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## Research Article

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## PHYTOCHEMICAL CONTENTS AND EFFECTS OF ETHANOL EXTRACTS OF *Centaurea senegalensis* D.C. AND *Vitellaria paradoxa* C.F. GAERTN. ON CASTOR OIL-INDUCED DIARRHOEA IN WISTAR RATS

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## ABSTRACT

Medicinal plants have been used throughout human history. Plants can synthesize a variety of chemical compounds that perform important biological functions and serve as a defense against predators. Being one of the leading causes of preventable death in developing countries, diarrhoea mainly affects children and infants. This work was aimed at screening the extractives phytochemically and estimating their antidiarrhoeal efficacy using standard procedures of the aerial part of *Centaurea senegalensis* collected from the University of Maiduguri Campus and the rootbark of *Vitellaria paradoxa* obtained from Hawul Town, Borno State. The results of phytochemical screening of both extracts showed the presence of alkaloids, cardiac glycosides, flavonoids, and tannins. Terpenoids were present only in *C. senegalensis*; anthraquinone and saponins were only found in *V. paradoxa*. The acute lethal dose of both extracts was determined as 2154.10 mg/kg b. wt. in rats. The extracts were evaluated for antidiarrhoeal activity employing the castor oil-induced diarrhoeal model in Wistar rats. *C. senegalensis* and *V. paradoxa*, respectively, showed significant dose-dependent reduction ( $p < 0.05$ ) in the percentage inhibition of the wet faeces at 300, 600, 1200 mg/kg b. wt. as: 71.23 and 57.59%; 96.95 and 83.31%; 100 and 98.50%. Diphenoxylate HCl also showed 100% protection. Thus, the great potency of crude extracts against diarrhoea could be ascribed to the presence of bioactive compounds in both extractives. Research into isolation, structure elucidation/characterization of the compound(s) with more efficacy is strongly recommended. Therefore, this study supports the use of these plants in African Traditional Medicine as remedy against diarrhoea.

**Keywords:** Asteraceae, Castor oil, *Centaurea*, Diarrhoea, Phytochemical, Sapotaceae *Vitellaria*

## INTRODUCTION

The discovery of medicinal properties in plants can be attributed to the empirical knowledge acquired by early humans through their interactions with various substances in their environment. As they explored their surroundings in search of food, they likely observed the palatability and nutritional value of certain materials, as well as the adverse effects caused by others [1]. The use of plant-based remedies in healthcare has a long history, dating back to ancient times. The advent of orthodox medicine, driven by advancements in science and civilization, initially eclipsed the traditional reliance on plant-based and natural remedies for healing [1,2]. In

Africa, the widespread use of traditional medicine, largely composed of medicinal plants, has been attributed to cultural and economic factors. Recognizing this, the World Health Organization (WHO) encourages African member states to promote and integrate traditional medical practices into their healthcare systems. Plants contain complex mixtures of phytochemicals, also known as secondary metabolites, which can interact individually, additively, or synergistically to produce beneficial health effects. Notably, medicinal plants differ from pharmacological drugs in that they often contain multiple chemicals that work together catalytically and synergistically to produce a combined effect that exceeds the total activity of their separate constituents [3,4]. According to the World Health Organization (WHO), a significant proportion of the global population, approximately 80%, relies on traditional medicine as a primary source of therapy. The versatility of plants in synthesizing a diverse array of bioactive compounds is well-documented, with over 12,000 such compounds isolated to date, representing a mere fraction of the estimated total [5,6]. These phytochemicals interact with the human body through mechanisms analogous to those of conventional pharmaceuticals, modulating various biological processes to exert their therapeutic effects. Consequently, herbal medicines share similarities with conventional drugs in terms of their pharmacological profiles, possessing both beneficial and potentially harmful properties. The use of plants in medicine encompasses a broad range of applications, including the maintenance and enhancement of physical, mental, and spiritual well-being, as well as the treatment of specific diseases and disorders [7]. Diarrhoea with its accompanying faecal urgency and incontinence stems from an imbalance between fluid absorption and secretion in the intestine, along with increased intestinal motility. [1,8]. Acute diarrhoea disease is one of the principal causes of infant deaths in developing countries [8,9]. Diarrhoeal diseases are a significant global health burden, accounting for an estimated 443,832 deaths in children under the age of five and an additional 50,851 deaths in children aged five to nine years [10]. The clinical presentation of diarrhoeal patients typically includes frequent episodes of loose or watery stools, often exceeding three times per day, accompanied by abdominal pain and cramping [11,12]. In the context of African Traditional Medicine, various plant species, such as *Vitellaria paradoxa*, have been traditionally employed to treat or manage diarrhoeal conditions. These plants have been used to alleviate symptoms and potentially address the underlying causes of diarrhoeal diseases, highlighting the importance of exploring their phytochemical constituents and potential therapeutic properties. *Centaurea senegalensis* (Syn. *Launaea taraxacifolia*) is an annual branched scabrid herb, locally known in English as Wild Lettuce, *Namiji dayi* in Hausa, *Kamgabi* in Kanuri, and *Sal kamga* in Babur-Bura languages. *Centaurea senegalensis* is valued in the indigenous system of medicine in Nigeria as a crude drug for the treatment of stomachache, kidneys, micturition, diuresis, dropsy, gout and pains [13]. Pharmacological study of *Launaea taraxacifolia* confer it antioxidant, hypolipidemic, hypotensive, hypoglycemic and free radical scavenging properties [14].

*Vitellaria paradoxa*, commonly known as the shea tree, or *Vitellaria*, is a tree of the Sapotaceae family. It is the only species in the genus *Vitellaria* and is indigenous to Africa [9]. The shea tree is a traditional African food plant. The plant is called *Shea-butter tree* in English, *Lulu* in Arabic, *Kadanya* in Hausa, *Okwuma* in Igbo, and *Akumalapa* in Yoruba languages [15]. In Africa, ground roots (root bark) and bark (stem bark) are used to treat diarrhoea, jaundice, and stomach ache, the bark infusion is used against dysentery [16]. Traditionally the plant is used to treat different diseases such as: Diarrhoea, dysentery, helminthic and other G.I.T infections, skin diseases and wound infections [17]. This study is aimed at validating scientifically the use of these plants extract in folklore medicine adopting castor oil-induced diarrhoeal model in rats.



**Figure 1.** Aerial view of: (a). *Centaurea senegalensis* (b). *Vitellaria paradoxa* tree

## MATERIALS AND METHODS

### Sample Collection and Identification

Fresh aerial part of *Centaurea senegalensis* was collected from the University of Maiduguri, Campus, and the root bark of *Vitellaria paradoxa* was collected from Hawul Local Government Area, Borno State, Nigeria in December 2018. The herbarium samples were identified and authenticated by a Taxonomist in the Department of Botany, Faculty of Life Sciences, University of Maiduguri, Nigeria, where the voucher specimen numbers: 18.CH.01A and 16.CH02A were both deposited. The plants were then separately air-dried in the shade and afterward ground into coarse powder using a wooden mortar and pestle.

### Procedure for Plant Extraction

Three hundred grams (300 g) of the air-dried powdered plant materials were independently extracted with 85% ethanol in water. The plant materials were independently extracted using refluxing method for a period of four hours; the extractives were individually filtered and the filtrate was air-dried under reduced pressure on an evaporating dish until dryness. The combined extracts were independently concentrated to obtain a gummy crude extract. Each crude extract was then subjected to qualitative phytochemical screening, acute toxicity study, and anti-diarrhoeal activity.

### Phytochemical Screening

The phytochemical evaluation was carried out using standard methods of analysis as earlier described by several authors [18-21].

### Experimental Animals

All experiments on laboratory animals followed the standard procedures for the treatment of animals according to the International Guiding Principles for Biomedical Research involving Animals [22] CIOMS, as certified by the Animal Ethics Committee of the Faculty of Pharmacy, University of Maiduguri with approval number FP/022018/TETFP03 and animals handled in compliance with the international guiding principles for biochemical research involving animals. Thirty (30) healthy Wistar strain rats of either sex used for this study, weighing between 200-250 g, were obtained from Animal House, Department of Veterinary Pharmacology, Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri. They were kept for two weeks in well-ventilated plastic cages in the Department of Pharmacology, Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Maiduguri, to acclimatized to the laboratory conditions before the experiment. The rats were fed with growers' mash (Sanders Feeds Nig. Ltd.) and allowed water *ad libitum*.

### Acute Toxicity Study

The oral acute toxicity study was conducted in accordance with Lorke's method [23]. The extracts were separately dissolved in buffered saline solution and given via intragastric route (*p.o*). All animals were observed on the day of treatment and surviving animal was monitored for signs of acute toxicity for 24 hours. Recovery and weight gain were seen as indications of having survived the acute toxicity.

The value of the LD<sub>50</sub> was calculated by the formula:  $LD_{50} = \sqrt{(D_0 \times D_{100})}$

D<sub>0</sub> = Highest value that showed no mortality

D<sub>100</sub> = Lowest dose that produced mortality.

### Castor Oil-Induced Diarrhoea in Rats

The castor oil-induced diarrhoea model was carried out using the method described by Shoba and Thomas [24]. The rats fasted for 12 hours prior commencement of the study and were randomly divided into five groups each containing three rats. The grouping is as follows: Group 1 was treated with 300 mg/kg b. wt. of extract, Group 2 was treated with 600 mg/kg b. wt. of extract, Group 3 was treated with 1200 mg/kg b. wt. of the extracts, Group 4 was treated with Normal saline 5 mL/kg b. wt. and Group 5 was treated with Diphenoxylate 5 mg/kg b. wt. After 30 minutes post-treatment, castor oil (2 mL/rat) was administered to all the groups. All treatments were given intragastrically. The animals were then placed in individual cages on a clean white paper. Four hours post-castor oil administration, the cages were inspected for the presence of characteristic diarrhoea droppings; absence of which was regarded as protection [23]. Diarrhoea was seen as the fluid material that stained the paper beneath the cages. The Percentage Protection (PP) was calculated as follows:

Equation 1: Percentage of wet faeces inhibition =  $(T_0 - T_1 / T_0) \times 100\%$

T<sub>0</sub> = number of wet faeces in control group, T<sub>1</sub> = number of wet faeces in test group.

The severity percentage of diarrhoea was calculated as:

Equation 2: Severity of diarrhoea =  $(\text{Diarrhoeal faeces (test group)} / \text{Total faeces}) \times 100\%$  [25].

### Statistical Analysis

The Statistical analysis was carried out by one-way ANOVA and the analyses are presented in Table 3. The significance of the difference between means was determined by the student-Newman-Keuls multiple comparisons test. The results were expressed as Mean $\pm$ SE. Comparisons between groups were made and results on castor oil-induced diarrhoea were analyzed and were also regarded as significant when  $p < 0.05$ .

### RESULTS AND DISCUSSION

The extracts from the aerial part of *Centaurea senegalensis* and the rootbark of *Vitellaria paradoxa* yielded 36.0 g (12.05%) and 65.3 g (221.77%) respectively. The results of the phytochemical screening of the hydroethanol aerial part extract of *Centaurea senegalensis* and that of the rootbark of *Vitellaria paradoxa* are presented in Table 1. The results of the acute oral toxicity ( $LD_{50}$ ) level of the aerial part extract of *Centaurea senegalensis* and the rootbark extract of *Vitellaria paradoxa* were each calculated as 2154.10 mg/kg b. wt. as shown in Tables 2a,b below; while the results of the castor oil induced-diarrhoeal effect of the hydroethanol aerial part extract of *Centaurea senegalensis*, that of the root-bark extract of *Vitellaria paradoxa* and diphenoxylate on albino rats are shown in Table 3.

**Table 1.** Phytochemical contents of the ethanol extracts of the aerial part of *Centaurea senegalensis* and rootbark of *Vitellaria paradoxa*

| Phytochemicals     | <i>Centaurea senegalensis</i> | <i>Vitellaria paradoxa</i> |
|--------------------|-------------------------------|----------------------------|
| Alkaloids          | +                             | +                          |
| Anthraquinones     | -                             | +                          |
| Cardiac glycosides | +                             | +                          |
| Flavonoids         | -                             | +                          |
| Saponins           | NT                            | +                          |
| Tannins            | +                             | +                          |
| Terpenoids         | +                             | -                          |

**Key:** + = Present, - = Absent, NT = Not tested.

**Table 2a.** Acute toxicity level of the crude aerial part extract of *Centaurea senegalensis paradoxa* administered orally to rats

| Phase I |         |                     |              | Phase II |       |                     |              |
|---------|---------|---------------------|--------------|----------|-------|---------------------|--------------|
| S/N     | Group   | Dose (mg/kg b. wt.) | No. of death | S/N      | Group | Dose (mg/kg b. wt.) | No. of death |
| 1       | A       | 10                  | 0            | 1        | I     | 1600                | 1            |
| 2       | A       | 10                  | 0            | 2        | II    | 2900                | 0            |
| 3       | A       | 10                  | 0            | 3        | III   | 5000                | 0            |
| 4       | B       | 100                 | 0            |          |       |                     |              |
| 5       | B       | 100                 | 0            |          |       |                     |              |
| 6       | B       | 100                 | 0            |          |       |                     |              |
| 7       | C       | 1000                | 0            |          |       |                     |              |
| 8       | C       | 1000                | 0            |          |       |                     |              |
| 9       | C       | 1000                | 1            |          |       |                     |              |
| 10      | Control | N/S (1ml/kg)        | 0            |          |       |                     |              |
| 11      | Control | N/S (1ml/kg)        | 0            |          |       |                     |              |
| 12      | Control | N/S (1ml/kg)        | 0            |          |       |                     |              |

N/S = Normal saline

**Table 2b.** Acute toxicity level of the crude rootbark extract of *Vitellaria paradoxa* administered orally to rats

| Phase I |         |                     |              | Phase II |       |                     |              |
|---------|---------|---------------------|--------------|----------|-------|---------------------|--------------|
| S/N     | Group   | Dose (mg/kg b. wt.) | No. of death | S/N      | Group | Dose (mg/kg b. wt.) | No. of death |
| 1       | A       | 10                  | 0            | 1        | I     | 1600                | 1            |
| 2       | A       | 10                  | 0            | 2        | II    | 2900                | 0            |
| 3       | A       | 10                  | 0            | 3        | III   | 5000                | 0            |
| 4       | B       | 100                 | 0            |          |       |                     |              |
| 5       | B       | 100                 | 0            |          |       |                     |              |
| 6       | B       | 100                 | 0            |          |       |                     |              |
| 7       | C       | 1000                | 0            |          |       |                     |              |
| 8       | C       | 1000                | 0            |          |       |                     |              |
| 9       | C       | 1000                | 1            |          |       |                     |              |
| 10      | Control | N/S (1ml/kg)        | 0            |          |       |                     |              |
| 11      | Control | N/S (1ml/kg)        | 0            |          |       |                     |              |
| 12      | Control | N/S (1ml/kg)        | 0            |          |       |                     |              |

N/S = Normal saline

**Table 3.** Effect of aerial part extract of *Centaurea senegalensis* and rootbark extract of *Vitellaria paradoxa* on castor-oil induced diarrhoea in Wistar rats 5mL/kg b. wt..

| Treatment (mg/kg b. wt.)     | Mean no. of unformed faeces      |                              | Percentage protection (%) |                    |                        | Percentage severity (%) |
|------------------------------|----------------------------------|------------------------------|---------------------------|--------------------|------------------------|-------------------------|
|                              | <i>C. senegalensis</i> (Mean±SE) | <i>V. paradoxa</i> (Mean±SE) | <i>C. senegalensis</i>    | <i>V. paradoxa</i> | <i>C. senegalensis</i> | <i>V. paradoxa</i>      |
| 300                          | 6.33±0.58 <sup>a</sup>           | 9.33±1.15 <sup>a</sup>       | 71.23                     | 57.59              | 9.83                   | 14.50                   |
| 600                          | 0.67±0.58 <sup>b</sup>           | 3.67±2.52 <sup>b</sup>       | 96.95                     | 83.31              | 1.04                   | 5.70                    |
| 1200                         | 0.00±0.00 <sup>b</sup>           | 0.33±0.58 <sup>c</sup>       | 100                       | 98.50              | 0.00                   | 0.52                    |
| Normal saline (5 mL)         | 22.00±3.00 <sup>c</sup>          | 22.00±3.00 <sup>d</sup>      | 0.00                      | 0.00               | 34.20                  |                         |
| Diphenoxylate HCl (5 mg)     | 0.00±0.00 <sup>b</sup>           | 0.00±0.00 <sup>e</sup>       | 100                       | 100                | 0.00                   |                         |
| Total no. of unformed faeces | 64.33                            |                              |                           |                    |                        |                         |

Means with different superscripted letters along the same column are significantly different ( $p < 0.05$ ), CO = Castor oil,  $n = 3$ .

The One-way ANOVA was used for the analysis; difference between means was determined by student-Newman-Keuls multiple comparisons test.

The compounds found in plants are of many kinds, but most are in four major biochemical classes: the alkaloids, glycosides, polyphenols, and terpenes [26]. The results of phytochemical screening of both the aerial part of *Centaurea senegalensis* and rootbark of *Vitellaria paradoxa* showed the presence of alkaloids, cardiac glycosides, flavonoids, and tannins. Terpenoids were present in *C. senegalensis*; anthraquinone and saponins were only found in *V. paradoxa*. These phytochemicals could be responsible for the antidiarrhoeal activities displayed by the extracts. The presence of these secondary metabolites: alkaloids, saponins, sterols, and terpenes is also responsible for the antidiarrhoeal property of the extracts under consideration [27]. Other Workers have attributed the antidiarrhoeal properties of some medicinal plants due mainly to their phytochemical constituents like alkaloids, flavonoids, phytosterols, and tannins [1,10,28,29].

The results of oral acute toxicity levels ( $LD_{50}$ ) of both the aerial part and rootbark ethanol extracts of *C. senegalensis* and *V. paradoxa* were calculated as 2154.07 mg/kg b. wt. in Wistar rats.

Since research has shown that castor oil is often administered orally to induce diarrhoea in rats [30], this study used the castor oil-induced diarrhoea model for the evaluation of the antidiarrhoeal property of *C. senegalensis*

and *V. paradoxa*. One of the active components of castor oil (ricinoleic acid) is responsible for its diarrhoea-inducing property. It stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandins, which in turn stimulate motility and secretion. However, like other opioid drugs, diphenoxylate acts by slowing intestinal contractions, allowing the body to consolidate intestinal contents and prolong transit time. Thus, allowing the intestine to draw moisture out of them at a normal or high rate and therefore stop the formation of loose and liquid stools.

Similar to our earlier findings [1], these results showed that the extractives met some part of the criteria for acceptance of a drug as an antidiarrhoeal [1]; these criteria include inhibition of the production of wet or unformed faeces in animals and inhibition of gastrointestinal propulsive action [30].

*C. senegalensis* and *V. paradoxa* as medicinal plants are not exceptional; they provided significant protection ( $P < 0.05$ ) in all the doses of the extracts: 300, 600, and 1200 mg/kg showed antidiarrhoeal activity demonstrating 71.23, 96.95, and 100%; and 57.59, 83.31, and 98.50% respectively. More so, the percentage protection exhibited by *C. senegalensis* at 1200 mg/kg b. wt. to that of the standard drug (diphenoxylate HCl 5 mg/kg b. wt.), which also demonstrated 100% protection. Even though the precise mechanism of hyper-secretion affected by these extracts is unknown to the authors; however, the reduction in faecal wetness strongly suggests that it may act by inhibiting/suppressing gastrointestinal hyper-secretion in rats [25]. Relatively, the extract of *C. senegalensis* at the highest dosage of 1200 mg/kg b. wt. and the standard drug (5 mg/kg b. wt.) both exhibited the least severity index of 0.00%. The antidiarrhoeal activity of the ethanol extracts of *C. senegalensis* and *V. paradoxa* induced model could not be unrelated to the phytochemicals therein, and as well having a similar mechanism of anti-secretory actions and re-establishment of osmotic balance in rats [31].

## CONCLUSION

Conclusively, the extracts can be said to be moderately safe and that both extracts have similar phytochemical contents with relatively good antidiarrhoeal effects at the tested doses. However, the extract of *Centaurea senegalensis* at the highest dosage can be compared with the standard drug (diphenoxylate hydrochloride). Therefore, the use of these plants in folklore medicine in the management of diarrhoea and diarrhoeal related symptoms is scientifically validated. Moreover, there is need for the isolation identification and characterization of the bioactive compound(s) responsible for this pharmacological activity.

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