Evaluation of Anti-diarrhoeal and Anti-ulcer Properties of Fractions of *Triumfetta cordifolia* A. Rich (Tiliaceae) Fruit in rats

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**ABSTRACT:** *Triumfetta cordifolia* fruit is said to have many ethnobotanical uses, among them is their use in gastrointestinal disorders. The aim of the work is to investigate the antidiarrhoeal and antiulcerogenic properties of the fruit fractions in rats. The crude ethanol extract was dissolved in distilled water and partitioned with n-hexane, chloroform, ethyl acetate and n-butanol. 50 mg/kg of each of the fractions was investigated for antidiarrhoeal and antiulcerogenic effects in rats. Yohimbine, cimetidine and propranolol were used as positive controls. The fractions of the fruit, showed varying significant (p<0.001), degrees of antidiarrhoeal and antiulcerogenic effects. It was observed that n-butanol and aqueous fractions had more effects on experimentally-induced ulcers and diarrhoea than chloroform and n-hexane fractions. The results on experimentally-induced ulcer and diarrhoeal models showed that the fractions possess these properties. These effects may in part be due to their actions on cholinergic and adrenergic mechanisms as well as their secondary metabolites.

**Keywords:** *Triumfetta cordifolia*, ulcer, diarrhoea, fractions, n-hexane, n-butanol, aqueous, chloroform.

**INTRODUCTION**

*Triumfetta cordifolia* A. Rich (Tiliaceae) is an erect, perennial herb with hairy but sometimes smooth stem commonly found in Florida, Bermuda, central and South America as well as in West Africa (Howard, 1989). In Nigeria, it is mainly found in the Southern region where it grows in sandy to clay soils with pH values between 5.5 to 8.0. In Akwa Ibom State, the root is used for venereal diseases, liver and kidney ailments while the fruit is usually macerated in water or local alcohol for the treatment of diarrhoea, dysentery, ulcer and delayed labour (Idongesit Linus personal communication). The acute toxicity of extracts of this plant was earlier reported by Onilede (2005). To determine the medicinal properties of *Triumfetta cordifolia* A. Rich fruit as acclaimed by the local people of Uyo Local Government Area of Akwa Ibom State, the antidiarrhoeal and antiulcerogenic properties of the fruit were investigated on experimentally-induced diarrhoea and ulcer models in rats.

**MATERIALS AND METHODS**

**Plant Material**

The fruits of *Triumfetta cordifolia* were collected from Afaha Oku in Uyo Local Government Area of Akwa Ibom State in October, 2005. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey of the Department of Botany, University of Uyo, where the specimen voucher was deposited.

**Preparation of Extract**

The fresh fruit (4 kg) was air-dried for four weeks after which it was reduced to powder using pistle and mortar. The pulverized plant material was then
macerated in glass tank using 99% ethanol for 72h. The crude ethanolic extract was then concentrated to dryness in vacuo at 40°C. 100g of the ethanolic extract of the fruit was dissolved in 80 ml of distilled water and partitioned successive with n-hexane, chloroform, ethyl acetate and n-butanol. Partitioning with each of these solvents was done at least three times to ensure maximum separation (partitioning) of the constituents of the extract. The fractions were evaporated to dryness in vacuo at 40°C, monitored by TLC and subjected to pharmacological tests.

**Phytochemical Screening**
Phytochemical screening of the fruit was done according to the methods of Clarke (1975), Odebiyi and Sofowora (1978) and Trease and Evans (1989). Tests for alkaloids, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones and terpenes were carried out.

**Animals**
Albino rats (weighing 140 – 200g) were used. They were housed in standardized environmental conditions (22 ± 2.5°C, relative humidity 80-85%, 12 h light / 12h dark cycle) and fed with standard rodent diet (Unfailing Veterinary Service, Uyo, Nigeria) and water ad libitum. Approval for the use of animals in the study was obtained from the Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

**Effect of *T. cordifolia* fruit fractions on intestinal propulsion in rats**
The effects of *T. cordifolia* fruit fractions on intestinal propulsion in unanaesthetised rats was tested using the charcoal meal method of Capasso et al., (1976); Nwafor and Bassey (2007). They were fasted for 24h but allowed free access to water. They were randomized and placed in six cages of six animals per cage. Group 1 was administered with 10% Tween 80 (5 ml/kg) by gavage; groups 2-5 were treated with 50 mg/kg (p.o) of aqueous, n-butanol, chloroform and n-hexane fruit fractions of *Triumfetta cordifolia* respectively. Group 6 was administered with 5 mg/kg of yohimbine subcutaneously. After 1 h, each rat received 2 ml castor (p.o) and was then observed for consistency of faecal matter and the frequency of defaecation for 3 h.

**Effect of *T. cordifolia* fruit fractions on castor oil – induced diarrhoea**
Diarrhoea was induced in rats according to the method of Nwodo and Alumanah (1991); Nwafor and Bassey (2007). Animals were fasted for 24h but allowed free access to water. They were randomized into six groups of six rats each. Group 1 (control) received 10% Tween 80 (5 ml/kg) by gavage; groups 2-5 were treated with 50 mg/kg (p.o) of aqueous, n-butanol, chloroform and n-hexane fractions of *Triumfetta cordifolia* respectively. Group 6 received yohimbine (5mg/kg.sc.). After 1 h, each rat received 2 ml of castor oil (p.o). Thirty minutes later, the rats were killed by cervical dislocation and exsanguinated, the small intestine was ligated at both pyloric sphincter and at the ileocaecal junctions. The entire small intestine was dissected out, its contents were expelled into a graduated measuring cylinder and the volume of the contents was recorded.

**Effect of *T. cordifolia* fruit fractions on castor oil – induced fluid accumulation**
Fluid accumulation was induced in rats according to the method of DiCarlo et al., (1994); Nwafor and Bassey (2007). Animals were fasted for 24h but allowed free access to water. They were randomized into six groups of six rats each. Group 1 (control) received 10% Tween 80 (5 ml/kg) by gavage; groups 2-5 were given 50mg/kg of aqueous, n-butanol, chloroform and n-hexane fractions (p.o) respectively. Group 6 received yohimbine (5mg/kg.sc.). After 1 h, each rat received 2 ml castor (p.o). Thirty minutes later, the rats were killed by cervical dislocation and exsanguinated, the small intestine was ligated at both pyloric sphincter and at the ileocaecal junctions. The entire small intestine was dissected out, its contents were expelled into a graduated measuring cylinder and the volume of the contents was recorded.

**Effect of *T. cordifolia* fruit fractions on indomethacin –induced gastric ulceration in rats**
The rats were randomized into six groups of six rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment (Alphin and Ward, 1967). Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na2CO3); groups 2-5 were pretreated with 50 mg/kg of aqueous, n-butanol, chloroform and n-hexane fractions of *Triumfetta cordifolia* (p.o) respectively while Group 6 received cimetidine (100 mg/kg p.o.) 1 h later, group 2-6 were administered with indomethacin. 4 h after indomethacin administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic
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examination was carried out with a hand lens and the presence of ulcer lesion was scored (Nwafor et al., 1996). Ulcer index (Ul), preventive ratio (PR) and degree of ulceration (DU) of each of the groups pretreated with extract fraction was calculated using standard methods (Zaidi and Mukerji, 1958; Nwafor et al., 2000).

**Effect of* T. cordifolia* fruit fractions on ethanol - induced gastric ulceration in rats**

The above procedure (as in indomethacin - induced ulceration) was repeated using ethanol (2.5 ml/kg). The rats were randomized into six groups of six rats each. Food was withdrawn 24 h and water 2 h before the commencement of experiment. Group 1 (control) received only ethanol (2.5 ml/kg p.o.; groups 2-5 were pretreated with 50 mg/kg (p.o.) of aqueous, n-butanol, chloroform and n-hexane fruit fractions of *Triumfetta cordifolia* respectively; group 6 received propranolol (40 mg/kg; p.o) dissolved in distilled water). 1 h later, groups 2-5 were administered with ethanol. Four hours after ethanol administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10 % formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of lesions were scored (Nwafor et al., 2005).

**Statistical analysis**

Multiple comparisons of Mean ± S.E.M were carried out by one way analysis of variance (ANOVA), followed by Tukey – Krammar multiple comparisons post test p=0.05.

**RESULTS**

**Small intestinal propulsion**

Different fractions of Triumfetta cordifolia inhibited the intestinal propulsion from 24.20 % to 48.2 %. The degree of inhibition was in this pattern: Aqueous > n -butanol > n- hexane > chloroform. These inhibitions were less than that exhibited by a standard drug (diphenoxylate 73.48 %)

**Castor oil – induced diarrhoea**

Extract fractions of Triumfetta cordifolia inhibited castor oil - induced diarrhoea in varying degrees ranging from n-butanol > chloroform fraction (40.91 %). The least inhibition exhibited by chloroform fraction was higher than that of yohimbine, an α2 receptor blocker.

<table>
<thead>
<tr>
<th>Treatment/route of administration (p.o)</th>
<th>Dose (mg/kg)</th>
<th>Mean intestinal length (cm)</th>
<th>Mean distance moved by charcoal (cm)</th>
<th>% inhabitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10% Tween 80)</td>
<td>-</td>
<td>99.00±3.19</td>
<td>63.30±1.63</td>
<td>36.06</td>
</tr>
<tr>
<td>n-hexane</td>
<td>50</td>
<td>97.87±5.54</td>
<td>60.02±0.23</td>
<td>38.67</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
<td>103.47±3.88</td>
<td>78.43±0.27</td>
<td>24.20</td>
</tr>
<tr>
<td>n-butanol</td>
<td>50</td>
<td>90.15±9.49</td>
<td>48.52±0.81</td>
<td>46.18</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>91.32±7.89</td>
<td>47.30±0.75</td>
<td>48.20</td>
</tr>
<tr>
<td>Diphenoxylate</td>
<td>5</td>
<td>94.27±2.79</td>
<td>25.00±0.57</td>
<td>73.48</td>
</tr>
</tbody>
</table>

Values represent Mean ± S. E. M. (n=6) p.o = per oral
Significance relative to control: *p<0.001)
n.s = not significant
Table 2:
Effect of different fractions of *Triumfetta cordifolia* on castor oil-induced diarrhoea in rats.

<table>
<thead>
<tr>
<th>Treatment/route of administration (p.o)</th>
<th>Dose (mg/kg)</th>
<th>Mean faecal matter</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10% Tween 80)</td>
<td>-</td>
<td>11.00±0.69</td>
<td>-</td>
</tr>
<tr>
<td>n-hexane</td>
<td>50</td>
<td>6.00±1.10*</td>
<td>45.45</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
<td>6.50±0.47*</td>
<td>40.91</td>
</tr>
<tr>
<td>n-butanol</td>
<td>50</td>
<td>2.50±0.37*</td>
<td>77.27</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>5.67±0.61*</td>
<td>48.45</td>
</tr>
<tr>
<td>Yohimbine (sc)</td>
<td>5</td>
<td>7.83±0.67</td>
<td>31.82</td>
</tr>
</tbody>
</table>

*Values represent Mean ± S. E. M. (n=6) p.o = per oral
Significance relative to control: *p<0.001
n.s = not significant*

Table 3:
Effect of different fractions of *Triumfetta cordifolia* on castor oil-induced intestinal fluid accumulation in rats.

<table>
<thead>
<tr>
<th>Treatment/route of administration (p.o)</th>
<th>Dose (mg/kg)</th>
<th>Mean volume of intestinal fluid (ml)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10% Tween 80)</td>
<td>-</td>
<td>3.32±0.10</td>
<td>-</td>
</tr>
<tr>
<td>n-hexane</td>
<td>50</td>
<td>2.92±0.01*</td>
<td>12.05</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
<td>3.68±0.19*</td>
<td>-10.84</td>
</tr>
<tr>
<td>n-butanol</td>
<td>50</td>
<td>2.75±0.01*</td>
<td>17.17</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>2.80±0.01*</td>
<td>15.66</td>
</tr>
<tr>
<td>Yohimbine(Sc)</td>
<td>5</td>
<td>3.15±0.10 ns</td>
<td>5.12</td>
</tr>
</tbody>
</table>

*Values represent Mean ± S. E. M. (n=6), p.o = per oral; Sc = subcutaneous.
Significance relative to control: *P< 0.001; n.s = not significant

Table 4:
Effect of different fractions of *Triumfetta cordifolia* on indomethacin-induced ulceration in rats.

<table>
<thead>
<tr>
<th>Treatment/route of administration (p.o)</th>
<th>Dose (mg/kg)</th>
<th>Degree of ulceration (%)</th>
<th>Ulcer index</th>
<th>Preventive ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (indomethacin)</td>
<td>60</td>
<td>100</td>
<td>10.75±0.03*</td>
<td>-</td>
</tr>
<tr>
<td>n-hexane</td>
<td>50</td>
<td>94.60</td>
<td>10.17±0.12*</td>
<td>5.40</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
<td>78.30</td>
<td>8.42±0.06*</td>
<td>21.67</td>
</tr>
<tr>
<td>n-butanol</td>
<td>50</td>
<td>38.10</td>
<td>4.08±0.03*</td>
<td>62.05</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>60.40</td>
<td>6.39±0.14*</td>
<td>40.56</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100</td>
<td>1.42</td>
<td>0.95±0.03*</td>
<td>99.16</td>
</tr>
</tbody>
</table>

*Values represent mean ± S. E. M. (n=6); p.o., = per oral.
Significance relative to control: *P<0.001.

**Castor oil – induced intestinal fluid accumulation**
The effect of different fractions of *Triumfetta cordifolia* on castor oil-induced intestinal fluid accumulation is as shown on Table 3. The fractions showed minimal inhibitions on castor oil-induced fluid accumulation which ranged from chloroform (-10.84 %) to n-butanol (17.17 %). Relative to yohimbine, the fractions showed greater inhibitory effects.

**Indomethacin - induced gastric ulceration**
The fractions significantly (p<0.001) protected the animals from indomethacin-induced ulceration. This protection was in polarity-dependent fashion. Aqueous (93.50 %) > n-butanol (61.90 %) > chloroform(21.70 %) > n-hexane (5.40 %) (Table 4).
Ethanol induced gastric ulceration
Triumfetta cordifolia fractions pretreatment on ethanol-induced gastric ulceration, showed a non-polar dependent protection. This protection was statistically significant (p<0.001) Table 5.

<table>
<thead>
<tr>
<th>Treatment/route of administration (p.o)</th>
<th>Dose (mg/kg)</th>
<th>Degree of ulceration (%)</th>
<th>Ulcer index</th>
<th>Preventive ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ethanol)</td>
<td>-</td>
<td>100</td>
<td>4.00±0.012*</td>
<td>-</td>
</tr>
<tr>
<td>n-hexane</td>
<td>50</td>
<td>75.00</td>
<td>3.00±0.02*</td>
<td>25.00</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
<td>75.00</td>
<td>3.00±0.03*</td>
<td>25.00</td>
</tr>
<tr>
<td>n-butanol</td>
<td>50</td>
<td>37.50</td>
<td>1.50±0.02</td>
<td>62.50</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>55.00</td>
<td>2.20±0.03*</td>
<td>45.00</td>
</tr>
<tr>
<td>Propranolol</td>
<td>40</td>
<td>62.50</td>
<td>2.50±0.50</td>
<td>37.50</td>
</tr>
</tbody>
</table>

Values represent mean ± S. E. M. (n=6); p.o = per oral. Significance relative to control: *P< 0.001.

Reserpine – induced gastric ulceration
The fractions significantly protected the rats from reserpine-induced ulceration in rats (p<0.001). This protection is in non-polar dependent manner. (Table 6).

<table>
<thead>
<tr>
<th>Treatment/route of administration (p.o)</th>
<th>Dose (mg/kg)</th>
<th>Degree of ulceration (%)</th>
<th>Ulcer index</th>
<th>Preventive ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (reserpine)</td>
<td>8</td>
<td>100.00</td>
<td>5.08±0.015*</td>
<td>-</td>
</tr>
<tr>
<td>n-hexane</td>
<td>50</td>
<td>91.90</td>
<td>4.67±0.28a</td>
<td>8.07</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
<td>68.90</td>
<td>3.50±0.12b</td>
<td>31.10</td>
</tr>
<tr>
<td>n-butanol</td>
<td>50</td>
<td>25.20</td>
<td>1.28±0.13b</td>
<td>74.80</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>68.90</td>
<td>3.50±0.01b</td>
<td>31.10</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100</td>
<td>90.20</td>
<td>4.58±0.22b</td>
<td>9.84</td>
</tr>
</tbody>
</table>

Values represent Mean ± S. E. M. (n = 6); p.o = per oral. Significance relative to control: aP<0.01; bP<0.001.

DISCUSSION

The extract fractions of *Triumfetta cordifolia* inhibited in non-uniform manner intestinal propulsion, castor oil - induced diarrhoea and fluid accumulation in rats. These degrees of inhibition were in this descending order: Aqueous > n-butanol > n-hexane > chloroform for intestinal propulsion; n-butanol > aqueous > n-hexane > chloroform for castor oil-induced diarrhoea, and n – butanol > aqueous > n-hexane > chloroform for intestinal fluid accumulation respectively.

Spontaneous movement is inherent in non-vascular smooth muscle (myogenic) as a fundamental characteristics and is modified in - situ by hormonal and neuronal influences (Golenhofen, 1970; Nwafor et al., 2005). Spontaneous movement of gastrointestinal muscle is regulated by the cycles of depolarization and repolarization. It is known that acetylcholine binds to muscarinic receptors on smooth muscle, thereby triggering a sequence of events resulting ultimately in smooth muscle contraction (Nwafor and Okwuasaba, 2005). That the fractions had inhibitory effects on the spontaneous contractility of gastrointestinal muscle indicated in part that it may be mediating its effect through cholinergic mechanism.

Drugs affecting motility, frequency and consistency of diarrhoea also affect secretion (DiCarlo et al., 1994; Nwafor and Okwuasaba, 2001). The intraluminal fluid accumulation induced by castor oil was enhanced by yohimbine, an α2 - adrenergic blocker, suggesting the involvement of adrenergic mechanism.

All the results therefore suggest that the extract fractions produced an inhibitory action on gastrointestinal functions, motility and secretion and this is partly mediated by muscarinic and adrenergic receptor systems. The extract fractions also inhibited in varying degrees indomethacin, ethanol and reserpine-induced ulcerations in rats. The degrees of inhibition are in this descending order: n-butanol > aqueous > chloroform > n – hexane for indomethacin; n-butanol > aqueous > chloroform Ξ n-hexane for ethanol, and n-butanol > aqueous Ξ chloroform > n-butanol for reserpine respectively.

Indomethacin is a known ulcerogen especially in an empty stomach (Bhargava et al., 1973), the evidence of indomethacin - induced ulceration is mostly on the glandular (mucosal) part of the stomach (Evbuonwa
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and Bolarinwa, 1990; Nwafor and Bassey 2007). 'Although the mechanisms underlying the ulcerogenicity of indomethacin are not completely understood, it has been known that inhibition of prostaglandin synthesis may be important (Hiruma – Lima et al., 2006; Vane, 1971). This view is supported by the fact that prostaglandins normally serve as protective functions in stomach by maintaining gastric microcirculation (Vane, 1971; Ferreira and Vane, 1974) and causes gastric secretion of bicarbonate (Garner et al., 1979) and mucus (Menguy and Desbailllets, 1967).

Numerous studies have demonstrated that the effect of absolute ethanol on the gastric mucosa is related to production of free radicals, the increase of lipoperoxidation and the decrease of the levels of non protein SH compounds in the gastric mucosa (Mizui and Doteuchi, 1986). It is also established that disturbances in gastric secretion, damage to the gastric mucosa, alterations in permeability, gastric mucus depletion and free radical production are observed after the administration of ethanol (Salim, 1990).

Reserpine in said to elucidate ulcers through the mobilization of superoxide and hydroxyl radicals (oxygen – derived free radicals (Salim, 1989). The fractions reduced reserpine-induced ulceration in rats. It has been proposed that mucosal protection induced by non - prostanoitd compounds may be mediated through the mobilization of endogenous prostaglandins (Konturek et al., 1989). It is possible that one of the mechanisms of antiulcerogenic effects of the extract may be due to its ability to mobilize prostaglandins in gastric mucosa by increasing its microcirculation or through the inhibition of oxygen – derived free radicals formation in the gastric mucosa. All the results therefore suggest that the extract fractions produced inhibitory action on gastrointestinal functions, motility and secretion, and this effect is mediated in part through cholinergic and adrenergic mechanisms.

The phytochemical analysis of the extract fractions revealed the presence tannins, alkaloids, flavonoid and cardiac glycosides. Flavonoids and tannins are some of most important botanical compounds with anti-ulcer and gastroprotective activities (Wahida et al., 2007). Different mechanisms have been proposed to explain the gastroprotective effect of flavonoids, including an increase in mucosal prostaglandin content and a decrease in histamine secretion from mast cells by the inhibition of histidine decarboxylase. Flavonoids are free radical scavengers that are known to play an important role in preventing ulcerative and erosive lesions of gastrointestinal tract (Borrelli and Izzo, 2000; Konan and Bacchi, 2007) Tannins with its protein precipitating and vasoconstrictory effects could be advantageous in preventing ulcer development (Aguwa and Nwako, 1988). Tannins being astrigent, may have precipitated microproteins on site of the ulcer thereby forming an impervious protective pellicle over the lining to prevent absorption of toxic substances and resist the attack of proteolytic enzymes (Nwafor et al., 1996).

In conclusion therefore, the fractions may in part be mediating their actions through the cholinergic and adrenergic mechanisms or through the action of its active metabolites.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Miss Sifonobong Akpan, Mr. Nsikan Udo and Mr. Aniefiok Ukpong, all of the Department of Pharmacology & Toxicology, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

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