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Research Article

Muscarinic Receptor Mediated Spasmolytic Effects of Crude Extract and Fractions Prepared from *Solenostemon rotundifolius* Leaves on an Isolated Rabbit Jejunum

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Abstract

Solenostemon rotundifolius leaves have widely been used in ethnomedicine for the management of diarrhea and other diseases of the gastrointestinal tract. In this study, the spasmolytic effects of its leaf extract and fractions on normal and acetylcholine induced rhythmic contractions of an isolated rabbit jejunum were investigated to possibly validate this traditionally acclaimed anti- diarrheal property. Crude extract was prepared from the plant's leaves and 7 fractions were obtained following fractionation of this crude extract. About 2-3 cm length of jejunum isolated from a stunned rabbit was suspended vertically in a 30 ml organ bath containing tyrode solution and bubbled with air. After equilibration, various doses of the extract, fractions and atropine were separately administered to the piece of tissue to determine their effects. Effects of graded doses of acetylcholine on the piece of tissue were also determined and repeated separately in the presence of atropine and the test extract and fractions with adequate washing preceding each administration. Results obtained showed that the effect of the crude extract on the piece of isolated tissue was inhibitory as graded doses like atropine, produced a dose dependent relaxation of the tissue, lowering the amplitude of contractions in each case with 0.33 mg/ml producing 71.42 percent inhibitory effect and 1.00 mg/ml producing an inhibitory activity of 91.67 percent. The effects of all the fractions were also inhibitory but fraction 5 produced the highest relaxation effect. Acetylcholine when applied caused dose dependent contractions which were significantly inhibited by the crude extract and fractions in a manner which compared favourably with that of atropine. The order of strengths of inhibitions of the test agents on acetylcholine induced contractions is: F5>F4>F3>CRUDE>F6>F1>F2>F7. We therefore conclude that *Solenostemon rotundifolius* leaf extract and fractions, having shown significant spasmolytic effects on an isolated piece of jejunum, may contain active components with anti-diarrheal potentials and be of value in the management of the disease.

Keywords: Acetylcholine; Fraction; Inhibitions; Tissue; *Solenostemon rotundifolius*; Spasmolytic

Introduction

The fact that large number of medicinal plants is domiciled in Africa of which Nigeria is part may be the reason for the current level of dependence on herbal medicine in the region for the management of diseases. Indeed global statistics have shown about 80% dependence on the healing potentials of medicinal plants [1], a figure projected to increase in the near future due to the high cost and many side effects of synthetic drugs on one part and current exploits of herbs on the other [2]. Scientific validation of ethnomedicinal claims on medicinal plants has also of late given a boost to new drugs discovery and development. This rational drug discovery may involve a combination of botanical, phytochemical, biological and molecular techniques as key activities in the systematic processes leading to the emergence of the eventual new drug [3].

The smooth muscles of the gastrointestinal tract play major role in the movement of materials along the tract, but the degree of movement must be balanced by the combined effects of the parasympathetic and sympathetic arms of the autonomic nervous system. Over activity of the former may cause excessive contractions leading to above normal bowel movement (diarrhea), often characterized by the removal of frequent watery stools. The role of

acetylcholine in gastrointestinal motility via muscarinic receptor binding is also well established [4], hence limiting excessive gastrointestinal muscle contractions via antagonism of acetylcholine activity may be an effective means of managing diarrhea. The spontaneous rhythmic contractions of the rabbit jejunum also make it an ideal tissue for studying the antidiarrheal effects of drugs including plants extracts. In the study, the medicinal plant of study is *Solenostemon rotundifolius.*

S. rotundifolius belongs to the *Lamiaceae* (*Labiatae*) family, a group of aromatic flowering plants with about 236 genera and 6900- 7200 species [5] many of which are cultivated for their medicinal, ornamental and culinary properties. *S. rotundifolius* is commonly called Hausa potato in Nigeria [6-8]. Leaves, flowers, seeds, stem and roots of some plants from *Lamiacea* family have potentials to be analgesic, antipyretic, anti-fungal, anti-spasmodic, anti-oxidant, anti-microbial, anti-diabetic, anti-asthmatic, anti-diarrhoeal and antiseptic agents [5]. In addition, boiled leaves of S. rotundifolius are also used in ethnomedicine for the treatment of dysentery while results of earlier investigations suggest that the crude extract of *S. rotundifolius* may possess anti-diarrheal activity [6,9]. Anti-diarrheal agents may act on the gastrointestinal smooth muscles to inhibit motility and transit time of intestinal contents. It was against this background that this study was aimed at investigating the effect of *S. rotundifolius* leaf extract and its fractions on the rhythmic contractions of the rabbit jejunum, with a view to validating its anti-diarrheal usefulness and to possibly reveal the mechanisms of the suggested anti-diarrheal activity.

Materials and Methods

Chemicals and drugs

Among drugs used were acetylcholine and atropine (Sigma-Aldrich Drugs and Chemicals Company, USA) while chemicals were sodium chloride, potassium chloride, calcium chloride, sodium hydrogen carbonate, sodium dihydrogen phosphate, magnesium chloride and glucose (BDH Company, UK).

Plant materials

Fresh leaves of *S. Rotundifolius* were collected from National Root Crops Research Institute (NRCRI) Umudike, Nigeria and were identified at the Genetic Resource Unit of NRCRI as leaves of *S. rotundifolius*.

Extract preparation

Extract from the plant material was prepared according to the method used by Ijioma., *et al.* with slight modification [4]. The collected leaves were air dried under shade in an open laboratory space for 14 days and pulverized into fine powder in a locally fabricated milling machine powered by a petrol motor (Honda Company, Japan). Three hundred (300) grams of the powdered material was macerated in 1000 ml of ethanol by intermittently shaking the mixture within 48 hours. Filtration was then carried out to obtain the extract in solution which was first concentrating in a rotary evaporator at 50^0C and completely dried in a hot air oven maintained at 40^0C to obtain a crude dark pasty solid extract, which weighed 39.72g and represented a percentage yield of 13.24%. The extract was preserved in the refrigerator until needed.

Bioassay-guided fractionation of the ethanol extract from *S. rotundifolius* leaves

The method of separation in column chromatography based on the distribution of the components in a mixture between a fixed (stationary) and a moving (mobile) phase proposed by Abbot and Andrew (1970) was adopted. The stationary phase was the column of adsorbent, through which the mobile phase, the liquid moved on. Silica gel, 100-200 mesh, was used as the stationary phase while gradient solvent system of combination of petroleum ether, chloroform and methanol was used as the mobile phase. For column chromatography, sample slurry of the extract was prepared by dissolving 34 g of the ethanol extract of *S. rotundifolius* in 50 ml of chloroform and was thoroughly mixed with 100g of silica gel (100-200 mesh) and left for 24 hours to dry. Wet packing method was used to prepare the silica gel column. Silica gel (400g) was taken in petroleum ether in the ratio of 1:2, stirred vigorously with glass rod and was poured into a column of 5 cm diameter and 100 cm height pre-plugged at the bottom with cotton wool. The column was left for 30 minutes to stabilize. Sample slurry was made in petroleum ether, the least polar solvent of the eluting solvent system. Slurry was carefully applied to the top of the packed column and was covered with dry silica gel granules to avoid spattering of the eluting solvent on the sample. The solvent system was carefully poured into the column by the side wall and the column tap was gently opened to allow elution at a flow rate of one drop per four seconds. An elution volume of 50ml each was collected in pre-weighed labelled 100ml beakers.

Gradient solvent system of petroleum ether, chloroform and methanol were used in the combinations shown below to elute the entire compounds from the sample. Thirty-nine fractions of 50ml each were collected (Table 1).

The recovered fractions from the column chromatographic separation were subjected to analytical thin-layer chromatography (TLC) and fractions with difference in retention factor (R_F) value of not more than 0.1 - 0.15 were pooled together.

For the preparation of thin layer chromatography plates, silica gel slurry was made and was used to coat one surface of the glass plate to form a thin layer on the plate. The coated plates were left in the open laboratory to dry for three hours at room temperature before being activated in the oven at 100^0C for 1 hour. The plates were then ready for use.

Solution of fractions of column chromatography was made using chloroform as solvent. A small capillary tube was used to collect some fraction from the beaker and spotted 1.5cm above the base of the plate. The spotted plate was put in TLC tank containing 100ml of the mixture of solvents used for elution. Since there were no literature reports on the plant under investigation a suitable solvent system for the separation has to be established experimentally. A large number of solvent systems were tried so as to obtain reasonable difference in the RF values of the components of the bioactive fractions. The following solvent system was found suitable: petroleum ether: chloroform (80:20), for fractions 6-14; petroleum ether: chloroform: methanol (50:40:10) for fractions 15-29;

S/N	Petroleum ether (ml)	Chloroform (ml)	Methanol (ml)
1	100	0	0
2	90	10	0
3	80	20	0
4	70	30	0
5	60	40	0
6	50	50	0
7	40	60	0
8	30	70	0
9	20	80	0
10	10	90	0
11	0	100	0
12	0	95	5
13	0	90	10
14	0	85	15
15	0	80	20
16	0	75	25
17	0	70	30
18	0	65	35
19	0	60	40
20	0	55	45
21	0	50	50
22	0	45	55
23	0	40	60
24	0	35	65
25	0	30	70
26	0	25	75
27	0	20	80
28	0	15	85
29	0	10	90
30	0	5	95
31	0	0	100

Table 1: Solvent proportion.

chloroform: methanol (60:40) for fractions 30-39. The plates were put in the development chamber and care was taken to ensure that the spot was above the level of solvent to prevent the spotted material from dissolving in the solvent rather than undergoing chromatography. The cap of the developing chamber was carefully replaced to avoid disturbing the movement of the solvent. The solvent was left to advance up to 80-85% of the plate by capillary action, the plate was removed and the position of the solvent front was marked with a pencil. The developed plate was allowed to dry in the open laboratory; it was transferred to an iodine tank for colour development. The distance travelled by the spot was measured. The retention factor (RF)of each spot was determined.

$$R_F = \frac{\text{Distance moved by spot}}{\text{Distance moved by solvent}}$$

Fractions with R_F difference not greater than 0.1 - 0.15 and those with the same solvent system were pooled together. In the situation were there were multiple components in a fraction evident by the presence of more than one R_F value in a spotted material, the mean of all the R_F values was taken (Table 2).

Fractions	Retention factor (R_F)	Total Weight of recovered pooled fractions (mg)	Label
6	0.933	1.942	F1
7	0.878		
8	0.872		
9	0.798		
10	0.815		
11	0.925		
12	0.917		
13	0.894		
14	0.887		
15	0.922	3.832	F2
16	0.838		
17	0.839		
18	0.883		
19	0.927		
20	0.898	5.061	F3
21	0.893		
22	0.839		
23	0.861		
24	0.880		
25	0.836	8.884	F4
26	0.818		
27	0.818		
28	0.766		
29	0.796		
30	0.840	2.705	F5
31	0.741	7.756	F6
32	0.879		
33	0.839		
34	0.724		
35	0.906	7.859	F7
36	0.880		
37	0.880		
38	0.880		
39	0.920		

Table 2: Pooling of fractions.

Experimental Animals

Adult rabbits (2.5-2.8 kg) procured from the College of Veterinary Medicine Michael Okpara University of Agriculture Umudike, Abia State, Nigeria were used. The animals were housed in adequately ventilated cages and fed with finisher mash (Vital Feeds, Nigeria) and water *ad libitum* but starved for 24 hours prior to experiment. Experiments were carried out in accordance with international guidelines for care and use of laboratory animals and with the ethical principles of Michael Okpara University of Agriculture, Umudike. Approval was sought for and obtained from the ethics committee of the Department of Physiology and Pharmacology in the University (MOUAU/VPP/EC/2016/004), where the experiments were carried out.

Preparation of rabbit jejunum for *in vitro* study

Each rabbit used for the study was sacrificed by cervical dislocation before the abdominal cavity was immediately opened to isolate the jejunum which was transferred into a beaker containing tyrode solution maintained at 37^0C (pH 7.4) and continuously bubbled with air. The constituents of tyrode solution per liter were NaCl (8g), KCl (0.2g), $CaCl_2$ (0.2g), $NaHCO_3$ (1g), NaH_2PO_4 (1g), $MgCl_2$ (0.1g) and Glucose (2g). About 3cm length of the jejunum was suspended vertically in a 30ml organ bath containing tyrode solution also bubbled with air and allowed to equilibrate for 30 minutes before basal rhythmic contractions were recorded. Dose-response relationships were established for acetylcholine, the crude extract and fractions 1-7 of the extract. The effects of acetylcholine in the presence of atropine, crude extract and fractions were also established. A minimum time of 1 minute was allowed for tissue responses to separate drug applications before being washed 2 times with tyrode solution. Concentrations of test agents were presented as final bath concentrations (FBC) calculated as:

$$FBC = \frac{C_1V_1}{V_2}$$

Where C_1 = Initial concentration

V_1 = Volume of test substance added

V_2 = Volume of the tissue bath (30ml)

FBC values were expressed in milligrams per ml (mg/ml).

Percentage inhibition for each dose of extract tested was calculated as:

$$\text{Percentage inhibition} = \frac{\text{Basal amplitude} - \text{test amplitude}}{\text{Basal amplitude}} \times 100$$

Statistical analysis

The soft ware package used for data analyses was SPSS Version 20.0 (IBM SPSS Inc, Chicago, IL) and level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. The probability level of less than 5% ($p<0.05$) was considered statistically significantly different between the test and control groups as well as among test groups for measured values.

Results

Effect of *S. rotundifolius* crude extract, fractions and atropine on the amplitude of contraction

The effect of the crude extract on the piece of isolated rabbit jejunum was inhibitory as graded doses produced a dose dependent relaxation of the smooth muscles of the jejunum, lowering the amplitude of rhythmic contractions in each case. While the lowest dose applied (0.33 mg/ml) produced a 71.42 percent inhibitory effect, the highest dose (1.00 mg/ml) produced an inhibitory activity of 91.67 percent. The effect of the crude extract also compared favorably with that of atropine ($0.6x10^{-4}$ mg/ml) which produced an inhibitory effect of 53.36 percent (Table 3, Figure 1). The activities of all fractions obtained from the crude were also inhibitory, producing similar effects as the crude extract (Tables 4-10). However fraction 5 produced the highest relaxation effect of the piece of isolated tissue compared to the other fractions (Figure 1).

Final bath concentration (mg/ml)	Basal amplitude of contraction (mm)	Amplitude in response to the crude extract (mm)	Percentage inhibition (%)
0.33	14.00 ± 0.00	4.00 ± 0.41^c	71.42
0.67	13.00 ± 0.00	3.00 ± 0.00^b	76.92
1.0	12.00 ± 0.00	1.00 ± 0.00^a	91.67
Atropine ($0.6x10^{-4}$)	11.00 ± 0.00	5.13 ± 0.25^d	53.36

Table 3: Effect of graded doses of *S. rotundifolius* crude extract on basal amplitude of contraction of the rabbit jejunum.

Final bath concentration (mg/ml)	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses of F1 (mm)	Percentage inhibition (%)
0.33	10.50 ± 0.58	5.50 ± 0.58^c	47.62
0.67	10.75 ± 0.50	4.0 ± 0.00^b	62.79
1.0	9.25 ± 0.50	2.00 ± 0.00^a	78.37
Atropine ($0.6x10^{-4}$)	11.00	5.13 ± 0.25^c	53.36

Table 4: Effect of graded doses of F1 on basal amplitude of contraction.

Final bath concentration (mg/ml)	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses of F2 (mm)	Percentage inhibition (%)
0.33	17.25 ± 0.50	11.25 ± 0.50^c	34.48
0.67	16.75 ± 0.29	10.00 ± 0.00^c	40.29
1.0	11.63 ± 0.48	3.00 ± 0.00^a	74.20
Atropine ($0.6x10^{-4}$)	11.0 ± 0.00	5.13 ± 0.25^b	53.36

Table 5: Effect of graded doses of F2 on basal amplitude of contraction.

Final bath concentration mg/ml	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses ofF3 (mm)	Percentage inhibition (%)
0.33	10.63 ± 0.48	5.00 ± 1.15^c	52.33
0.67	11.75 ± 0.50	3.00 ± 0.00^b	75.03
1.0	10.00 ± 0.00	1.50 ± 0.58^a	85.83
Atropine ($0.6x10^{-4}$)	11.00 ± 0.00	5.13 ± 0.25^c	53.36

Table 6: Effect of graded doses of F3 on basal amplitude of contraction.

Final bath concentration mg/ml	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses ofF3 (mm)	Percentage inhibition (%)
0.33	10.63 ± 0.48	5.00 ± 1.15^{c}	52.33
0.67	11.75 ± 0.50	3.00 ± 0.00^{b}	75.03
1.0	10.00 ± 0.00	1.50 ± 0.58^{a}	85.83
Atropine ($0.6x10^{-4}$)	11.00 ± 0.00	5.13 ± 0.25^{c}	53.36

Table 6: Effect of graded doses of F3 on basal amplitude of contraction.

Final bath concentration (mg/ml)	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses ofF4 (mm)	Percentage inhibition (%)
0.33	7.15 ± 0.25^{a}	2.60 ± 0.25^{b}	63.64
0.67	18.25 ± 0.29^{e}	4.13 ± 0.25^{c}	77.37
1.0	8.75 ± 0.29^{b}	1.00 ± 0.29^{a}	88.57
Atropine ($0.6x10^{-4}$)	11.00 ± 0.00^{c}	5.13 ± 0.25^{d}	53.36

Table 7: Effect of graded doses of F4 on basal amplitude of contraction.

Final bath concentration (mg/ml)	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses of F5 (mm)	Percentage inhibition (%)
0.33	8.00 ± 0.41^{a}	2.13 ± 0.29^{b}	73.37
0.67	11.25 ± 0.29^{c}	2.00 ± 0.25^{b}	82.22
1.0	10.00 ± 0.00^{b}	1.10 ± 0.25^{a}	89.00
Atropine ($0.6x10^{-4}$)	11.00 ± 0.00^{c}	5.13 ± 0.25^{c}	53.36

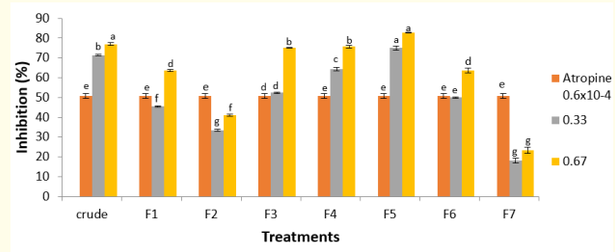
Table 8: Effect of graded doses of F5 on basal amplitude of contraction.

Final bath concentration (mg/ml)	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses of F6 (mm)	Percentage inhibition (%)
0.33	10.00 ± 0.00	4.88 ± 0.25^{c}	51.20
0.67	11.00 ± 0.00	4.00 ± 0.41^{b}	63.63
1.0	10.50 ± 0.00	2.93 ± 0.49^{a}	72.10
Atropine ($0.6x10^{-4}$)	11.00 ± 0.00	5.13 ± 0.25^{d}	53.36

Table 9: Effect of graded doses of F6 on basal amplitude of contraction.

Final bath concentration (mg/ml)	Mean Basal amplitude of contraction (mm)	Mean Amplitude in response to graded doses of F7 (mm)	Percentage inhibition (%)
0.33	11.00 ± 0.00^{a}	9.13 ± 0.25^{c}	17
0.67	14.50 ± 0.58^{d}	11.25 ± 0.29^{d}	22.41
1.0	14.00 ± 0.00^{c}	6.00 ± 0.00^{b}	57.14
Atropine ($0.6x10^{-4}$)	11.00 ± 0.00^{a}	5.13 ± 0.25^{a}	53.36

Table 10: Effect of graded doses of F7 on basal amplitude of contraction.



Treatments with same letters are not significantly different at P>0.05 from one another compared to their control.

Figure 1: Comparative result of the percentage inhibition of spontaneous rhythmic contraction of the rabbit jejunum.

Effect of *S. rotundifolius* crude extract, fractions and atropine on acetylcholine induced contraction on the rabbit jejunum

The application of acetylcholine to the piece of isolated tissue produced dose dependent contractions of the smooth muscle tissue with peak contraction achieved following the application of 1.0 x 10^{-4} mg/ml (Table 11). This activity of acetylcholine was significantly inhibited by the crude extracts and fractions in a manner which was similar to that of atropine following separate trials on the piece of smooth muscle tissue. Fraction 5 inhibited acetylcholine activity most while fraction 7 showed the least effect. The order of inhibitions of the agents on acetylcholine induced contractions is: F5>F4>F3>CRUDE>F6>F1>F2>F7 (Table 12).

Final bath concentration (x 10^{-4} mg/ml)	Basal amplitude of contraction (mm)	Amplitude in response to graded doses of Acetylcholine (mm)	Percentage rise in amplitude (%)
0.6	9.0	16.0	77.78 ± 0.00
1.0	10.0	20.5	105.00 ± 0.00
1.3	10.0	20.0	105.00 ± 0.00

Table 11: *In vitro* response of an isolated rabbit jejunum to acetylcholine application.

Treatments (Final bath concentrations in mg/ml)	Basal amplitude (mm)	Amplitude in response to treatment (mm)	Percentage activity of Ach (%)
Acetylcholine (1.0 x10^{-4}mg/m)	10.0	20.5	105.00 ± 0.00^{f}
Atropine (0.6x10^{-4}) + Ach (1.0 x 10^{-4}mg/ml)	5.0	8.0	60.00 ± 0.10^{e}
Crude + Ach (1.0 x 10^{-4}mg/ml)	9.0	16.0	43.75 ± 0.20^{b}
F1 + Ach (1.0 x 10^{-4}mg/ml)	6.0	9.0	50.00 ± 0.10^{d}
F2 + Ach (1.0 x 10^{-4}mg/ml)	6.0	9.5	63.33 ± 0.70^{e}
F3 + Ach (1.0 x 10^{-4}mg/ml)	7.0	10.0	42.86 ± 0.76^{b}
F4 + Ach (1.0 x 10^{-4}mg/ml)	5.0	7.0	40.00 ± 0.55^{b}
F5 + Ach (1.0 x 10^{-4}mg/ml)	6.0	8.0	33.33 ± 0.50^{a}
F6 + Ach (1.0 x 10^{-4}mg/ml)	7.5	11.0	46.67 ± 0.84^{c}
F7 + Ach (1.0 x 10^{-4}mg/ml)	7.5	12.5	66.67 ± 0.54^{e}

Table 12: Response of an isolated rabbit jejunum to acetylcholine in the presence of atropine, crude extract and fractions of *S. rotundifolius.*

Figure 2: Effect of graded doses of *S. rotundifolius* on basal amplitude of contraction.

Figure 3: Effect of acetylcholine on basal amplitude of contraction.

Figure 4: Effect of acetylcholine in the presence of graded doses of *S. rotundifolius* on basal amplitude of contraction.

Discussion

Muscarinic receptors (of M2 and M3 subtypes) are established binding sites for acetylcholine molecules to produce contractions of smooth muscles necessary for onward movement of intestinal contents. Activation of these receptors triggers phosphoinositide hydrolysis, Ca2+ mobilization, inhibition of adenylcyclase activity and potentiation of Ca2+ dependent non-selective conductance [10,11]; culminating in Ca2+ release from intracellular storage sites in the GIT smooth muscle cells and eventual muscle contractions [12]. The mechanism of acetylcholine activities on the GIT smooth muscle is well established [13].

Current reports support the use of isolated smooth muscle tissues like the jejunum as being ideal for evaluating the effects of drugs on the gastrointestinal tract. This is because such set ups allow the isolated tissue to function as a whole system such that physiological outcomes (contraction or relaxation) may approximate to outcomes in *in vivo* state [14,15].

In this study, *S. rotundifolius* crude extract and fractions significantly inhibited spontaneous contractions of the rabbit jejunum in a dose dependent manner, suggesting that the extract may contain agents with inhibitory effects. In a previous communication, we showed that *S. rotundifolius* contain high amounts of tannins and flavonoids [16] which have been implicated in gastrointestinal smooth muscles relaxation [17]. Although all fractions of the crude extract produced inhibitory effects on the piece of tissue, their activities differed following comparative analysis. The higher inhibitory activity produced by fraction 5 may be due to higher amounts of phytochemical substances in the fraction just as decrease in the concentrations of these phyto-constituents may be responsible for decreased spasmolytic activities of other fractions. Like atropine,

the extracts may have produced GIT inhibitions by antagonizing acetylcholine effect at the muscarinic receptor level. It is established that one of the mechanisms involved in gastrointestinal smooth muscle relaxation is the blocking of excitatory cholinergic pathway due to acetylcholine action [18,19] and the effects produced by the crude extract and fractions may have been via this anticholinergic pathway, hence the spamolytic effects of the crude extract and its fractions observed in this study may have been mediated via the muscarinic receptors. Similar conclusions were drawn in related studies on plant extracts following *in vitro* trials on smooth muscles tissues [20,21].

Conclusion

The crude extract and fractions of *S. rotundifolius* have shown significant smooth muscle relaxant effects on isolated gastrointestinal smooth muscle preparation which may have been mediated via muscarinic receptors due to interactions of its active components with muscarinic receptors in the gastrointestinal tract to inhibit acetylcholine induced contractions. The presence of phytochemicals like alkaloids, flavonoids, tannins and saponins in the extracts may be responsible for the observed pharmacological effects. If the results obtained in this study can be extrapolated to man, then extracts from *S. rotundifolius* may be of value in the management of diseases caused by excessive gastrointestinal spasms.

Authors' Contributions

Harbor and Ijioma handled the technical aspect of the work and wrote the first draft of the manuscript. Harbor did the literature work and statistical analysis. Ijeh and Ohaeri supervised, read through the work and made corrections. All authors did final reading of the work and approved same.

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