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 ($CHCl_3$, 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 LD_{50} 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

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

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

MATERIALS AND METHODS

Plant Material

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

Animals

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

Drugs.

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

Phytochemical Screening

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

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

The ethanol leaf extract portion (30g) was suspended in water (500ml) and partition exhaustively with solvent of increasing polarity chloroform, ethyl acetate and n-butanol respectively. The various partition portions of the extracts were concentrated in *vacuo* (10, 11). The partition portion of the extracts were subjected to phytochemical screening using standard protocols (12/3).

Toxicity Studies on S. angustifolia (LD_{50})

A total of 13 mice were used for the experiment. In the first phase, three doses of the extract were administered to three groups each containing three mice. In the second phase, more specific doses were administered to each group containing one mouse. The median lethal dose (LD_{50}) value was determined as the geometric mean of the highest non-lethal dose and the lowest lethal dose of which there is 1/1 and 0/1 survival (13)

Pharmacological Studies on Isolated Rabbit Jejunum

The method described by (20) and modified by (14) was adopted. The four adult rabbits obtained were starved overnight prior to the experiment. The animals were sacrificed by a blow on their head, exsanguinated and their abdomen cut open. Segments of their jejunum 3.0cm long were cut and placed separately in to 25ml organ baths containing Tyrode's solution of 136.8mMNaCl, 2.7mMKCl, 1.3mMCaCl, 12mM$NaHCO_3$,0.5mM$MgCl_2$, 0.14mMNa_2HPO_4, 5.5mMglucose well aerated and maintained at 37^oC. An initial tension of 1.0g was applied to the tissue and a 60min period of stabilization was observed. During this period, the physiological solution was changed every 15min after which the effect of acetylcholine at final bath concentration of ($6.4x10^{-3}$M) was evaluated and the tissue was equilibrated for 60mins before use. Dose response curve for acetylcholine ($4.0x10^{-3}$-$6.4x10^{-3}$) bath concentrations was obtained. The contractile responses of the spasmogen were recorded on the kymograph paper by means of a frontal writing lever in Ugobasile unirecorder 7050(GMBH, German). The tissue was washed three times with physiological solution and allowed to rest before the addition of the subsequent spasmogen. The direct effect of different portion of the extracts ($4.0x10^{-3}$-$6.4x10^{-3}$) bath concentrations were investigated after allowing the tissue to rest for 30 sec. Similarly, the effect of the other portion of the extracts were also investigated on submaximal dose of acetylcholine (Fig.1), so as to study the effect 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					
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	White ppt	-	+	-	+	-	-
	Methanol's	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	-	++	+	+	++	-	

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

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

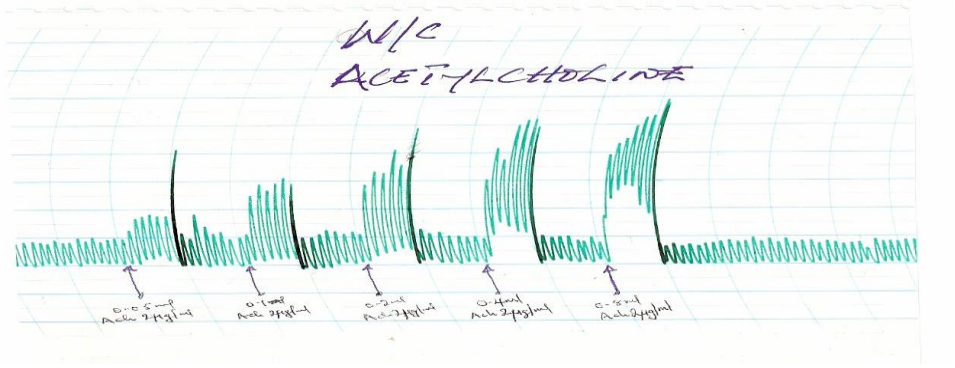
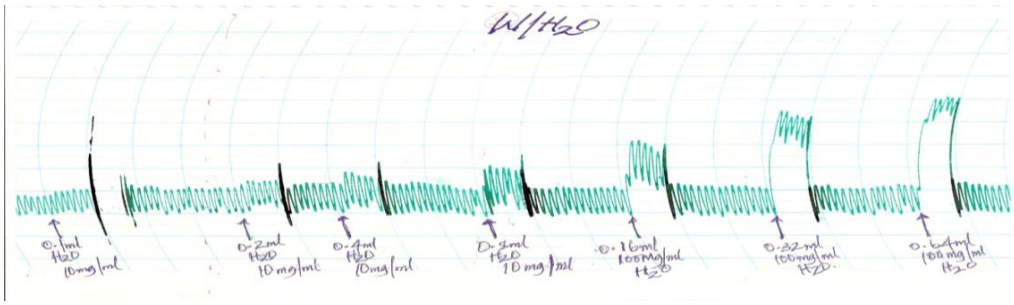


Fig: 1 Effect of contraction produce by Acetylcholine on isolated rabbit jejunum.

Fig: 2 Effect of contraction produced by the aqueous whole plant extract on isolatedrabbit jejunum



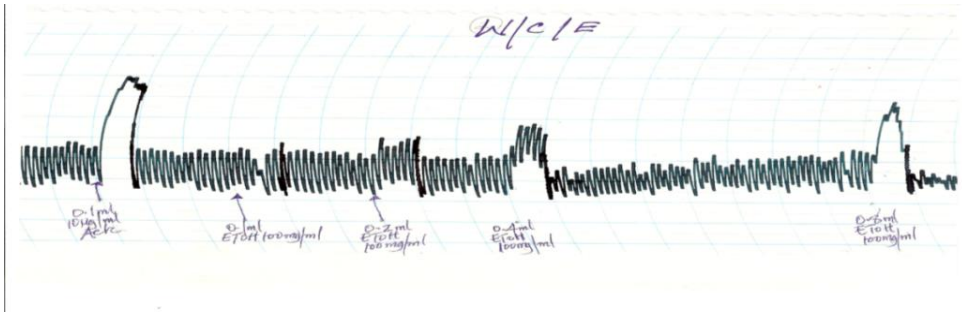


Fig: 3 Effect of contraction produced by ethanol whole plant extract on the isolated rabbit jejunum.

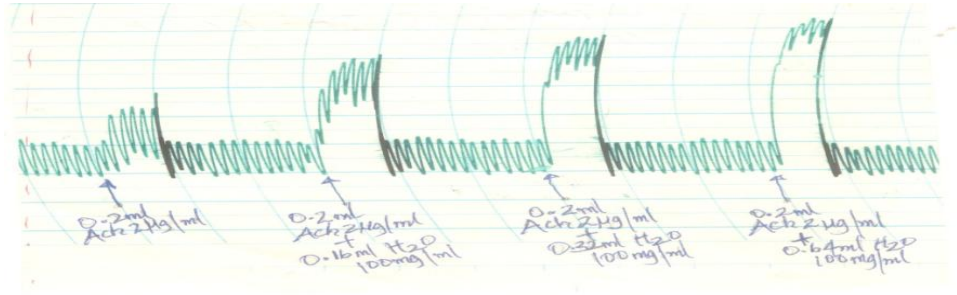


Fig.4 Effect of contraction produced byaqueous whole plant extract pre contracted with Acetylcholine on rabbit jejunum

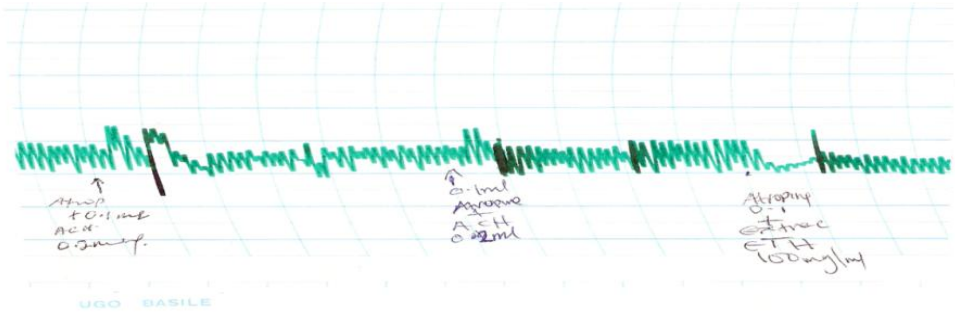


Fig:5 Effect of contraction produced by Atropine on tissues pre –contracted with aqueous portion of the whole plant extract on rabbit jejunum.

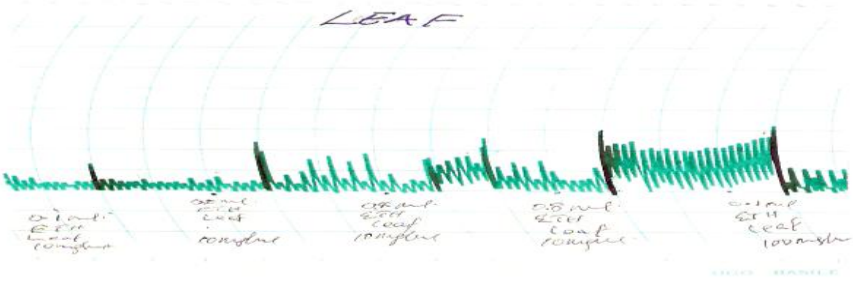


Fig: 6 Effect of contraction produced by the Leaf extract on isolated rabbit jejunum.

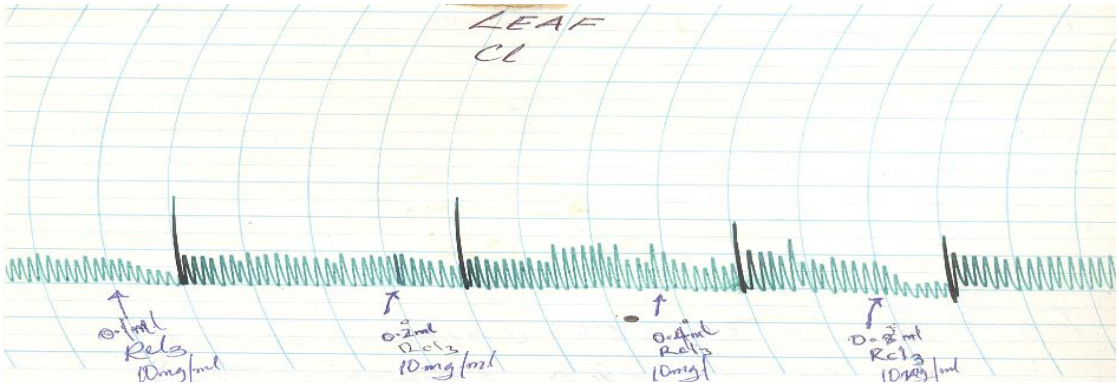


Fig:7. Effect of contractionproduced bythe Leaf (chloroform) extract on isolated rabbit jejunum.

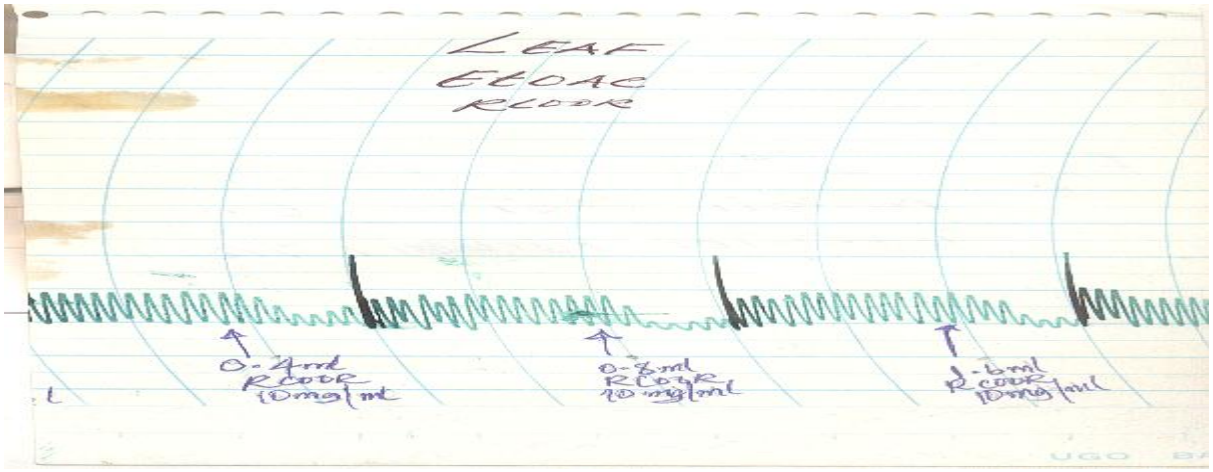


Fig:8 Effect ofcontraction produced by the Leaf (Ethylacetate) extract on isolated rabbit jejunum.

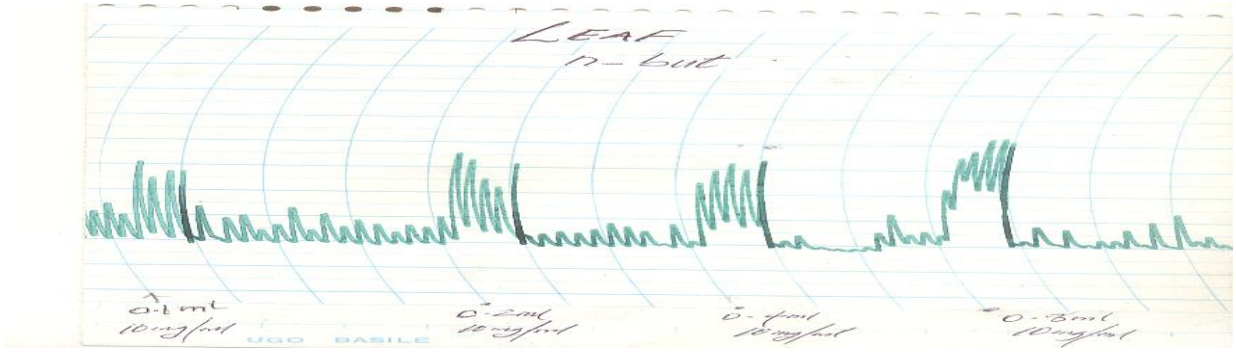


Fig:9. Effect of Rhythmic contraction produced by the Leaf (n-butanol) extract on isolated rabbit jejunum.

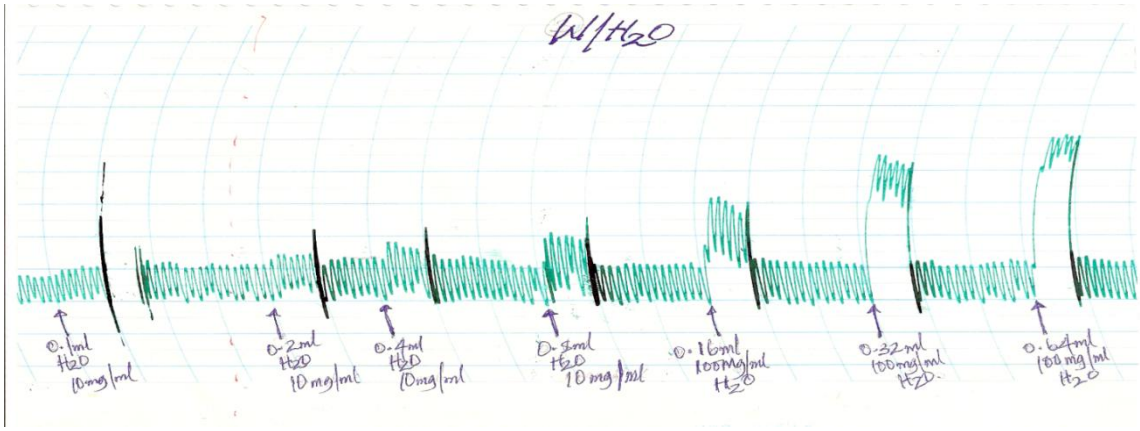


Fig: Effect of contraction produced by Leaf (aqueous)residue extract on isolated rabbit jejunum..

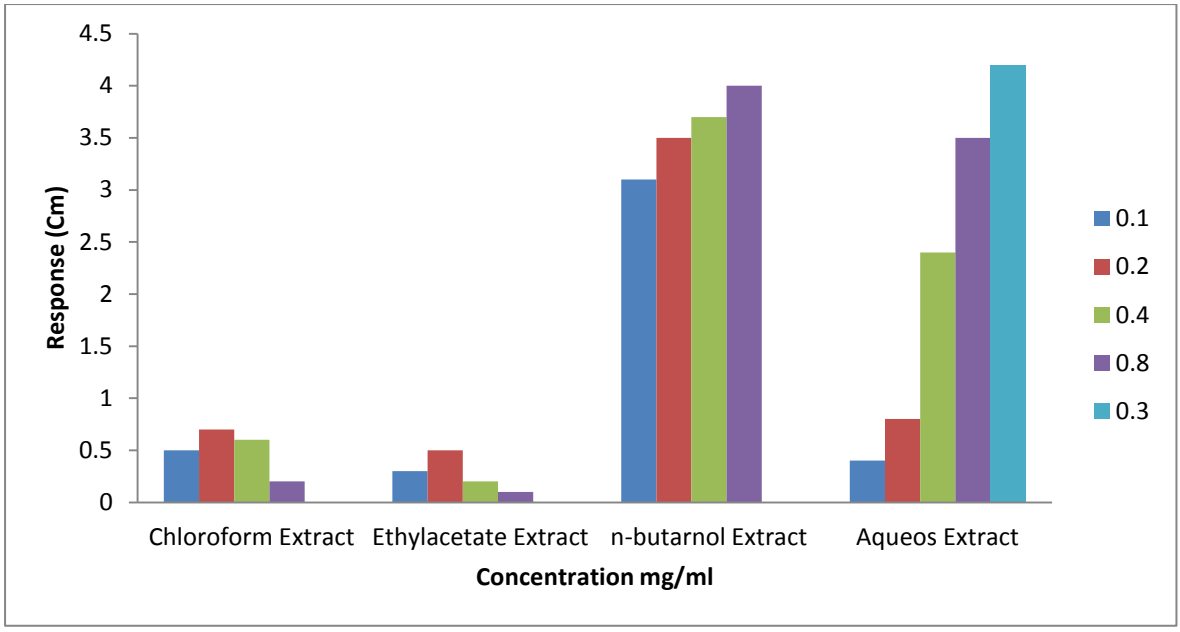


Fig: 10 Effect of chloroform, ethylacetate, n-butanol and aqueous extract of the leaf on rabbit ileum.

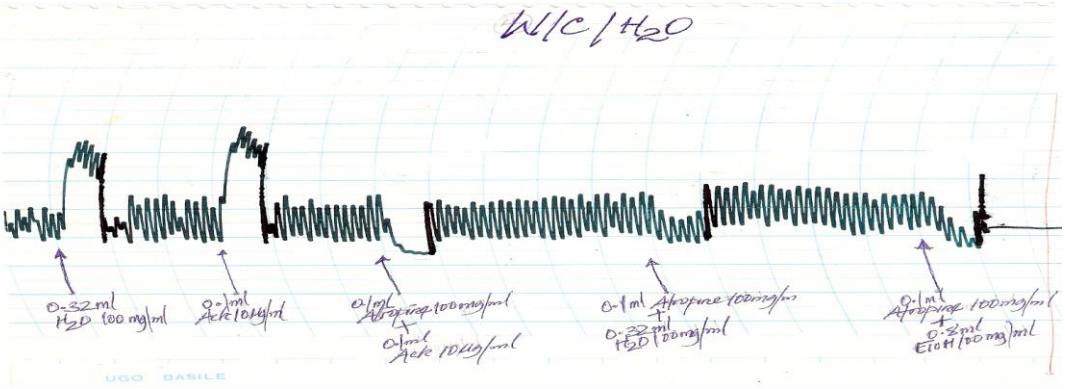


Fig:11 Effect of Atropine on tissue Pre-contracted with aqueous Leaf extract on isolated rabbit jejunum

RESULTS

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

DISCUSSION

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

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

CONCLUSION

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

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