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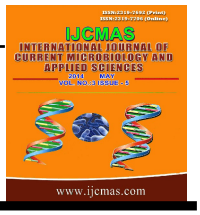

Original Research Article

Phytochemical Constituents and Effect of Combined Ethanolic Leaf Extract of *Costus afer* and *Cleome Rutidosperma* on Lipid Profile and Some Haematological Parameters in Wistar Rats

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Keywords

Costus afer, Cleome rutidosperma haematological parameters, lipid profile

A B S T R A C T

In the present study *costus afer* (CA) and *cleome rutidosperma* (CR) was screened for the of presence of plant products and the effect of combined ethanolic leaf extract of *costus afer* and *cleome rutidosperma* on some haematological and serum lipid parameters in rats during a 28 day administration of the doses of 50mg/kg and 100 mg/kg body weight orally was investigated. Preliminary phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, cardiac glycosides, tannins and carbohydrates. The parameters evaluated include serum lipids, red and white blood cell indices. The results show that the extract administered significantly decreased ($p<0.05$) packed cell volume, haemoglobin concentration and lymphocytes at the dose of 50mg /kg and 100 mg/kg body weight when compared with control.. Also, the extract significantly increased ($p<0.05$) white blood cell count and neutrophils at all doses administered when compared with control. Moreover, the extract significantly reduced ($p<0.05$) total cholesterol concentration and increase ($p>0.05$) triglycerides concentration in the serum when compared with controls. The results of this study suggest that the combined leaf extract could be used in boosting immune system and may have beneficial effect on serum cholesterol concentration.

Introduction

Various organic compounds are derived from plants which are important in combating different diseases that we are constantly exposed to. Knowing these phytochemicals and their specific uses will go a long way in treating diseases in the medical as well as pharmaceutical field (Enwuru, 2008). Plants have provided mankind with herbal remedies for many diseases for many centuries till date. They continue to play a major role in primary healthcare as therapeutic remedies in developing countries. The role of plants in folklore medicine is attributed to the presence of phytochemicals; which are non nutritive plant chemicals that have disease preventing or curative properties.

C. afer, which is commonly called bush sugar cane or monkey sugar cane (Nyananyo, 2006).

C. afer which belongs to the family *Zingiberaceae* is a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in moist or shady forest of West and Tropical Africa (Iwu, 2009). *C. afer* is a useful medicinal plant that is highly valued for its anti-diabetic, anti-inflammatory and anti-anthritic properties in South-East and South-West Nigeria (Soladoye and Oyesika, 2008).

Cleome rutidosperma grows principally at low altitudes in humid conditions *Cleome rutidospermalinn*(family-cleomaceae) is use to relieve earache, pain, skin disease. In Ghana, Gabon and DR Congo leaf sap is applied to cure earache and deafness. The plant is used in the treatment of paralysis epilepsy, convulsion and spasm (Anuradha, *et al*., 2013*).*

According to Tiwari and Rao (2002) polyherbal therapies have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves, that work together in a dynamic way to produce therapeutic efficacy with minimum side effects. It is in this light that this work was designed to investigate the effects of combined extracts from two widely used plants *Costus afer* and *Cleome rutidosperma* employed traditionally in the management of some maladies.

Due to the importance of Haematological parameters and lipid profile in the normal functioning of human system and or as a tool of diagnosis, *Costus afer* and *Cleome rutidosperma*, a commonly used plant in Nigerian folklore medicine was chosen to investigate its effect on haematological parameters and lipid profile of normal albino rats

Materials and Methods

Animals

Twelve (12) adult healthy male albino rats, weighing between 150 and 200g were used in this study. The rats were obtained from the animal house of the Niger Delta University, College of Health Sciences, Bayelsa State and housed in standard cages. They were then allowed free access to standard feed (growers mash) and water for a period of two weeks to acclimatize to the cage environment prior to the commencement of the experiment. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Chemicals

All chemicals used were of analytical standard

Preparation of extracts

Fresh leaf of costus afer and cleome rutidosperma were collected within Amassoma, Yenagoa, Bayelsa State. collected from a residential farmyard in Niger Delta University (N.D.U),Amassoma, Wilberforce Island, Bayelsa State, Nigeria and was botanically identified and deposited at the Herbarium of department of biological science, in Niger Delta University (N.D.U), Amassoma, Wilberforce Island, Bayelsa State, Nigeria.These were washed with distilled water, shade dried and

pulverized. The leaves of Costus afer and Cleome rutidosperma were thoroughly washed with distilled water to remove debris and contaminants, they were then dried in an oven at 40℃ until a constant weight was reached, and then pulverized using an electric blender (Blender, 462 Nakai Japan).

200g of the powdered mixture (i.e 100g each of Costus afer and Cleome rutidosperma) was extracted in 600ml of absolute ethanol for 24 hours at room temperature with constant shaking using a flask shaker (Model, Denly A-500). The extract was filtered with Whatman No 1 filter paper and the resulting filtrate evaporated to dryness using a rotatory evaporator at 40℃ , the resultant concentrate was then reconstituted in distilled water to give the required doses used in the study.

Phytochemical screening:

Phytochemical tests were conducted on the sample of leaves of *Costus afer* and *Cleome rutidosperma* to determine the presence of alkaloids, anthraquinone, tannins, terpenoids, saponins, flavonoids, cardiac glycosides and carbohydrates using standard protocols (Sofowora, 1993; Trease and Evans, 1993).

Experimental design and procedures

Experimental design

Twelve rats of the wistar strain weighing between 100-200g were distributed into three groups of four (4) animals each.
Group 1 served as the normal control group and received standard feed and water, while group 2 and 3 were the test groups and they received 50mg/kg body weight and 100mg/kg body weight of the combined leaf extract respectively.

Biochemical Analysis

Sample Collection

After the experimental period, animals in different groups were sacrificed. By the end of each experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. 5ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 minutes before centrifuging at 800g for 5 minutes. The supernatant was used for the lipid analysis. The remaining blood sample was put in an EDTA bottles for haematological determinations.

Haematological estimation

Estimation of total cholesterol and triglycerides was done by cholesterol oxidase- phenol aminoantipyrine method (Rifa and Warnick, 2006).The haemoglobin (Hb) level was measured by the cyanomethaemoglobin method. (Baker *et al.*,1998). Packed cell volume (PCV) was measured using microheamatocrit method and total White Blood Cell (WBC) count was estimated by visual method (Cheesbrough, 2000).

Statistical Analysis

The result obtained from the study were analysed using the statistically package for social science (spss) version 16.0 for windows. one way ANOVA followed by Post-hoc Turkey was used to compare mean ± S.D, and values were considered significant at $p< 0.05$.

Results and Discussion

Phytochemical screening of the dry leaves of the plants showed trace amounts of

tannins (+) and saponins (+) in *costus afer* and trace amounts of glycoside (+) in both plants, and abundant amount of saponins (++), tannins (++) in *cleome rutidosperma* while flavonoids (++) and alkaloids (++) were present abundantly in both plants (Table 1).

Result of the study in table 2 and 3 showed that the rats in groups two and three administered 50mg/kg and 100mg/kg body weight of extract had a significant difference ($p<0.05$) in lipid profile and haematological parameters compared to the control.

There was a significant increase ($p<0.05$) in WBC count and neutrophil and a decrease ($p<0.05$) in PCV, HB and Lymphocyte compared to the control.(Table 2).There was also a significant($p<0.05$) decrease in cholesterol and increase($p>0.05$) in Triglyceride compared to the control.(Table 3)

Table.1 Phytochemical constituents of leaves of *Costus afer* and *Cleome rutidosperma*

Phytochemicals	Tannins	Saponins	Glycoside	Alkaloids	Flavonoid
Costus afer	+	+	+	++	++
Cleome rutidosperma	++	++	+	++	++

Table.2 Effect of Combined ethanolic Leaf Extract of *Costus afer* and *Cleome rutidosperma* on Haematological Parameters in rats

GROUP(S)	**PCV (%)**	**HB (g/dL)**	**WBC (L)**	**NEUT (%)**	**LYMPH (%)**
1(control)	47.0 ± 1.82^{a}	17.1 ± 0.41^{a}	5.62 ± 0.17^{a}	56.0 ± 1.82^{a}	40.5 ± 0.57^{a}
2(50mg/kg) CA and CR	32.6 ± 0.83^{b}	13.0 ± 0.30^{b}	9.60 ± 0.62^{b}	58.5 ± 1.29^{b}	36.2 ± 1.7^{b}
3(100mg/kg) CA and CR	27.6 ± 0.45^{c}	11.4 ± 0.39^{c}	11.6 ± 0.75^{c}	68.7 ± 1.70^{c}	26.7 ± 0.95^{c}

Data mean±SD (n = 4). Mean in the same column with different superscript letter(s) are significantly different $p<0.05$ (one way ANOVA followed by Post-hoc Turkey).

Table.3 Effect of Combined ethanolic Leaf Extract of *Costus afer* and *Cleome rutidosperma* on Lipid Profile in rats

GROUP(S)	**TRIG (umol/L)**	**CHOL (umol/L)**
1(control)	0.83 ± 0.12^{a}	2.55 ± 0.20^{a}
2 (50mg/kg) CA and CR	0.85 ± 0.05^{a}	2.05 ± 0.12^{b}
3 (100mg/kg) CA and CR	0.86 ± 0.09^{a}	1.70 ± 0.08^{c}

Data mean±SD (n = 4). Mean in the same column with different superscript letter(s) are significantly different $p<0.05$ (one way ANOVA followed by Post-hoc Turkey)

The robust phytochemistry of *costus afer* and *cleome rutidosperma* makes it a potent therapeutic agent as is justified by its popularity in African folk medicine.

Assessment of plasma lipid profile is required for the state of wellbeing of every individual as cardiovascular diseases and coronary heart diseases are silent, serial killers of our age. The assessment of plasma haematological parameters can be used to determine how toxic a compound can be to the blood parameters. Some Phytochemicals may have deleterious effects on the blood cells and to this end, it was necessary to determine the effect of the leaf extracts of *costus afer* and *cleome rutidosperma* on lipid profile and haematological parameters.

This study shows presence of saponin, alkaloid, flavonoids and cardiac glycosides in leaves of *costus afer* and *cleome rutidosperma* which could be responsible for their medicinal purposes.

Assessment of haematological parameters can not only be used to determine the extent of deleterious effect of extracts on the blood of an animal, but it can also be used to explain blood relating functions of a plant extract or its products (Yakubu *et al*., 2007).

There was a significant increase ($p<0.05$) in WBC count and neutrophil and a decrease ($p<0.05$) in PCV, HB and Lymphocyte compared to the control (Table 2).

Reports about WBC counts have pointed out that whereas increased count of WBC is supposed to be helpful in boosting immune system (Adedapo *et al*., 2007; Mohajeri, 2007), a decreased count of WBC shows the suppression of leucocytes and their production from bone marrow (Odesanmi *et al*., 2010; Jimoh *et al*., 2008; Osuigwe *et al*., 2007;Adedapo *et al*., 2004). Therefore, an increased count of WBC in combined ethanolic leaf extract of various concentration treated animals, as observed in the present study, suggests that the combined leaf extract of CA and CR might have a good potential to boost the immune system. Adedapo *et al*., (2007), Onyeyilli *et al*., (1992) reported, that reduction in RBC, Hb and PCV is an indication of either the destruction of RBC or their decreased production, which may lead to anemia. On the contrary an increase in the count of RBC, Hb and PCV is suggestive of polycythemia and positive erythopoisis (Iranloye, 2002; Mansi and Lahham, 2008; Kuppast *et al*., 2009; Okpuzor *et al*., 2009). Hence, a significant reduction of PCV and Hb of the combined leaf extract of CA and CR treated animals is suggestive of anaemia.

There was also a significant ($p<0.05$) decrease in cholesterol and increase ($p>0.05$) in Triglyceride compared to the control (Table 3). High blood cholesterol concentration is one of the important risk factors for cardiovascular disease (Adebayo *et al*., 2005) Thus the reduction in serum total cholesterol concentration effected by the extract is beneficial and may reduce the risk of cardiovascular disease because agents that have the ability to lower cholesterol concentration in the blood have been reported to reduce vascular resistance by improving endothelial function (Adebayo *et al*., 2005). Similar alterations in lipid as well as haematological profiles were reported in various other plant extracts such as *Bulbine natalensis* (Yakabu and Afolayan, 2009), *Bougainvillea spectabilies leaves* (Adebayo *et al*., 2005) and *Fadogia agrestis* stem (Yakabu *et al.,* 2007). This

work also agrees with the work done by (Omodamiro and Nwankwu, 2013) on effect of Voacanga leaves extract on serum lipid profile and haematological parameters. it is also similar to work by Mishra and Vandon (2012) on the effect of *B. spectabilis* on hematological parameters in rats.

In conclusion the results of this study suggest that the combined leaf extract could be used in boosting the immune system and may have beneficial effect on serum cholesterol concentration.

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