



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_12293
Title of the Manuscript:	INFLUENCE OF METHANOLIC LEAF EXTRACTS OF <i>Vernonia amygdalina</i> ON CASTOR OIL INDUCED DIARRHEA IN RATS
Type of article	Original Research Article

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
Please Authors should format their manuscript with the TEMPLATE provided by the journal. It is very easy and nice. Please take note of the fonts, the font sizes, and the arrangements of text in the template. AUTHORS WROTE "NOTED" TO SOME COMMENTS MADE EARLIER BUT DID NOT DO ANYTHING ABOUT IT. PLEASE ADDRESS THOSE COMMENTS Repetitions should be avoided. There are still too many repetitions. It does not make the article interesting to read For instance In the abstract Diarrhea is a common cause of death in developing countries and second most common cause of infant deaths worldwide. The effect of a methanolic leaf extract of Bitter leaf (<i>Vernonia amygdalina</i>) and Lemon grass (<i>Cymbopogon citratus</i>) on Castor oil-induced diarrhea in male albino rats was investigated. Fifty (50) white male albino rats ranging between 120-227g were induced with castor oil for development of diarrhea. They were divided into two sub-groups of twenty-five (25) subsequently divided into groups; Five groups of rats with induced diarrhea were treated with 2 mg/kg loperamide, 100, 200, and 400 mg/kg <i>Vernonia amygdalina</i> and <i>Cymbopogon citratus</i> extract, or vehicle respectively. The cumulative frequencies of wet and formed stools were noted on the 3rd - 6th hour. It was observed that 400mg/kg body weight of the extracts reduced the fecal spot compared to 200mg/kg and 100mg/kg body weight. <i>Vernonia amygdalina</i> and <i>Cymbopogon citratus</i> extracts were found to be non-toxic. The phytochemical screening of the methanolic extract of <i>Vernonia amygdalina</i> and <i>Cymbopogon citratus</i> revealed the presence of some bioactive components which may be responsible for the antidiarrhea properties observed in this study. The above findings suggest that the methanolic extracts of <i>Vernonia amygdalina</i> and <i>Cymbopogon citratus</i> contain active constituents that have antidiarrhea activities that may lead to its use in treating diarrhea. In the methodology Lemon grass (<i>Cymbopogon citratus</i>) leaves was obtained from Mararaba market, a suburb of Abuja, Nigeria, it was identify and authenticated by Mrs Tarfa D. Florence, The Head of Department, Medicinal Chemistry and Quality Control, National Institute of Pharmaceutical Research and Development, Abuja, Nigeria. Voucher specimen number of the plant NIPRD/15/014. These leaves were dried for fifteen days at ambient laboratory temperature (20-28°C). It was milled to a fine powder using commercial blender (Blendtec), and stored in the dark at laboratory temperature in closed containers until required. The powdered sample of the leaves of <i>Cymbopogon citratus</i> were weighed using electric balance 24.1g <i>Cymbopogon citratus</i> were used for the methanolic extraction, using soxhlet apparatus. The Methanolic extract was dried invacuo using rotary evaporator. Bitter leaf (<i>Vernonia amygdalina</i>) was obtained from Masaka market a suburb of Abuja, Nigeria, it was	NOTED I HAVE DONE ALL THE CORRECTIONS THE REVIEWER SUGGESTED IN THE MANUSCRIPT NOTED



SDI FINAL EVALUATION FORM 1.1

identify and authenticated by Mrs Tarfa D. Florence, The Head of Department, Medicinal Chemistry and Quality Control, National Institute of Pharmaceutical Research and Development, Abuja, Nigeria. Voucher specimen number of the plant is NIPRD/09/014. These leaves were dried for fifteen days at ambient laboratory temperature (20-28°C). It was milled to a fine powder using commercial blender (Blendtec), and stored in the dark at laboratory temperature in closed containers until required. The powdered sample of *Vernonia amygdalina* leaves were weighed using electric balance. 22.8g *Vernonia amygdalina* were used for the methanolic extraction, using soxhlet apparatus. The Methanolic extract was dried *invacuo* using rotary evaporator.

Table 1: Experimental design

GROUP	DESCRIPTION	TREATMENT (<i>Cymbopogon citratus</i>)
A	Control	Normal saline
B	Standard control	Administered with 2 mg/kg body weight of loperamide
C	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 100mg/kg body weight extract for 6 hours
D	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 200mg/kg body weight extract for 6 hours
E	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 400mg/kg body weight extract for 6 hours

Table 2: Experimental design

GROUP	DESCRIPTION	TREATMENT (<i>Vernonia amygdalina</i>)
A	Control	Normal saline
B	Standard control	Administered with 2 mg/kg body weight of loperamide
C	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 100mg/kg body weight extract for 6 hours
D	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated

NOTED

NOTED AND CORRECTED



SDI FINAL EVALUATION FORM 1.1

		with 200mg/kg body weight extract for 6 hours
E	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 400mg/kg body weight extract for 6 hours

Key words: It would be much more preferred if the key words are prominent words in the study but are not in the title. This widens the scope for search of this article.

2.2 Phytochemical screening

I think you do not need all these. It has been summarized by your earlier statement. Please delete

Test for alkaloids

1.0ml of the methanolic extracts, few drops of dragendorf reagent were added. An orange colour developed, indicating the presence of alkaloid.

Test for Flavonoids

1.0ml of the methanolic extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids

Test for Tannins

Ferric chloride test:-1.0ml of the methanolic extract was diluted with 4.0ml of distilled water (in ratio 1:4) and few drops of 10% ferric chloride solution were added. Blue or green precipitate or colouration formed, indicating the presence of tannins.

Test for saponin

To 0.5ml of the methanolic extract, 5.0ml distilled water was added vigorously for 2 minutes. Then few drops of olive oil were added. Formation of emulsion showed the presence of saponin.

Test for steroids

Two ml of acetic anhydride was added to 0.5ml of the methanolic extracts with 2 ml H₂SO₄. The colour changed from violet to blue indicating the presence of steroids.



SDI FINAL EVALUATION FORM 1.1

Test for terpenoids (Salkowski test)

0.5ml of the methanolic extracts was mixed in 2 ml of chloroform and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inner face was formed indicating the presence of terpenoids.

Test for Anthraquinones:

0.5ml of the methanolic extract was boiled with 10ml H₂SO₄ and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for a greenish-yellow for *Vernonia amygdalina* and *Cymbopogan citratus* extracts.

Test for Cardiac Glycolyses (Keller-Killani Test):

1ml of the methanolic extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated deoxysugar characteristics of cardenolides.

What do authors mean by “Infection of Diarrhea”. Do they mean “induction of Diarrhea”?

Separate units from figures e.g. 100mg/kg should be 100 mg/kg

“third, fourth and fifth group group received 100mg/kg, 200mg/kg and 400mg/kg methanol extract of *Vernonia amygdalina* and *Cymbopogan citrates* (i.p)”. Were the drugs given concurrently or these were two separate treatments? If it is two separate treatments, please correct it in the whole write-up

Results
 Avoid repetition

Phytochemical screening. Tables 3 &4 can be combined into one table
 Number of fecal spot: Table 5 & 6 can be combined into one table

Conclusion
 Delete this from the conclusion: “The methanolic extract of *Vernonia amygdalina* and *Cymbopogon citratus* neutralized the ricinoleic acid responsible diarrhea inducing property of castor oil under the normal treatment period of diarrhea. Therefore, it can be concluded that “

Modify this portion of the conclusion to be more definite: “*Vernonia amygdalina* and *Cymbopogon citratus*



SDI FINAL EVALUATION FORM 1.1

may possess anti-diarrhea properties”.

Add this to the conclusion: The extract would therefore be effective in the management of diarrhea