

Full Length Research Paper

Evaluation of *Sorghum bicolor* leaf base extract for gastrointestinal effects

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The leaf base of *Sorghum bicolor* (Family: Gramineae, Poaceae) was cold-macerated with 70% v/v methanol. The aqueous methanolic extract was further fractionated into non-polar, medium polar and very polar components using hexane, ethylacetate and water (aqueous), respectively. The gastrointestinal effects of these extracts were tested on intestinal motility (transit) in mice, castor oil-induced diarrhoeal model in rats, isolated rabbit jejunum, guinea pig ileum and rat stomach fundus strip. The oral and intraperitoneal LD₅₀ values for the extracts were determined in mice and rats. The aqueous methanolic extract (100 – 400 mg/kg i.p) significantly ($P < 0.05$) and dose-dependently decreased the intestinal motility, inhibited castor oil-induced diarrhoea, produced concentration-dependent relaxation of rabbit jejunum with half maximal effective concentration (EC₅₀) of 0.21 mg/ml. This extract also produced both non-myogenic and slight relaxation effects on guinea pig ileum and a contraction on rat stomach fundus strips. Both aqueous and ethylacetate fractions also reduced intestinal motility. However, ethylacetate fraction caused greater reduction than the aqueous fraction. The oral LD₅₀ value for the aqueous methanolic extract in both rats and mice was found to be ≥ 2000 mg/kg while the intraperitoneal values are 1414.2 mg/kg in rats and 1341.6 mg/kg in mice. The intraperitoneal value for both aqueous and ethylacetate fractions is ≥ 2000 mg/kg in mice. The study provided scientific bases for the traditional use of *S. bicolor* for treatment of gastrointestinal related problems such as diarrhoea.

Key words: *Sorghum bicolor*, gastrointestinal, motility, diarrhoea, jejunum, ileum, fundus.

INTRODUCTION

Sorghum bicolor (Linn.) Pers. (Family: Gramineae; Poaceae) is a cultivated annual grass resembling corn in appearance. It is grown in all tropical and warm temperate countries (Encyclopedia Americana, 1995). Ethno-medicinal reports on the plant showed a world-wide use of different parts of *S. bicolor* plant in folklore medicine (Grieve, 1931; Watt and Breyer-Brandwijk, 1962; Perry, 1980; Duke and Wain, 1981; Morton, 1981; Chiej, 1984; Grieve, 1984; Okokoh, 1999). The reports include the use of *S. bicolor* plant for treatment of gastrointestinal related problems such as diarrhoea, fluxes and stomach ache (Watt and Breyer-Brandwijk, 1962; Perry, 1980; Morton, 1981; Duke and Wain, 1981). It has been shown that

diarrhoea ranges from a mild and socially inconvenient illness to a major cause of malnutrition among children in developing countries and the cause of 4-5 million deaths throughout the world annually (Anonymous, 1979; Syder and Merson, 1982). It has also been shown that HIV/AIDS pandemic has been among the most serious natural disasters in recent centuries (Adeyi et al., 2006). According to Piot et al. (1999), HIV has become endemic in parts of Africa and is a major public health problem on the same magnitude as malaria, diarrhoea and malnutrition. Omalu et al. (2007) showed that most of the HIV/AIDS patients had chronic diarrhoea leading to severe weight loss. The leading cause of morbidity and mortality by diarrhoea in developing countries prompted the World Health Organisation (WHO) to constitute a diarrhoeal disease control programme (CDD) which includes studies of traditional medicinal practices, together with the evaluation of health education and prevention

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approaches to combat the problem of diarrhoea (Anonymous, 1979; Syder and Merson, 1982; Lutterodt, 1989). WHO has long recognised and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various diseases and ailments. These plants, which abound in our environment, are widely accepted by the population and serve as cheaper alternative to orthodox medicine (Sofowora, 1993; Akah and Nwambie, 1994). However, the report by Zhu et al. (2002) has shown that the rationale for the utilization of medicinal plants has rested largely on long term clinical experience with little or no scientific data on their efficacy and safety. Presently, there is paucity of information on the scientific justification for the use of *S. bicolor* plant parts in the treatment of gastrointestinal problems such as diarrhoea, fluxes and stomach ache. Therefore, the objective of this study is to provide the scientific basis for its application in folklore medicine and for possible drug development.

MATERIALS AND METHODS

Plant collection and extraction

The dry mature leaves of *S. bicolor* were collected from Maganawa town, Sokoto State, Nigeria between November and January, 2006. The plant was authenticated by a Plant Taxonomist, Mr. Ibrahim Muazzam of Herbarium Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The specimen was deposited in NIPRD's Herbarium with voucher specimen number 3815. The dark red portions of the leaves, which are attached to the suckers of the plants, were cut out from the entire leaves (the portion of the leaves especially claimed to be used ethnomedicinally). This portion was pulverized in a mortar. Two hundred grams (200 g) of the pulverized sample was cold-macerated in 5 L of 70% v/v methanol over 96 h period on a shaker (GFL D 3006 mgH, Germany) to ensure maximum extraction. The extract was then filtered using clean cotton wool. The filtrate was placed on water bath to allow evaporation of the solvents and consequent concentration of the extract for subsequent studies. A yield of 23.6% w/w extract was obtained.

The aqueous methanolic extract (10.15 g) was further fractionated into non-polar, medium polar and very polar components using hexane, ethylacetate and water (aqueous) respectively to determine the component(s) producing the pharmacological effects observed with the aqueous methanolic extract before fractionation. The hexane fraction was greenish, fatty/oily and gave only a yield of 0.5% w/w (probable indication of presence of only very small quantities of non-polar components in the extract). Ethylacetate fraction gave a yield of 95.9% (w/w) (constituting the major component) and appeared shiny, deep brownish-black, clumped up but not sticky. Aqueous component gave a yield of 3.6% w/w and appeared deep brownish, clumped up and lightly sticky.

Animals

Swiss albino mice (15.7 - 25.2 g), Wistar rats (152.5 - 314.0 g), guinea pigs (345.0 - 418.0 g) and rabbits (2.2 - 2.8 kg) of both sexes were used for the investigations. They were obtained from the Animal Facility Centre, Department of Pharmacology and

Toxicology, NIPRD, Abuja. The experimental animals were separated for two weeks in the experimental room for acclimatization. They were housed in appropriately designed cages suitably bedded with wood shavings. The animals were maintained under normal environmental temperature (26-28°C), approximately 12 h day and night illumination cycle. The animals were fed *ad libitum* with standard NIPRD formulated feed and had free access to water except where starvation was needed in the course of the experiment. The animals were grouped according to their body weight and sex to achieve approximately uniform conditions among the groups. All investigations were performed in accordance with the 'Principles of laboratory animal care' (NIH Publication # 85-23, 1985).

Drugs and chemicals

Acetylcholine, Histamine, 5-Hydroxytryptamine (5-HT), Carbachol (all from Sigma, USA), Castor oil (Bell Sons, England), Loperamide (Xepa-S Pattinson, Malaysia), D-Glucose Monohydrate (Dextrose, LNL, Nigeria), Sodium Chloride, Potassium Chloride, Sodium Dihydrogen Orthophosphate, Sodium Chloride, Potassium Chloride, Sodium Dihydrogen Orthophosphate, Sodium Hydrogen Bicarbonate, Calcium Chloride, Magnesium Chloride, Magnesium Sulphate, Potassium Dihydrogen Orthophosphate (all from BDH, Poole, England) were the drugs and chemicals used for the investigations.

Acute toxicity studies

The modified method of Lorke (1983) was adopted to estimate the median lethal dose (LD_{50}) of the aqueous methanolic extract, ethylacetate and aqueous fractions in Swiss albino mice and Wistar rats of both sexes. Extract administration was done in biphasic manner via oral and intraperitoneal routes. The first phase involved administration of widely differing doses of the extract/fractions (100-2,000 mg/kg i.p. and p.o.) to determine the toxicity range. The doses administered in the second phase were further narrowed down based on the toxicity range observed in the first phase and involved administration of more specific doses (800-1,200 mg/kg i.p.) to new sets of experimental animals. The animals were observed 24-72 h post treatment for behavioural changes such as ataxia, nervousness, dullness, alertness, excitement and death. The (LD_{50}) was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused 0% lethality.

Intestinal transit test

The test was done according to the method of Akah et al. (1998). Swiss albino mice (15.7-25.2 g body weight) of both sexes were used. They were starved of feed for 24 h prior to the experiment but allowed free access to water. The mice were divided into five ($n = 5$). The first group received normal saline (20 ml/kg i.p.) to serve as the negative control. Groups two, three and four mice received the extract at doses of 100, 200, 400 mg/kg i.p. respectively. Carbachol (1 mg/kg i.p.) was given to the fifth group to serve as the reference standard. 10 min. post treatment, 0.5 ml of a 5% charcoal suspension in 10% tragacanth powder was administered orally to each mouse. The mice were sacrificed 30 min later and their abdomen cut open. The percentage of distance traveled by the charcoal plug in the small intestine (from the pylorus to the caecum) was determined for both the treated and control groups (Akah, 1989).

This study was repeated using the ethylacetate (100, 200, 400 mg/kg i.p.) and aqueous (100, 200, 400 mg/kg i.p.) fractions as well. Normal saline (20 ml/kg i.p.)-treated group was used as the negative control while atropine (0.1 mg/kg i.p.)-treated group was used as the reference standard.

Castor oil-induced diarrhoeal test

The method of Pulok et al. (1998) was adopted. Adult Wistar rats (157.9-314.0 g) of either sex were used. They were fasted for 18 h. They were then divided into five groups (of five rats each). The first group received normal saline (20 ml/kg i.p.) to serve as the negative control while graded doses of the extract (100, 200, 400 mg/kg i.p.) were given to groups two, three and four respectively. Loperamide (10 mg/kg i.p.) was given to the fifth group as a reference standard. 1 h post treatment, 1 ml of castor oil was given orally to rats in all the groups. The rats were then observed for defecation over a 4 h period following castor oil administration. The presence of characteristic diarrhoeal droppings was noted on transparent paper surface spread beneath every cage. The severity of diarrhoea was given as scores based on the faecal consistency as follows: ++ = 2 (very watery); + = 1 (moderately watery); - = 0 (not watery; Di Carlo et al., 1994 modified).

Studies on isolated rabbit jejunum

Adult rabbits (2.2-2.8 kg) of both sexes used for the study were starved of feed for 18 h. They were stunned by a blow on the head, sacrificed and the abdomen cut open. A segment of the jejunum (about 2-3 cm long) was removed and dissected free of adhering mesentery. The tissue was then suspended in 25 ml organ bath containing tyrode solution. The physiological solution constituted of: NaCl 90 g; 10% KCl 20 ml; 10% $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 5 ml; D-Glucose 10 g; NaHCO_3 10 g; 10% CaCl_2 20 ml; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1 ml dissolved in 10 L of distilled water and maintained at 37°C and aerated with air.

The effects of acetylcholine and the leaf base extract were tested on the strips of jejunum. The responses were recorded isometrically on microdynamometer set at sensitivity of 3.0 mV and speed of 24 mm/min. The concentration of the extract at which it was half maximally effective (EC_{50}) was also determined. This involved the conversion of the log organ bath concentration at which the percent of maximal effect was 50 to normal organ bath concentration.

Studies on isolated guinea pig ileum

Adult guinea pigs (345.0-418.0 g) of both sexes used for the study were starved of feed for 18 h. They were stunned by a blow in the head, sacrificed and the abdomen of each cut open. About 2 cm strip of the guinea pig ileum was removed and the adhering mesentery dissected out. This was mounted in a 25 ml organ bath containing aerated tyrode solution of the above composition and maintained at 37°C. The set up was connected to microdynamometer recorder set at sensitivity of 2.0 mV and speed of 24 mm/min.

Following equilibration, the effects of histamine, acetylcholine and graded concentrations of the extract were tested on the tissue.

Studies on isolated rat stomach fundus strip

Adult Wistar rats (152.5-210.0 g) of both sexes were used. They were sacrificed after a stun and the abdomen cut open to remove the stomach. The pyloric region was cut off from the fundus region of the stomach. A strip of the stomach fundus was then made and mounted in aerated Krebs solution constituted of NaCl 69 g; 10% KCl 35 ml; 10% $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$ 29 ml; 10% KH_2PO_4 16 ml; D-Glucose 20 g; NaHCO_3 21 g; Molar CaCl_2 25.2 ml; added to 10 litres of distilled water. The set up was connected to microdynamometer recorder set at sensitivity of 3.0 mV and speed of 24 mm/min.

At equilibration, the effects of acetylcholine, histamine, 5-Hydroxytryptamine (5-HT) and the extract were tested on the tissues. The

concentration of the extract at which it was half-maximally effective (EC_{50}) was also determined.

Statistical analysis

The results of the studies were expressed as mean \pm SEM. The differences between the control and treated means were analysed using one-way analysis of variance (ANOVA). Student t-test was used where ANOVA showed significant difference. Statistical significance was established at P-values < 0.05. Results were presented as tables, graphs and tracings as appropriate.

Compliance with good laboratory practice (GLP)

The studies were carried out according to Good Laboratory Practice (GLP) Regulations of Organization for Economic Cooperation and Development – OECD (UNDP/World Bank/WHO, 2001).

RESULTS

Acute toxicity studies (LD_{50})

No overt toxicity sign or death was observed in rats and mice 72 h post oral treatment with 100-2,000 mg/kg doses of aqueous methanolic extract of *S. bicolor* leaf base. The oral median lethal dose (LD_{50}) of the extract in rats and mice is therefore ≥ 2000 mg/kg p.o. No overt toxicity sign or death was observed in rats after 24 h of intraperitoneal (i.p.) treatment with the aqueous methanolic extract (100-2,000 mg/kg). However, within 48 h of the treatment, all the rats treated with 2,000 mg/kg i.p. dose became recumbent and died while those treated with 100-1,000 mg/kg i.p. doses neither showed toxicity signs nor death 72 h post i.p. treatment. For the estimation of the intraperitoneal median lethal dose (LD_{50}) in rats, assessment based on 24 h post treatment showed a median lethal dose (LD_{50}) $\geq 2,000$ mg/kg i.p. since no overt toxicity sign or death was observed in i.p.-treated rats after 24 h. However, an assessment based on 48 h post i.p. treatment observation gave a calculated median lethal dose of 1,414.2 mg/kg i.p. in rats. The mice treated with doses of the extract $\leq 1,200$ mg/kg i.p. showed neither toxicity signs nor death 24 h post treatment. At the dose of 1,500 mg/kg i.p., the mice were calm, dull, with increased respiratory rate. At this dose, mortality of 66.7 and 100.0% occurred within 24 and 48 h of i.p. treatment respectively. The mice treated i.p. with 2,000 mg/kg dose were calm, dull, recumbent with increased respiratory rate. A mortality of 100.0% occurred at this dose within 24 h. The calculated intraperitoneal medial lethal doses in mice are 1,248.0 and 1,341.6 mg/kg i.p. for 24 and 48 h post treatment observations respectively.

For the ethylacetate fraction of *S. bicolor* leaf base extract, 33.3 and 66.7% of 1000 and 2000 mg/kg i.p.-treated mice were dull, immobilised with increased respi-

Table 1. Inhibitory effect of 70% v/v methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on intestinal motility in mice.

Treatment	Mean body weight (g)	Mean Intestinal length (cm)	Mean distance travelled by charcoal (cm)	Movement of charcoal as % of intestinal length (%)
Control	19.82 ± 1.3	37.96 ± 1.1	14.30 ± 0.9	37.7
<i>S. bicolor</i>				
100 mg/kg i.p.	20.30 ± 1.3	5.78 ± 1.1	6.86 ± 1.1*	19.2
200 mg/kg i.p.	19.14 ± 1.5	34.76 ± 1.7	3.00 ± 1.5*	8.6
400 mg/kg i.p.	19.20 ± 0.8	36.60 ± 2.2	2.42 ± 0.7*	6.6
Carbachol (1 mg/kg i.p.)	22.63 ± 0.6	35.63 ± 1.2	28.03 ± 4.5**	78.7

*= $p < 0.05$; significant reduction in intestinal propulsion; **= $p < 0.05$; significant increase in intestinal propulsion (one-way ANOVA, Student t-test).

ration within 12 min post administration. All the mice later recovered and no further toxicity sign or death was observed 24, 48 and 72 h post intraperitoneal administration. The intraperitoneal LD₅₀ of ethylacetate fraction of *S. bicolor* leaf base extract in mice is therefore ≥ 2000 mg/kg.

For the aqueous fraction of *S. bicolor* leaf base extract, only 33.3% of mice treated intraperitoneally with the dose of 2,000 mg/kg were dull, immobilized with increased respiration within 10 min of administration. The mice also recovered and no further toxicity sign or death was observed 24, 48 and 72 h post i.p. administration. The intraperitoneal LD₅₀ of aqueous fraction of *S. bicolor* leaf base extract in mice is therefore ≥ 2000 mg/kg.

Effect on intestinal transit

The aqueous methanolic leaf base extract of *S. bicolor* (100-400 mg/kg i.p.) significantly ($P < 0.05$) decreased the propulsive movement of charcoal meal through the mice gastrointestinal tract when compared with the normal saline control. The observed effects were dose-dependent with percent charcoal movement of 19.2, 8.6 and 6.6% (equivalence of inhibitory percentages of 80.8, 91.4 and 93.4%) for 100, 200 and 400 mg/kg i.p. respectively. Carbachol (1 mg/kg i.p.) on the other hand significantly ($P < 0.05$) increased the intestinal propulsion with inhibitory percentage of 21.3% equivalent to 78.7% charcoal movement (Table 1).

The aqueous fraction of the 70% v/v methanolic extract of *S. bicolor* leaf base (100-400 mg/kg i.p.) also reduced charcoal meal movement at all the tested doses when compared with the normal saline control. The doses of 100, 200 and 400 mg/kg i.p. produced percent charcoal movement of 42.7, 46.3 and 50.5 respectively. This is equivalent to propulsion inhibitory percentages of 57.3, 53.7 and 49.5% respectively. The inhibitory effect decreased dose-dependently and was only significant ($P < 0.05$) at the dose of 100 mg/kg i.p. The highest inhibitory effect was also observed at the 100 mg/kg i.p dose (Table 2).

The ethylacetate fraction (100 – 400 mg/kg i.p.) also reduced charcoal meal movement at all the tested doses with percent charcoal movement of 36.9, 37.0 and 23.0 (equivalent to propulsion inhibitory percentages of 63.1, 63.0 and 77.0%) for doses of 100, 200 and 400 mg/kg i.p. respectively). The effect was not dose-dependent but was significant ($P < 0.05$) at all the tested doses. The dose of 400 mg/kg i.p. produced the highest inhibitory effect (Table 2).

Atropine (0.1 mg/kg i.p.) also reduced charcoal movement in comparison with the control, having inhibitory percentage of 26.4% (equivalent to 73.6% charcoal movement). However, the inhibitory effects of the aqueous and ethylacetate fractions of the extract were much higher than that of the tested dose of atropine (Table 2).

Effect on castor oil-induced diarrhoea

The aqueous methanolic extract of *S. bicolor* leaf base did not inhibit castor oil-induced diarrhoea at the dose of 100 mg/kg i.p. On the other hand, the doses of 200 and 400 mg/kg i.p. of the extract exhibited marked anti-diarrhoeal activity with 100% diarrhoeal inhibition. This effect compared favourably with standard anti-diarrhoeal drug, loperamide (10 mg/kg i.p.). The diarrhoeal onset time observed in the 100 mg/kg extract group was shorter than that of the normal saline control group (Table 3).

Effect on isolated rabbit jejunum

The aqueous methanolic extract of *S. bicolor* leaf base (0.04-2.56 mg/ml) produced a concentration-dependent relaxation of rabbit jejunum. This effect was in contrast to those of acetylcholine (0.004-0.016 μ g/ml) and histamine (0.4-0.8 μ g/ml), which caused contraction of the same tissues (Appendix I). The organ bath concentration of the extract at which it is half-maximally effective (EC₅₀) on rabbit jejunum was 0.21 mg/ml (an equivalence of log organ bath concentration of -0.67; Figure 1).

Table 2. Inhibitory effects of the aqueous and ethylacetate fractions of *S. bicolor* leaf base extract (100 – 400 mg/kg i.p.) on intestinal motility in mice.

Treatment	Mean body weight (g)	Mean intestinal length (cm)	Mean distance travelled by charcoal (cm)	Movement of charcoal as percentage of intestinal length
Control	28.63 ± 3.2	31.98 ± 3.0	24.78 ± 2.9	77.5
Aqueous fraction				
100 mg/kg i.p.	26.18 ± 3.7	31.83 ± 2.1	13.60 ± 1.9	42.7*
200 mg/kg i.p.	26.45 ± 3.3	38.60 ± 0.7	17.88 ± 3.9	46.3
400 mg/kg i.p.	26.98 ± 3.2	30.70 ± 2.8	15.5 ± 6.3	50.5
Ethylacetate fraction				
100 mg/kg i.p.	28.43 ± 1.4	40.10 ± 2.1	14.78 ± 1.1	36.9*
200 mg/kg i.p.	29.00 ± 1.3	37.23 ± 2.0	13.78 ± 1.9	37.0*
400 mg/kg i.p.	27.08 ± 2.5	41.10 ± 2.8	9.45 ± 2.9	23.0*
Atropine (0.1 mg/kg i.p.)	25.75 ± 4.4	36.65 ± 0.6	26.98 ± 2.5	73.6

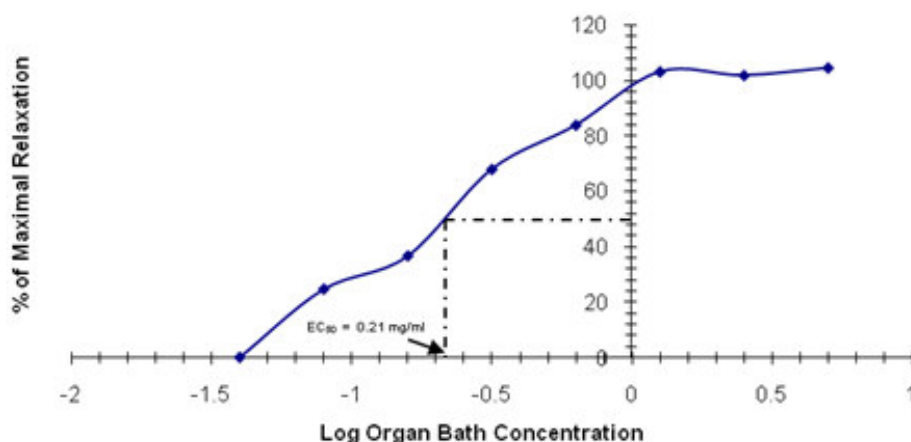
* = $p < 0.05$; Significant reduction in intestinal propulsion (one way ANOVA; Student t-test, $n = 5$).

Table 3. Effect of 70% v/v methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on castor oil-induced diarrhoea in rats.

Treatment	Mean diarrhoeal Onset time (min)	Diarrhoeal score				Inhibition (%)
		++	+	-	Total score	
Control	174.3 ± 25.6	3	1	0	7	-
<i>S. bicolor</i>						
100 mg/kg i.p.	158.0 ± 25.8	2	3	-	7	0.0
200 mg/kg i.p.	0.0 ± 0.0	-	-	5	0*	100.0
400 mg/kg i.p.	0.0 ± 0.0	-	-	5	0*	100.0
Loperamide (10 mg/kg i.p.)	0.0 ± 0.0	-	-	5	0*	100.0

* = $p < 0.05$; Statistical difference from control (student t-test).

Note: (++) = 2; + = 1; - = 0 multiplied by the number of animals with a particular severity of diarrhoea).

**Figure 1.** Half-maximal effective relaxation concentration (EC_{50}) of 70% v/v methanolic leaf base extract of *S. bicolor* (0.04 - 5.12 mg/ml) on rabbit jejunum. Data derived from Appendix I.

Effect on guinea pig ileum

The aqueous methanolic extract of *S. bicolor* leaf base

(0.04-5.12 mg/ml) did not produce any effect on smooth muscles of guinea pig ileum (Appendix II) except in one out of four tissue preparations studied in which a slight

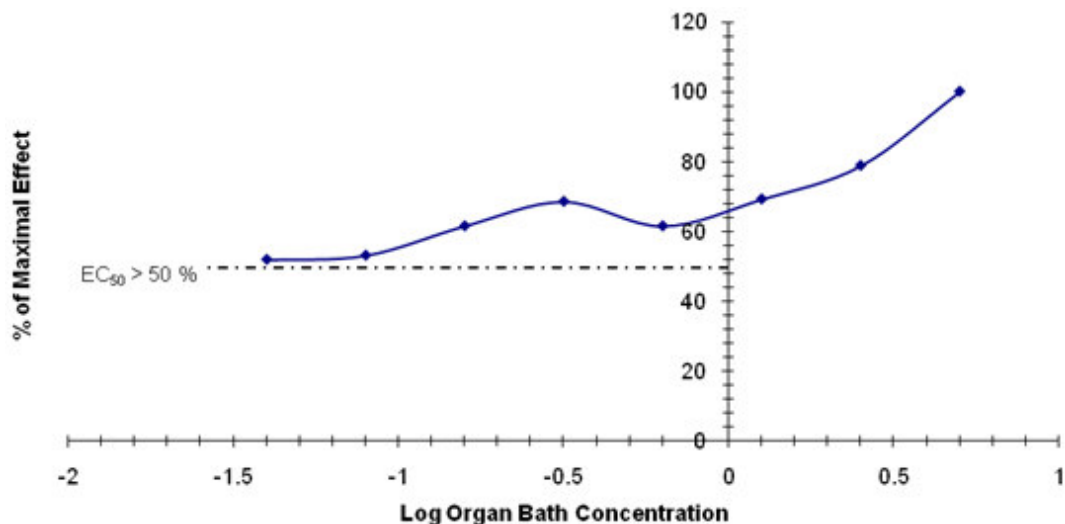


Figure 2. Half-maximal effective contractile concentration (EC_{50}) of 70% v/v methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on rat stomach fundus strip. Data derived from Appendix IV. Note: The graph showed that lower concentrations of the extract will be needed to produce contractile effects $\leq 50\%$ for subsequent calculation of the concentration of the extract at which it is half-maximally effective (EC_{50}).

relaxation was observed at extract concentrations of 1.28-5.12 mg/ml. Histamine (0.04-0.16 $\mu\text{g/ml}$) and acetylcholine (0.02-0.16 $\mu\text{g/ml}$) produced contraction of all the studied tissues (Appendix III).

Effect on isolated rat stomach fundus strip

The aqueous methanolic extract of *S. bicolor* leaf base (0.04 - 5.12 mg/ml) contracted the smooth muscles of rat stomach fundus strip. The effect was not concentration-dependent. Acetylcholine (0.04 - 0.16 $\mu\text{g/ml}$), histamine (0.08-0.16 $\mu\text{g/ml}$) and 5-hydroxy tryptamine (5-HT; 0.004-0.016 $\mu\text{g/ml}$) also contracted these tissues but in a concentration dependent manner (Appendix IV). The aqueous methanolic extract at the tested organ bath concentrations (0.04 - 5.12 mg/ml) produced percent of maximal contractions greater than 50% on isolated rat stomach fundus strip (Figure 2).

DISCUSSION

Evaluations carried out on *S. bicolor* leaf base extract revealed that its aqueous methanolic extract significantly ($P < 0.05$) and dose-dependently reduced the propulsive movement of charcoal meal through the mice gastrointestinal tract. This is indicative of reduction in peristaltic activity and ultimately reduction in gastrointestinal motility. This finding has implications on the rate of gastric emptying as well as intestinal movement and secretions.

The inhibitory effects on gastrointestinal motility were also observed on the aqueous and ethylacetate fractions of the leaf base extract. However, the ethylacetate fraction inhibited gastrointestinal motility more than the

aqueous fraction. This shows that the plant constituent(s) responsible for the anti-motility activity were not lost after fractionation. They, however seem to reside more in the ethylacetate fraction (medium polar) than in the aqueous portion (very polar). It was also observed that the propulsion inhibitory effects of both aqueous and ethylacetate fractions of the extract were much higher than that of the tested dose of atropine (0.1 mg/kg i.p.).

A delay in gastric emptying will prevent speedy evacuation of the stomach contents (Bertaccini et al., 1981; Akah et al., 1998). This probably explains the significant ($P < 0.05$) inhibition (100%) of castor oil induced diarrhoea by aqueous methanolic extract. This effect was comparable to that of loperamide (10 mg/kg i.p.). Castor oil is classified as a stimulant laxative (Dinesh et al., 1999). The diarrhoeal-inducing property of castor oil is known to be due to its active component ricinoleic acid (McKeon et al., 1999), which causes irritation that diminishes electrolyte permeability in the small intestine (Gaginella and Phillips, 1975; Zavala et al., 1998). Anti-motility drugs such as loperamide block the actions of castor oil and are used to relieve diarrhoea. It is therefore possible that the aqueous methanolic leaf base extract inhibited castor oil-induced diarrhoea via the mechanism of gastro-intestinal motility inhibition, which is spasmolytic effect. According to the report of Di Carlo et al. (1994), drugs affecting intestinal motility and secretion also possess anti-diarrhoeal activity. It could also be via anti-electrolyte permeability action. However, there are other anti-diarrhoeal mechanisms. Further studies will elucidate other possible anti-diarrhoeal mechanism(s) for *S. bicolor* extract.

S. bicolor leaf base extract produced a concentration-dependent relaxation of rabbit jejunum in contrast to

concentration-dependent contractility caused by acetylcholine and histamine on the same tissues. The extract did not produce any effect on smooth muscles of guinea pig ileum (except in one out of four preparations studied in which a slight relaxation was observed at extract concentrations of 1.28-5.12 mg/ml) while these same tissues were contracted by histamine and acetylcholine. Although the mechanism for the observed relaxation was not elucidated in the present study, the relaxation could be the cause of the anti-motility effect observed in the intestine and the subsequent anti-diarrhoeal activity observed earlier in the study. Dinesh et al. (1999) reported that advantage can be taken of agents that reduce intestinal motility, gastric secretory effect and are anti-spasmodic as adjunctive treatment in non-ulcer dyspepsia, irritable bowel syndrome and diverticular disease. Antispasmodics are of value for treating abdominal cramps associated with diarrhoea while anti-motility drugs relieve diarrhoea. This therefore suggests that the extract has the potential of being developed into anti-spasmodic and/or anti-motility agent. This corroborated the use of *S. bicolor* (especially the seed) in folklore medicine as a remedy for diarrhoea. The plant constituent(s) responsible for the anti-diarrhoeal activity of the plant might be present in both the seed and the leaf.

Conversely, the extract contracted the smooth muscles of rat stomach fundus strip in a manner that was not concentration-dependent as did acetylcholine, histamine and 5-hydroxytryptamine. The mechanism for this contractile effect on stomach fundus requires further elucidation. This will help to explain the reason for having contractile effect in the stomach (as was demonstrated on stomach fundus strip) and relaxation effect in the intestine (as was demonstrated on rabbit jejunum, guinea pig ileum and mice intestinal motility).

In conclusion, development of antimotility /anti-spasmodic/ anti-diarrhoeal drug(s) from *S. bicolor* should be explored because it may possess some advantages over already existing orthodox agents.

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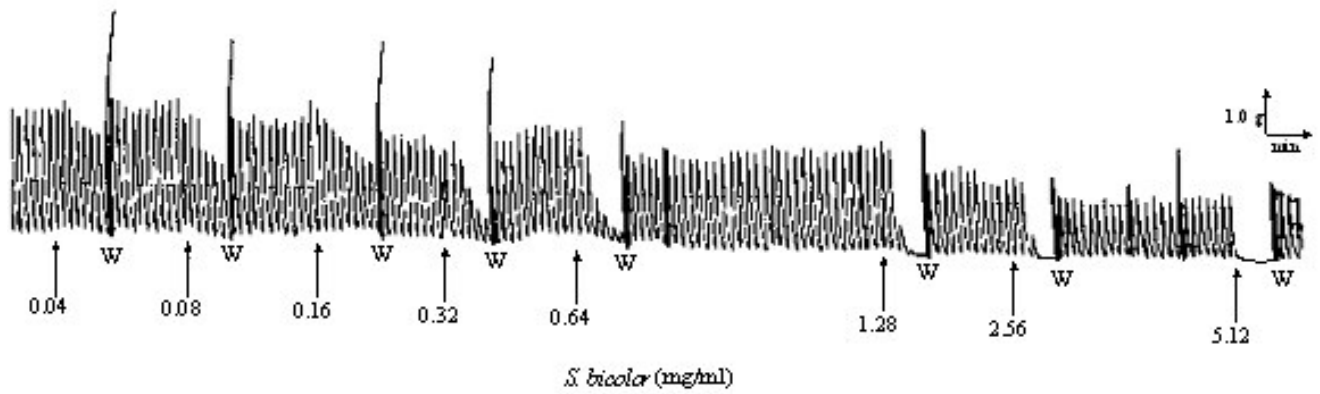
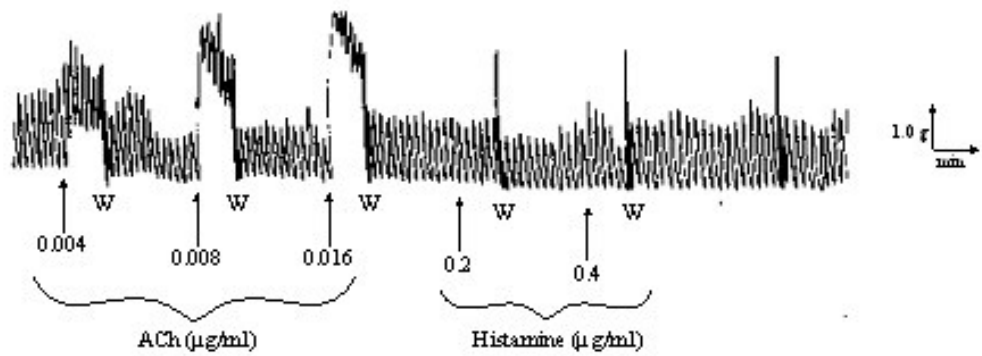
The authors are grateful to Dr U.S. Inyang, the Director General, National Institute for Pharmaceutical Research and Development (NIPRD) and his Management team for the grant awarded to support studies on *S. bicolor*. The technical assistance provided by Sunday Dzarma, John Kono, Musa Jet, Ibrahim Adamu, Mohammed Umaru, Dari Yaú is appreciated. Gratitude also goes to Ibrahim Muazzam, a Plant Taxonomist with NIPRD's Herbarium for the ethnobotanical information he provided on the plant of study.

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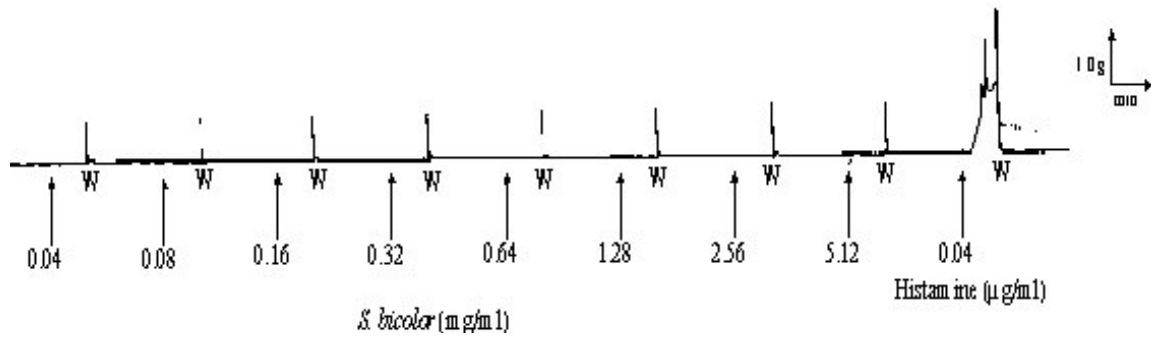
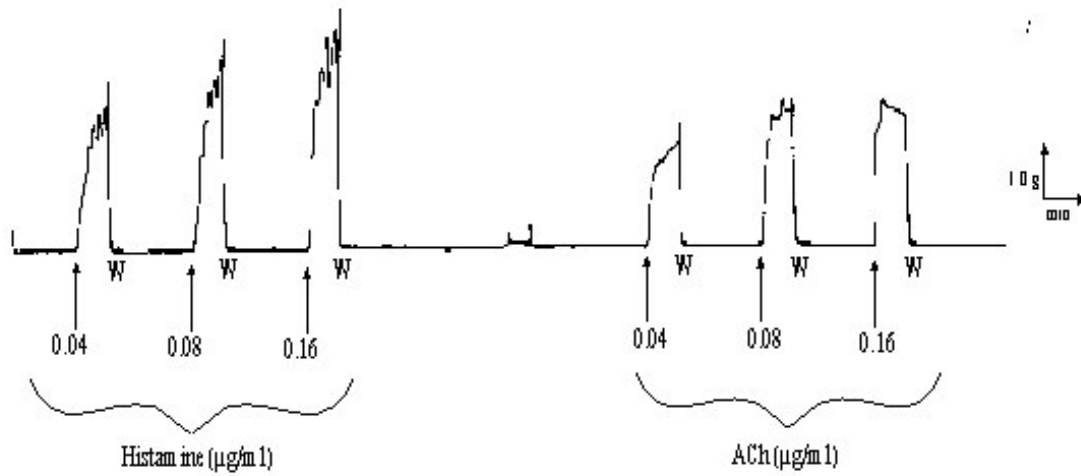
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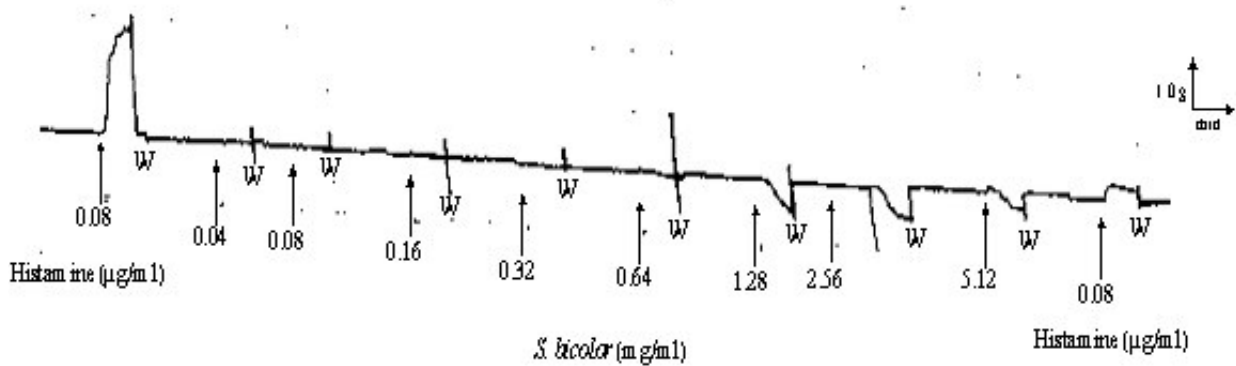
Appendix



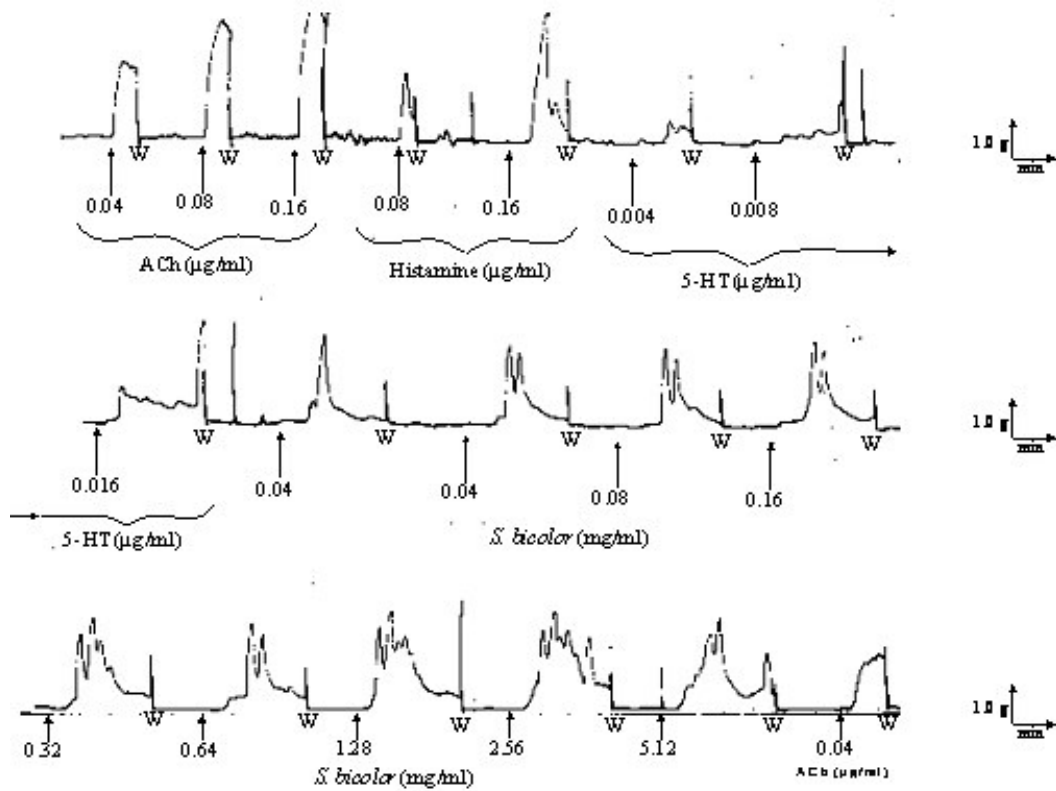
I. Relaxation effect of 70% v/v methanolic leaf base extract of *S. bicolor* (0.04 – 5.12 mg/ml) on isolated rabbit jejunum. Sensitivity = X3Mv; Speed = 24 mm/mi in; Ach = acetylcholine.



II. Non-myogenic effect of 70% v/v methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on isolated guinea pig ileum. (Sensitivity = X3Mv; Speed = 24 mm/mi in; Ach = acetylcholine).



III. Slight relaxation effect of 70% v/v methanolic extract of *S. bicolor* leaf base (1.28 – 5.12 mg/ml) on guinea pig ileum. Sensitivity = X3Mv; Speed = 24 mm/mi in; Ach = acetylcholine.



IV. Contractile effect of 70% v/v methanolic leaf base extract of *S. bicolor* (0.04 – 5.12 mg/ml) on rat stomach fundus strip. Sensitivity = X3Mv; Speed = 24 mm/mi in; Ach = acetylcholine.