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Lipid lowering, hypoglycemic and antioxidant activities of *Chromolaena odorata* (L) and *Ageratum conyzoides* (L) ethanolic leaf extracts in albino rats

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Abstract

The lipid lowering activity of ethanolic leaf extract of two medicinal plants, *Chromolaena odorata* and *Ageratum conyzoides* in male Albino rats were investigated. *Chromolaena odorata* and *Ageratum conyzoides* ethanolic leaf extract was administered at 25mg/kg body weight respectively and then a combination of the two extract at 12.5mg/kg body weight each concurrently to the animals for 14 days. Results show that the leaf extracts of the two medicinal plants singly significantly ($p \leq 0.05$) increased the mean body weights of the experimental animals 115.42±0.46g to 118.45±0.68g; 121.67±0.54g respectively. Total protein level was non-significantly ($p \leq 0.05$) increased from 9.76±0.04mg/dl to 9.87±0.06mg/dl; 9.83 ±0.12mg/dl respectively. Glucose concentration was reduced significantly ($p \leq 0.05$) from 65.32±3.56mg/dl to 61.68±2.12mg/dl and 62.34±1.28mg/dl respectively. The lipid profile of the experimental animals, total cholesterol, triacylglycerol, LDL, VLDL were significantly ($p \leq 0.05$) reduced, except HDL which significantly increased in the animals. Lipid peroxidation significantly reduced. The antioxidant activities of the extracts also showed that Superoxide dismutase (SOD), Catalase (CAT), Glutathione-s-transferase (GST), Glutathione peroxidase (GSH) and Vitamin C also increased ($p \leq 0.05$) significantly. A combination of the two medicinal plants extracts had a more significant ($p \leq 0.05$) effect on the parameters measured compared to when they were administered separately.

Keywords: Ethno-medicine, Lipids, Atherosclerosis, glucose, protein and phytochemicals

Introduction

Man has always been highly dependent on nature. Throughout history medicinal plants have featured in the life of man. They were always of paramount importance in the treatment of diseases for the people. The search for a cure was not only the instinct of self – preservation, but also for self-protection [1]. It has been reported that about some three hundred species of medicinal plants are used worldwide in the pharmaceutical, food, cosmetics and perfumery industries [2]. Traditional herbal medicine has made outstanding achievements in such area as bone-setting, mental disorders, sickle-cell, anemia, epilepsy, liver and kidney problems, diabetes, stroke, malaria and many others [3, 4]. Traditional remedies are made from leaves, barks of trees, roots, fruits, flowers and seeds, and are taken orally [4]. Depending on the herb and the intended treatment, parts of the plants may be used singly or in combination with other parts [5].

Two types of medicinal plant leaves *Chromolaena odorata* Linn and *Ageratum conyzoides* have been used by ethno-medicine practitioners in the South East of Nigeria to treat and manage stroke, heart disorders, diabetes, wounds, epilepsy and headaches [6].

Chromolaena odorata Linn also known as Siam weed is a perennial scrambling shrub, native to Central and South America and the Caribbean's. It belongs to the family Asteraceae and is used as a medicinal herb in the South East of Nigeria. In traditional medicine practice it is used as an anti-malaria remedy and can also be traditionally applied to wounds to stop bleeding [2]. The aqueous extract and the decoction from the leaves of this plant have been used throughout Vietnam for the treatment of soft tissue wounds, burn wounds, and skin infections [7]. The anti-cancer activity of leaf extracts of *Chromolaena odorata* on human and mouse cell lines has been reported [8].

Fresh leaves of *Chromolaena odorata* have been reported to contain essential oil which include 5, 6-diethenyl-1- methyl-cyclohexene (44.7%), β -guiane (11.9%), elemol (8.5%) and patchoulene (8.6%) [9]. In-vitro cytotoxicity screening against human cervical cell line (HeLa), human laryngeal epithelial carcinoma cells (HEp-2) and (NIH-3T3), mouse embryonic

fibroblast cancer cell lines with the essential oil showed they had significant cytotoxic effect with IC_{50} values of 60.3, 67.5 and 72.00 $\mu\text{g mL}^{-1}$ towards HeLa, HEP-2 and NIH 3T3 cancer cell lines, respectively [9]. A moderate cytotoxicity potential with IC_{50} of 700 μL^{-1} of the essential oil of *Chromolaena odorata* on human epidermis cell line has also been reported [10]. Non-significant cytotoxic activities with LC_{50} values of 324 and 392 $\mu\text{g mL}^{-1}$ of aqueous and ethanolic extracts respectively of *Chromolaena odorata* plant species collected from Nnewi Nigeria has been reported [6]. A significant free radical scavenging action against nitric oxide and hydroxyl radical of ethanolic and methanolic extract of leaves of *Chromolaena odorata* has been reported [11].

Ageratum conyzoides Linn is an ephemeral weed of crop fields which spreads across West Africa. *Ageratum conyzoides* Linn belongs to the family Asteraceae, and is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world. *A. conyzoides* has high phytochemical content which include flavonoids, alkaloids, coumarins, essential oils, and tannins. Many of these are biologically active. Essential oil yields that vary from 0.02% to 0.16% from a plant collected in India has been reported [12]. It has bioactivity with insecticidal and nematocidal activity [13]. *A. conyzoides* is widely utilized in traditional medicine by various countries; the most common use is to cure wounds and burns [14]. Traditional communities in India use the plant as a bacteriocide, antidiarrheic, and antilithic [15]. In Asia, South America, and Africa, aqueous extract of this plant is used as a bacteriocide [16, 17]. Traditional communities in Cameroon and Congo use this plant to treat fever, rheumatism, headache, and colic [18, 19]. The whole plant is used as an antidiarrheic [20]. In Brazil aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhea, rheumatism, spasms, or as a tonic [21, 22, 23]. Several investigators have reported on the pharmacologic efficacy of the plant. The inhibitory activity of ether and chloroform extracts against in-vitro development of *Staphylococcus aureus* has been reported [14]. Methanolic extract of the whole plant has been reported to show inhibitory action in the development of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [16]. Analgesic action in rats using aqueous extract of *A. conyzoides* leaves (100 to 400 mg/kg) has been reported [19]. Muscle relaxing activities of the plant, has been demonstrated, confirming its popular use as an antispasmodic [24]. Aqueous extract of the whole plant showed effective clinical control of arthrosis in patients [25].

The present study therefore is to investigate the comparative lipid lowering, hypoglycemic and antioxidant activities of *chromolaena odorata* (L) and *ageratum conyzoides* (L) ethanolic leaf extract in albino rats singly and when mixed together.

Materials and Methods

Plant leaves

Chromolaena odorata and *Ageratum conyzoides* leaves were collected from Abia State University, Uturu campus and were identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu. Voucher specimens were deposited at the departmental herbarium. The leaves were cleaned, sun dried for about eight days to a constant weight. The dried leaves were milled into fine powder using Arthur Miller milling machine and preserved in cellophane bags until when used.

Preparation of Leaf Extract

Ten grams (10g) of the powdered samples each of

Chromolaena odorata and *Ageratum conyzoides* were dissolved in 95% ethanol (1:10 w/v) by cold maceration for 24 hours. The mixtures were then filtered and freeze dried. The sample was then placed in airtight container and refrigerated until used.

Volume and concentration of extract

Chromolaena odorata: volume - 165ml, concentration - 19.5mg/ml

Ageratum conyzoides: volume - 142ml, concentration - 16.2mg/ml

Animal Studies and Experimental Design

A total of forty healthy male Albino rats of six weeks old and weighing between 110 to 125g were placed randomly into four groups of ten animals each.

Group 1 = served as control did not receive any plant leaves extract

Group 2 = served as test group for *Chromolaena odorata* and received 20mg/kg body weight of the extract.

Group 3 = served as test group for *Ageratum conyzoides* and received 20mg/kg body weight of the extract.

Group 4 = served as test group and received a combination of *Chromolaena odorata* and

Ageratum conyzoides (10mg of each) /kg body weight.

All the extract was administered orally by orogastric intubation for 14 days.

All the test animals were allowed water and feed *ad libitum* throughout the experiment.

Standard laboratory protocols for animal studies were maintained. Approval for animal studies was obtained from the Animal Ethics Committee of the College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria. Weights of the animals were recorded every seven days.

Collection of blood samples and serum preparation

At the 15th day, the animals were killed after overnight starving. Incisions were made into their thoracic cavity. Blood samples were collected by heart aorta puncture using a 10mL hypodermic syringe and allowed to clot in sample vials. The samples were centrifuged at 3000 rpm for 5 min. using the Bran Scientific and Instrument Company England centrifuge. The supernatant (serum) was harvested by simple aspiration with Pasteur pipette and stored in clean tubes at -4 °C until analysis.

Estimation of parameters

Determination of Total Cholesterol

Total cholesterol was determined by the enzymatic colorimetric cho-PAP method as described by [26]. High density lipoprotein (HDL) Very low density lipoprotein (VLDL), and Low density lipoprotein (LDL) were determined as described by [27]. Triacylglycerol and cholesterol were assayed as described by [28, 26, 29] using Biosystem kits. The measurements were performed according to the manufacturers' instruction.

Assay for Serum glucose and Total protein

Total protein was determined by the Lowry method [30]. Glucose was assayed using glucose enzymatic - colorimetric test kit, produced by Cypress diagnostics (Belgium). The test principle is based on the oxidation of glucose by glucose oxidase to gluconic acid and hydrogen peroxide. The hydrogen peroxide forms a red violet color with a chromogenic oxygen acceptor, phenol aminophenazone in the presence of

peroxidase. The color intensity is proportional to glucose concentration in the sample [31].

Assay for Antioxidant enzymes activity of test animals

The antioxidant enzymes: Catalase, Superoxide dismutase, Glutathione-s-transferase and Glutathione peroxidase were assayed as described by [32], using Biosystem kits. Vitamin C was assayed by the method as described by [33].

Determination of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) method as described by [34] was used to determine lipid peroxidation,

while Total cholesterol was determined according to the method described by [26].

Statistical Analysis

Values were represented as Mean \pm SD. Data obtained were subjected to one way Analysis of Variance (ANOVA) and group means were compared using Duncan's new multiple range tests. Differences were considered to be significant at ($p \leq 0.05$).

Results

Table 1: Effect of *Chromolaena odorata* and *Ageratum conyzoides* leaves extract on animal weight (g)

	Group 1 (Control)	Group 2 <i>C. odorata</i> (20mg/kg, b.w)	Group 3 <i>A. conyzoides</i> (20mg/kg b.w.)	Group 4 <i>C. odorata</i> and <i>A. conyzoides</i> (20mg/kg b.w.)
Body weight	115.42 \pm 0.46	118.45 \pm 0.68	121.67 \pm 0.54*	122.58 \pm 0.96*

*Values are mean \pm SD of triplicate determinations (n=10) Values with * are statistically significant ($p \leq 0.05$) compared with the control.

Table 2: Effect of *Chromolaena odorata* and *Ageratum conyzoides* leaves extract on Total protein and glucose levels (mg/dl)

Parameter	Group 1 (Control)	Group 2 <i>C. odorata</i> (20mg/kg b.w)	Group 3 <i>A. conyzoides</i> (20mg/kg b.w.)	Group 4 <i>C.odorata</i> and <i>A. conyzoides</i> (20mg/kg b.w.)
Total protein	9.76 \pm 0.04	9.87 \pm 0.06	9.83 \pm 0.12	12.57 \pm 0.15
Glucose	65.32 \pm 3.56	61.68 \pm 2.12*	62.34 \pm 1.28*	60.26 \pm 1.46*

*Values are mean \pm SD of triplicate determinations (n=10) Values with * are statistically significant ($p \leq 0.05$) compared with the control.

Table 3: Effect of *Chromolaena odorata* and *Ageratum conyzoides* leaves extract on fatty acid levels (mg/dL)

Parameter	Group 1 (Control)	Group 2 <i>C. odorata</i> (20mg/kg b.w)	Group 3 <i>A. conyzoides</i> (20mg/kg b.w.)	Group 4 <i>C.odorata</i> and <i>A. conyzoides</i> (20mg/kg b.w.)
Triacylglycerol	112.54 \pm 1.65	103.23 \pm 1.04*	104.52 \pm 0.98*	98.58 \pm 1.24*
Total Cholesterol	2.57 \pm 0.18	2.16 \pm 0.13	2.11 \pm 0.06*	2.02 \pm 0.15*
HDL	31.64 \pm 0.78	33.46 \pm 1.57	33.25 \pm 1.28*	34.18 \pm 1.11*
LDL	35.42 \pm 1.08	33.46 \pm 0.86	32.87 \pm 0.94*	32.16 \pm 1.03*
VLDL	4.53 \pm 0.18	4.16 \pm 0.04	4.06 \pm 1.20*	4.02 \pm 0.94*

*Values are mean \pm SD of triplicate determinations (n=10) Values with * are statistically significant ($p \leq 0.05$) compared with the control

Table 4: Effect of *Chromolaena odorata* and *Ageratum conyzoides* leaves extract on Lipid peroxidation (TBARS) mg/mL

Parameter	Group 1 (Control)	Group 2 <i>C. odorata</i> (20mg/kg.b.w)	Group 3 <i>A. conyzoides</i> (20mg/kg b.w.)	Group 4 <i>C. odorata</i> and <i>A. conyzoides</i> (20mg/kg b.w.)
Lipid peroxidation (TBARS) mg/mL	7.54 \pm 0.01	7.21 \pm 0.06	7.12 \pm 0.92	6.86 \pm 0.95*

*Values are mean \pm SD of triplicate determinations (n=10) Values with * are statistically significant ($p \leq 0.05$) compared with the control.

Table 5: Effect of *Chromolaena odorata* and *Ageratum conyzoides* leaves extract on antioxidant levels (U/L)

Parameter	Group 1 (Control)	Group 2 <i>C. odorata</i> (20mg/kg b.w)	Group 3 <i>A. conyzoides</i> (20mg/kg b.w.)	Group 4 <i>C. odorata</i> and <i>A. conyzoides</i> (20mg/kg b.w.)
SOD	0.26 \pm 0.07	0.45 \pm 0.41*	0.48 \pm 0.08*	0.56 \pm 0.12*
CAT	162.31 \pm 1.25	167.59 \pm 1.27*	166.24 \pm 0.89*	169.38 \pm 1.15*
GSH	4.63 \pm 0.93	4.87 \pm 0.14	4.76 \pm 0.16	4.98 \pm 0.18*
GST	1.68 \pm 0.02	2.13 \pm 0.12	2.35 \pm 0.06	2.48 \pm 0.75*
Vit C (mg/dl)	1.12 \pm 0.10	1.34 \pm 0.52	1.37 \pm 0.01	1.45 \pm 0.13*

*Values are mean \pm SD of triplicate determinations (n=10) Values with * are statistically significant ($p \leq 0.05$) compared with the control.

Discussion

The test animals fed the plant extracts showed significant ($p \leq 0.05$) increase in mean body weight (Table 1). The ethanolic extract of *Chromolaena odorata* and *Ageratum conyzoides* may have caused the significant increase of the mean body weight of the animals. Both plants are rich in phytochemicals which include flavonoids, terpenes and tannins [6, 12]. These phytochemicals may have caused increased protein synthesis, (Table 2) which may have caused the increase in the mean body weight of the experimental animals. Blood glucose level decreased ($p \leq 0.05$) significantly (Table 2). The reduction in glucose level may be due to the presence of phenols in the medicinal plants. Phenolic compounds are strong antioxidants [35]. Flavonoids are the largest group of

polyphenols that have been identified in vegetables, fruits and other plant parts and linked to reducing the risk of degenerative diseases. Phenols have been reported to show inhibitory activity on α -glucosidase activity. Phenolic compounds have been reported to inhibit pancreatic α -amylase and α -glucosidase [36]. The inhibition of α -glucosidase activity slows the breakdown of disaccharide to simple glucose, thereby reducing the rate at which glucose is absorbed into the blood stream [37]. It has also been reported that phenol-rich plant foods exhibit pancreatic α -amylase and α -glucosidase inhibitory activities in-vitro [37].

The lipid profile analysis (Table 3) shows that except HDL, all other measured lipid component reduced ($p \leq 0.05$) significantly. Cholesterol is a precursor of other steroids and

an important constituent of the cell membrane and bile acids [38, 39]. The transport of cholesterol to and from the liver is mediated by low and high density cholesterol. Fatty acid reduction by the medicinal plants shows they have the ability of lowering the lipid profile of the experimental animals. HDL is known as the 'good' cholesterol which is involved in the transport of other lipids to the liver for disposal in the bile; hence the significant ($p \leq 0.05$) increase could be beneficial [40]. The saponins have been reported to effect reduction in plasma concentration of cholesterol and lipids, hence may have contributed to the hypolipidemic activities of these medicinal plants. Phytosterols compete with dietary cholesterol (to which they are structurally related) for uptake in the intestine and facilitate its excretion from the body [38]. The levels of HDL-Cholesterol in the body have been linked as a factor in the etiology of cardiovascular diseases. Elevated serum cholesterol and LDL-cholesterol constitute risk factors in the development of cardiovascular diseases. Cholesteryl esters deposition during their transport in the blood vessels leads to hardening and narrowing of the vessels which causes cardiovascular diseases, especially atherosclerosis [41, 42]. The reduction of these lipids may have positive impacts on cardiovascular diseases.

Lipid peroxidation (Table 4) decreased ($p \leq 0.05$) significantly compared to control. The medicinal plants have been reported to be rich in phytochemicals which have antioxidant activities [6]. The significant reduction of lipid peroxidation could be due to the antioxidants providing protection to the cells from lipid. The oxidation of polyunsaturated lipids to aldehydes and peroxides leads to lipid peroxidations which are implicated in the development of diseases [43]. The aldehydes and peroxides are more dangerous than reactive oxygen species (ROS) because they are long-lived hence are capable of spreading to distant sites through blood circulation to cause more peroxidation of surrounding cells [44]. Malondialdehyde formation due to lipid peroxidation causes cytotoxicity and injury that leads to cell damage [45].

The antioxidants assayed in the experimental animals (Table 5) shows they all increased ($p \leq 0.05$) significantly compared to control. The nutritional composition of the two medicinal plants shows they are rich in phytochemicals; flavonoids, alkaloids and tannins which are phenolic compounds. Antioxidant activities in higher plants have been associated with phenolic compounds [46]. Polyphenols have antioxidant activities and antioxidant activities of plant foods correlates with the phenolic content [47]. SOD protects cells from ROS attack especially the superoxide radical [48]. SOD converts superoxide radical to hydrogen peroxide which is then converted to harmless water and oxygen [45]. GSH is involved in the reduction of lipid and hydrogen peroxide to eliminate oxidative stress [49]. GST has been reported to have the ability to conjugate and excrete toxic intermediates that are capable of causing diseases [50]. The significant ($p \leq 0.05$) increase of the antioxidants; glutathione S -transferase (GST) and glutathione peroxidase (GSH) could be beneficial to the animals and shows the plants have antioxidant activities.

Conclusion

The results of this study suggests that the ethanolic leaf extracts of *Chromolaena odorata* (L) and *Ageratum conyzoides* (L) lowered the concentration of glucose in the experimental animals; lowered the concentration of the lipids measured; Triacylglycerol, Total Cholesterol, LDL and VLDL, while the HDL was increased. The antioxidants measured also showed increase in their activities. A combination of the two plant extract administered to the animals concurrently

produced greater effects on the parameters measured. For effective pharmacologic activity we suggest a combination of the two plant extracts in the management of ailments. We therefore conclude that this result lays credence to the claims of alternative medicine practitioners that these medicinal plants lower blood glucose, reduce cholesterol level and up-regulate antioxidant activities in patients.

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