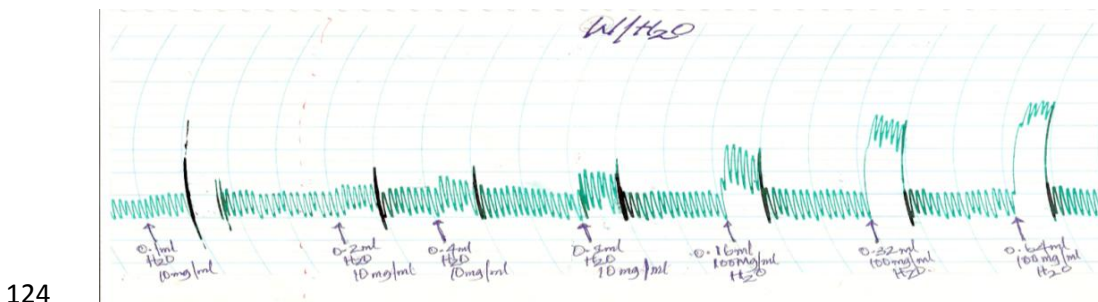
115 **Key:** - = Absent, + = Fairly present, ++ = Moderately present, +++ = Highly present.

116 Ps=Pet-ether, Es=Ethanol, Cl=Chloroform, Eta=Ethylacetate, n-But=n-Butanol, Aq=Aqueous

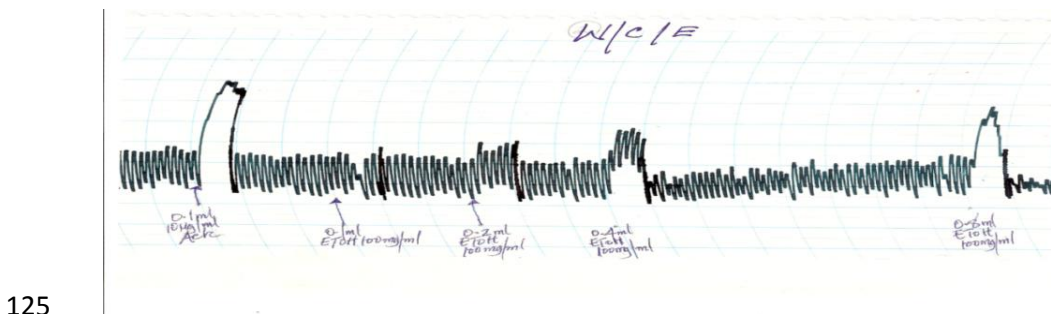


122 Fig: 1 Effect of contraction produce by Acetylcholine on isolated rabbit jejunum.

123 Fig: 2 Effect of contraction produced by the aqueous whole plant extract on isolated rabbit jejunum

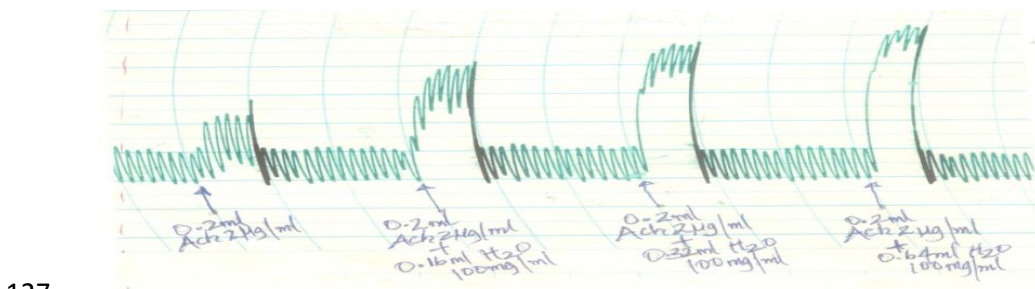


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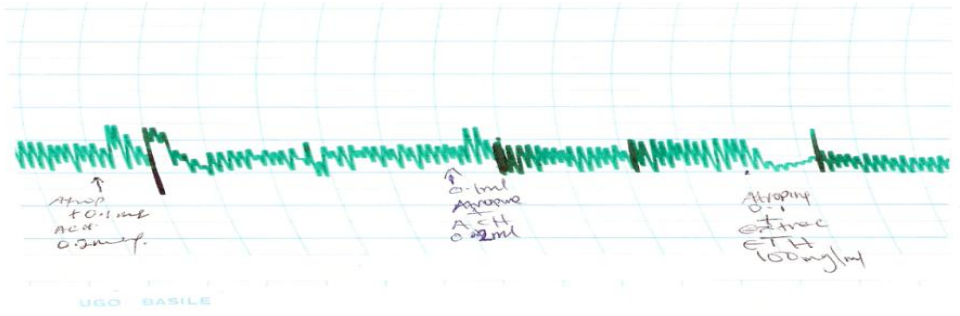
126 Fig: 3 Effect of contraction produced by ethanol whole plant extract on the isolated rabbit jejunum.



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128 Fig.4 Effect of contraction produced by aqueous whole plant extract pre contracted with Acetylcholine on rabbit jejunum

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131 Fig:5 Effect of contraction produced by Atropine on tissues pre -contracted with aqueous portion of
 132 the whole plant extract on rabbit jejunum.

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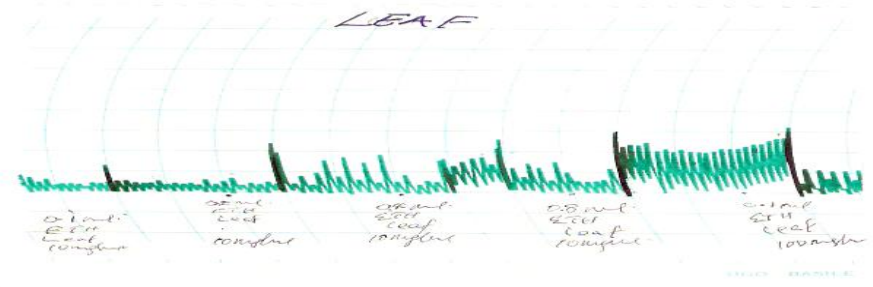
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139 Fig: 6 Effect of contraction produced by the Leaf extract on isolated rabbit jejunum.

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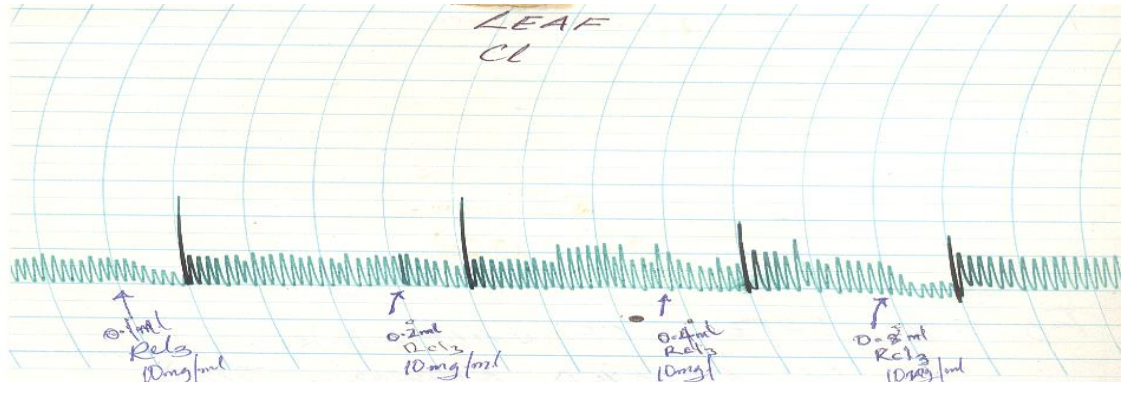
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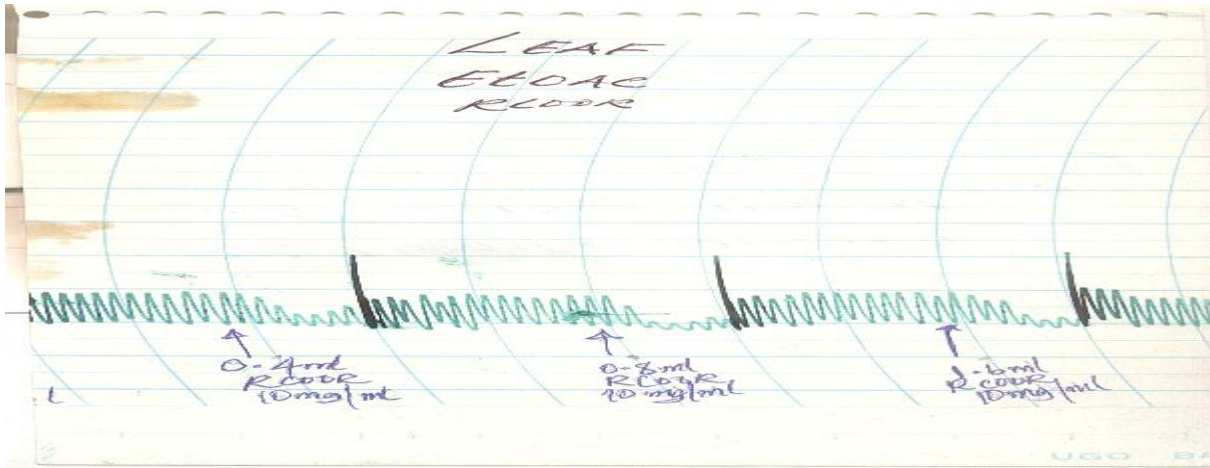
147 Fig:7. Effect of contraction produced by the Leaf (chloroform) extract on isolated rabbit
 148 jejunum.

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154 Fig:8 Effect of contraction produced by the Leaf (Ethylacetate) extract on isolated rabbit
 155 jejunum.

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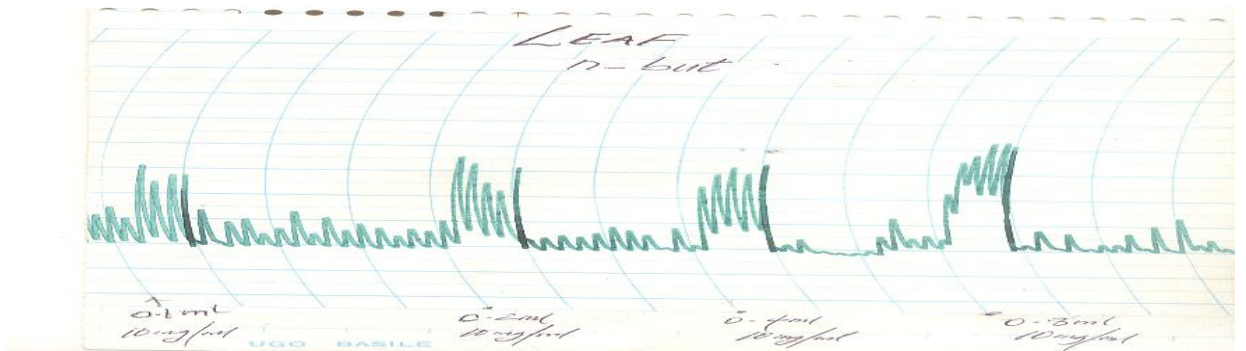
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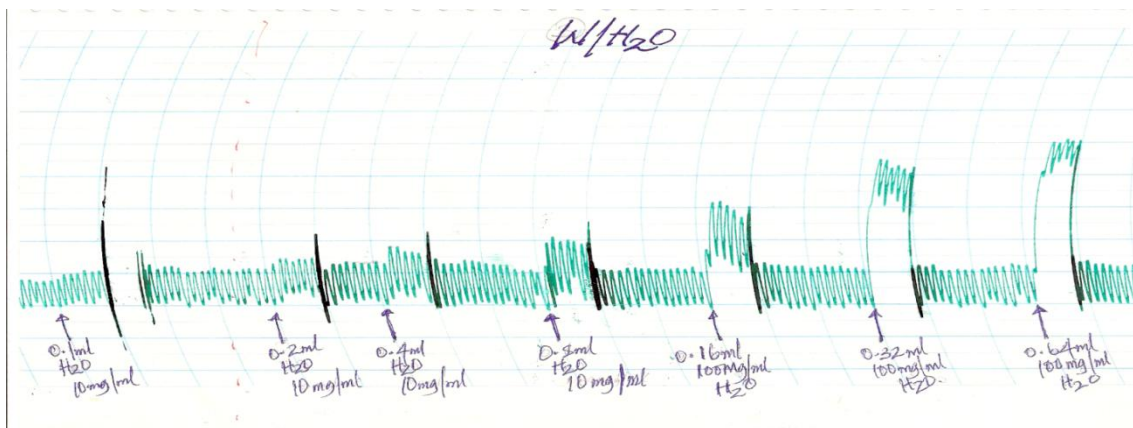
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162 Fig:9. Effect of Rhythmic contraction produced by the Leaf (n-butanol) extract on isolated
 163 rabbit jejunum.

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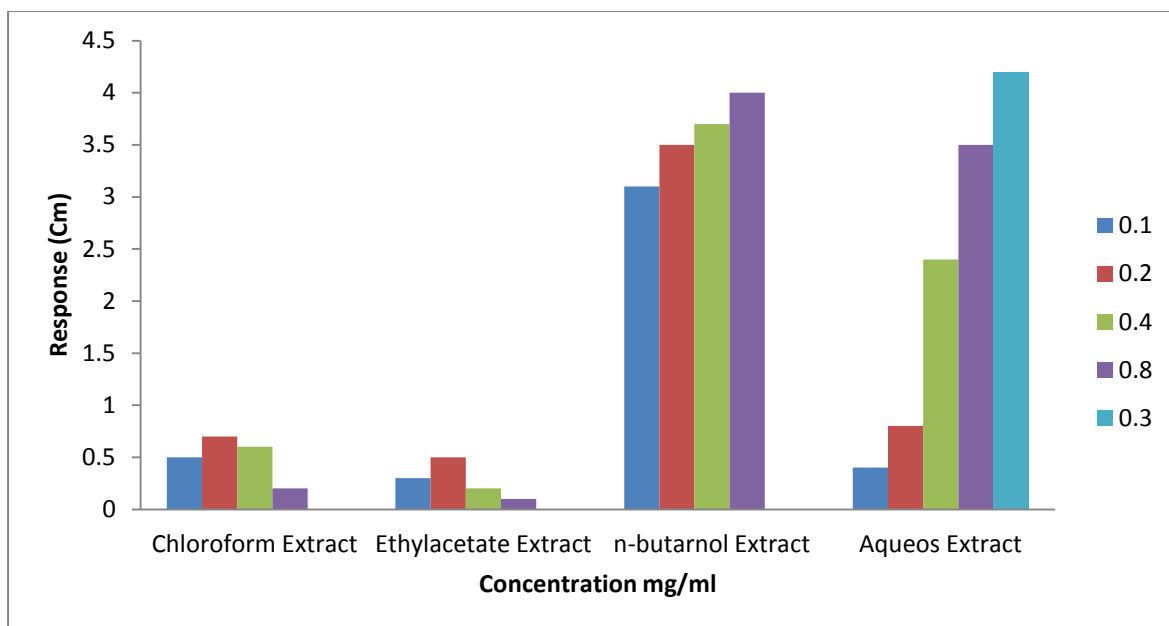


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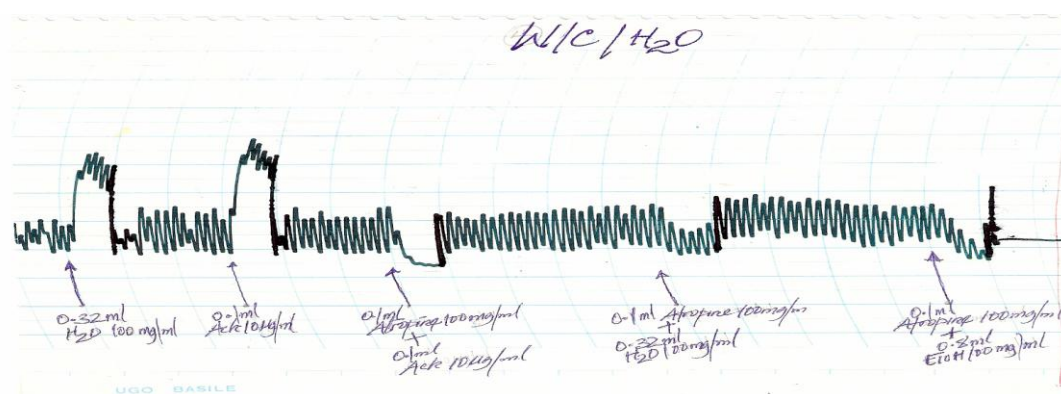
166 Fig: Effect of contraction produced by Leaf (aqueous)residue extract on isolated rabbit
 167 jejunum..

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 171 Fig: 10 Effect of chloroform, ethylacetate, n-butanol and aqueous extract of the leaf on rabbit
 172 ileum.



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 181 Fig:11 Effect of Atropine on tissue Pre-contracted with aqueous Leaf extract on isolated
 182 rabbit jejunum

183 **RESULTS**

184 The aqueous leaf extract pre contracted with atropine 0.1 – 0.8mg/ml was found to block the
 185 contraction amplitude of the spontaneous contraction of the smooth muscle (fig.5), but the
 186 pre contraction of the aqueous extract with acetylcholine produces a concentration dependent
 187 contraction of the isolated rabbit jejunum (fig.4). The pre contraction of the aqueous extract
 188 with Atropine and acetylcholine was also found to relax the ileum dose dependently (fig.11).

189

190 **DISCUSSION**

191 The result of phytochemical screening reveals the presence of terpenoids, steroids, saponins,
192 tannins, cardiac glycoside and carbohydrate (Table 1.). The standard solution of acetylcholine
193 at various concentrations produces contraction dependent on rabbit jejunum (Fig:1).The
194 result on (Fig: 2 and 3) shows that, the aqueous and ethanol portion of the whole plant
195 extracts induces concentration contraction dependent of the rabbit jejunum. The aqueous
196 portion of the extract pre contracted with acetylcholine potentiate the contraction of the
197 isolated rabbit jejunum dose dependently (Fig. 4). In Fig. 5 of the result above shows the
198 blocking effect of the contraction, this is as a result of Atropine pre contracted with ethanol
199 portion of the extract on the rabbit jejunum. Fig: 6 shows the induced dose dependent
200 contraction of the rabbit jejunum exhibited by the ethanolic portion of the leaf extract. The
201 chloroform and ethylacetate portion of the leaf extract exhibited dose relaxation respond at
202 high concentration while an inconsistency respond was observed by the n-butanol extract
203 even at a higher concentration on isolated rabbit jejunum (fig.7,8,9). The contractions
204 observed by the extracts on the tissues were similar to those produced by Acetylcholine (15,
205 16). The leaf extract portion pre-contracted with Atropine was also found to block the
206 response of the spasmogen contraction as seen in (fig.11). Acetylcholine induced contraction
207 of the smooth muscle, results from the activation of muscarinic receptors and the differences
208 in the muscarinic receptors are known to exist (17, 18).

209 The inhibitory effects of the extract induced contraction by the non-selective muscarinic
210 antagonist i.e. atropine observed in our study agrees with those of (19, 20). The attenuated
211 rhythmic contractions of the isolated tissue produced in our previous study by various extracts,
212 signifies that, the action might be mediated through the cholinergic receptors (15). The
213 medium inhibitory contraction of the extract on each of the spasmogen was observed to be as
214 result of the extract antagonizing the muscarinic receptors (21, 22). The extracts were
215 found to act through the musculotropic route on the rabbit jejunum. This further confirms its
216 activities via the musculotropic route (21, 14). The active principles presents in the extracts
217 are apparently acting on the tissue through the cholinergic receptors and hence are
218 responsible for the actions on the tissue (23, 24).

219

220 **CONCLUSION**

221 The study indicates that, the aqueous and ethanol portion of the whole plant extract contains
222 active components which can induce concentration dependent contraction of the rabbit
223 jejunum. The contractions observed in our study suggest that, they are inactivated in the
224 presence of other portion of the principles (fig: 9). The active principles contain in the plant
225 *S. angustifolia* are apparently mediated through muscarinic receptors other than MI
226 receptors. Therefore, the study has now justifies the use the plant by the folkloric healers in
227 the treatment of various gastrointestinal disorder in northern Nigeria, West Africa.

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