European Journal of Medicinal Plants
3(3): 369-380, 2013

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Preliminary Phytochemical Screening, Analgesic and Anti-inflammatory Properties of *Celosia isertii*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEE designed the study and wrote the first draft of the manuscript. Author ECA managed the literature searches; author EOL performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

Received 13th February 2013
Accepted 13th April 2013
Published 27th April 2013

ABSTRACT

Celosia isertii, a small genus of edible and ornamental plants in the amaranth family, Amaranthaceae, has been investigated for a number of properties, such as anti-diabetic activity, hepatoprotective property, anti-diarrheal property and lots more.
Aims: This study investigates the phytochemical compostion, analgesic and anti-inflammatory properties of the plant.
Methodology: Aqueous and ethanol extract of *Celosia isertii* was prepared and used for the experiment
Results: Phytochemical studies carried out on aqueous and ethanol extract of *Celosia isertii* leaves showed the presence of alkaloid, saponin, phenol, tannin, flavonoid, cardiac glycoside, steroid, phytosterol, triterpenoid and phlobatannin. The anti-inflammatory study carried out on the albino rats that had their paw injected with carrageenan injection to induce edema, showed that at 250 mg/kg plant extract, the anti-inflammatory activity of the ethanol extract was slightly less compared to diclofenac sodium (10 mg/kg), a known anti-inflammatory drug. While the analgesic study done on mice in which acetic acid was injected to induce pain, showed that at 250mg/kg of plant extract, the analgesic effect of

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diclofenac sodium (10 mg/kg), was greater than the aqueous extract, but less than the ethanol extract.
Conclusion: The results obtained in this study suggest that *Celosia isertii* has potent anti-inflammatory and analgesic properties, and therefore can be useful in painful inflammatory conditions.

Keywords: Anti-inflammatory; analgesic; phytochemicals and Celosia isertii.

1. INTRODUCTION

Nigeria is a country blessed with and rich in indigenous herbal resources which grow on their varied topography and under changing agro climatic conditions permitting the growth of almost 10,000 plant species, of which many are of medicinal value. It is a known and established fact about the important role medicinal plants play in the health of individuals in rural societies. This has given rise to their exploitations in the modern health care system. The medicinal value of these plants thus lies in some chemical substances that produce a definite physiological action on the human body [1,2]. The primary aim of sourcing for plant drug through any of the known strategies-through the ethnomedical survey programme is mainly, to detect the active (chemical) ingredients in the plants that exert some definite pharmacological effect in the body. The results of such investigation would most often, serve as a lead for the biological evaluation of these plants and would subsequently lead to the production of chemotherapeutically plant derived drug.

Celosia is a small genus of edible and ornamental plants in the amaranth family, Amaranthaceae. The plants are well known in East Africa's highlands and are used under their Swahili name, mfungu and ayovwe in the Delta central region of Niger Delta in Nigeria. It is also known as Lagos spinach, quail grass, Soko and feather cockscomb. It is a broad annual leaf vegetable, found in the countries of Central and West Africa, and is one of the leading leafy green vegetables in Nigeria, where it is known as 'soko yokoto', meaning *"make husbands fat and happy"*, [3]. In Spain it is known as *"Rooster comb"* because of its appearance. It grows widespread across Mexico, where it is known as *"Velvet flower"*, northern/ southern America, the West Indies and Southeast Asia where it is cultivated as a nutritious leafy green vegetable. Amongst the Urhobo's it has been used for the treatment of inflammations, fever, itching, hyperglycemia and bacterial infections. In Asia, it has been investigated and found to have anti-diabetic properties [3], anti-inflammatory properties [4], anti-diarrheal properties [5], antioxidant properties [6,7,8], as well as anti-hyper cholesterolemia properties [9]. Also the seeds are bitter, useful in blood diseases and mouth sores. This study also investigated the analgesic and anti-inflammatory properties of aqueous and ethanol extract of *Celosia isertii* leaves.

2. MATERIALS AND METHODS

2.1 Plant Material

Celosia isertii leaves were collected from the wide growing habitat within campus three of Delta State University Abraka. The leaves were removed from the stalk and air dried at room temperature (22±1ºC), to a constant weight after which it was grounded with sterilized machine and sieved to fine powder and made into extracts used for the experiment.

2.2 Preparation of the Extracts

The powder was weighed (600g). The powder was divided into two equal part and soaked in 1500ml distilled water and 1500ml of ethanol respectively, for 72hrs. The extract was obtained using an electrical evaporator extraction apparatus (rotary evaporator). The solvent was extracted at a temperature of 45ºC and pressure of 60cm of water. Paste- like extract was obtained and oven dried to complete solid and grinded to smooth powdered form.

2.3 Qualitative Phytochemical Analysis

The powdered material and extract of the plants were subjected to different kinds of chemical tests to investigate the presence of secondary metabolites such as saponins, tannins, flavonoids, phenol, anthraquinones cyanogenic glycosides, cardiac glycosides and alkaloids using standard procedures [10,11,12,13,14].

2.4 Test for Proteins

2.4.1 Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

2.5 Test for Carbohydrates

2.5.1 Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

2.6 Iodine Test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

2.7 Test for Phenols and Tannins

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

2.8 Test for Flavonoids

2.8.1 Shinoda test

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

2.9 Alkaline Reagent Test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

2.9.1 Test for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

The residue was dissolved in few drops of diluted acetic acid; 3 ml of acetic anhydride was added followed by few drops of Concentrated H_2SO_4. Appearance of bluish green colour showed the presence of phytosterol.

2.9.2 Test for triterpenoids

10mg of the extract was dissolved in 1 ml of chloroform, 1 ml of acetic anhydride was added followed by addition of 2 ml of Concentrated H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

2.9.3 Test for phlobatannins

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

2.9.4 Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

2.9.5 Test for glycosides

Liebermann's test: Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, that is, glycone portion of glycoside.

2.9.6 Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

2.9.7 Test for steroid

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then

2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

2.9.8 Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

2.9.9 Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.10 Quantitative Determination of the Chemical Constituent

2.10.1 Preparation of fat free sample

2 g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hours.

2.10.2 Determination of total phenols by spectrophotometric method

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

2.10.3 Alkaloid determination using Harborne (1973) method

5 g of the sample was weighed into a 250ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.10.4 Tannin determination by Van-Burden and Robinson (1981) method

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.I M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

2.10.5 Saponin determination

The samples were ground and 20 g of each were put into a conical flask and 100cm^3 of 20% aqueous and ethanol. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55^{0}C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

2.10.6 Flavonoid determination by the method of Bohm and KocipaiAbyazan (1974)

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.11 Animals

Twenty matured male albino rats and mice weighing between 150-200g and 20-28g were used for the experiment. Rats were housed with *ad libitum* access to feed and water in a well- ventilated animal unit provided by the Department of Physiology, Delta State University, Abraka (21 ± 5ºC, humidity 60 %, 12hr light/ dark cycle). The rats were fed standard chow (Growers marsh Feed Ltd, Sapele). Permission for the use of animals and animal protocol was obtained from the Research ethics committee of Delta State University, Abraka. And the laboratory animal ethics guideline was followed.

2.11.1 Time course study in carrageenan induce paw edema rats

The hind paw edema volume was measured by volume displacement method using plethysmometer by immersing the paw till the level of lateral malleolus at various time interval (0,1,2,3, and 5h) after 0.1g/10ml carrageenan injection, each measurement was repeated three times then averaged. The anti-inflammatory activity was calculated at each time of observation as percent inhibition of oedema in the animals treated with substances under test, in comparison with the vehicle-treated animals. The percent inhibition of oedema [18,19] was calculated using the formula.

$$\% \text{ inhibition} = \frac{(vo - vt)}{vo} x\ 100$$

Where Vt is the volume of edema at corresponding time, and Vo is the volume of edema of vehicle treated rats at the same time. Result was expressed as percentage inhibition of edema by comparing with the vehicle treated control group

2.11.2 Acetic acid induce writhing in mice [19]

A group of mice was also injected (intraperitonealy) with 0.1ml/1mlof 0.3%(v/v) acetic acid, the mice exhibiting the writhing movement (stretching of hind limbs and bending of trunk) were selected for the study. The mice were randomly divided into four groups (5mice/group).

The mice were administered with Aqueous 250mg/kg and Ethanol 250mg/kg of *Celosia isertii* and diclofenac sodium 10mg/kg (orally), 1hr prior to acetic acid injection the number of writhing movement were counted for 30mins following acetic acid injection.

2.12 Statistical Analyses

The result of this study were expressed as mean $\pm$ SEM, and were analyzed by one way analyses of variance (ANOVA) using statistical package for social science (SPSS, 16). Difference between the means were tested with post Hoc- Tukey's test for multiple comparison and significance was considered when $p< 0.01$. Student's dependent t-test was used to analyze the significant difference between the groups.

3. RESULTS AND DISCUSSION

Table 1. Qualitative analysis of the aqueous and ethanol extract of *Celosia isertii* leaves

Chemicals	Aqueous extract	Ethanol extract
Alkaloids	+ + +	+ + +
Saponins	+ + +	+ + +
Tannins	+ + +	+ + -
Flavonoids	+ + +	+ + +
Phenol	+ + +	+ + -
Anthraquinones	+ + -	+ + -
Cardiac glycosides	+ + +	+ + +
Steroid	+ + -	+ + -
Terpenoids	+ + -	+ + -
phytosterol	+ - -	+ + -
triterpenoids	+ + +	+ + -
Phlobatannins	+ + -	+ + +

Keys: + + + Abundantly present
+ + - Moderately present
+ - - Present in trace amount

Table 2. Quantitative analysis of the aqueous and ethanol extract of *Celosia isertii* leaves

Chemicals	Aqueous extract	Ethanol extract
Alkaloids	1.5%	0.9%
Saponins	9.0%	10.3%
Tannins	2.3%	3.18%
Flavonoids	2.8%	3.6%
Phenol	6.0%	6.9%

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities similar to the study done by Aiyegroro and Okoh [20]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids (Tables 1 and 2).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [21]. They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [22]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [23]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. [1]. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be anti-microbial substances against wide array of microorganisms in vitro [22]. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall, [24].

Triterpenoids are terpenoid derivatives of triterpene molecules. They may have useful anti-cancer properties, [25]. They also are effective antioxidant and show strong anti- cancer activities [26,27].

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation [28]. Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [29].

Steroids have been reported to have antibacterial properties, [30,31] and they are very important compounds especially due to their relationship with compounds such as sex hormones [32].

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [33]. Several workers have reported the analgesic [34], antispasmodic and antibacterial [35] properties of alkaloids.

Glycosides are known to lower the blood pressure according to many reports [35]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

The effect of the extracts of *Celosia isertii*, Diclofenac sodium and control are presented in Tables 3 and 4. In dose response study in carrageenan-induced edema, ethanol extract of *Celosia isertii* treated group exerted more response of inhibition. However, the extent of edema inhibition in rats treated with Aqueous 250mg/kg of *Celosia isertii* showed lesser inhibition as compared to diclofenac sodium (10mg/kg) treated group but ethanol extract 250mg/kg showed also a slightly lesser inhibition as compared to diclofenac sodium (10mg/kg) treated group.

Table 3. Effect of *Celosia isertii* (Aqueous and ethanol) leaf extract on Carrageenan induce hind paw edema in rats

Treatment	Dose/ concentration	Mean volume of paw edema (ml) at various time interval				
		0hr	1hr	2hr	3hr	5hr
Control	1ml/kg	2.9±0.4	4.5±0.2	5.9±0.3	6.7±0.2	7.5±0.2
Diclofenac sodium	10mg/kg	1.9±.01	3.3±0.1	2.9±0.2	2.0±0.4*	1.7±0.1*
Extract (aqueous)	250mg/kg	2.3±0.1	3.9±0.2	3.7±0.1	3.5±0.2*	3.3±0.1*
Extract (ethanol)	250mg/kg	2.0±0.1	3.9±0.2	3.0±0.1	2.4±0.3*	1.5±0.1*

Values are presented as Mean ±SEM, n=5, Significant level, P<0.01 calculated by comparing with vehicle treated and control group.*

Table 4. Showing dose concentration and % inhibition of inflammation

Treatment group	Dose/ concentration	% inhibition of inflammation
Control	1ml/kg	-
Aqueous Extract	250mg/kg	39.27
Ethanol Extract	250mg/kg	53.45
Diclofenac sodium	10mg/kg	57.09

Table 5. Effect of *Celosia isertii* on acetic acid induced writhing in mice

Treatment dose(mg/kg. p.o)	Number of writhing			
	0 – 10min	10 – 20min	20 – 30min	Total
Control (1ml/kg)	18.22±1.2	21.33±1.2	8.00±0.6	47.55±2.9
Diclofenac sodium(10mg/kg)	8.20±1.0**	11.66±1.2**	3.16±1.2**	23.48±2.1**
Aqueous (250mg/kg)	10.00±1.0	17.5±0.6*	5.1±0.6*	32.76±2.2*
Ethanol (250mg/kg)	9.60±1.1**	10.66±1.2**	3.0±0.4**	23.26±2.1**

*Values are presented as Mean ±SEM, n=5, Significant level *P<0.01 was calculated comparing with vehicle treated and control group.*
**when control was compared with aqueous group.*
***when the comparism was with the other groups.*

In time course study rat treated with *Celosia isertii* (ethanol 250mg/kg) and diclofenac sodium (10mg/kg) elucidated maximum inhibition of edema formation at the fifth hour after carrageenan injection

While for the work on the analgesic properties of the extract we made use of mice induce with acetic acid and observe the writhing. Pretreatment with *Celosia isertii* (Aqueous 250mg/kg and Ethanol 250mg/kg) prevented acetic acid induced writhing movement in mice, the inhibitory effort of diclofenac sodium (10mg/kg) on acetic acid induced writhing was greater than Aqueous 250mg/kg but less than ethanol 250mg/kg as shown in the Table 5.

5. CONCLUSION

The results revealed the presence of medicinally important constituents in the plant *Celosia isertii*, and several studies carried out by numerous authors are supportive of this view. Therefore, the plant *Celosia isertii*, could be seen as a good source of bioactive chemical compounds which can be of great value in drug production. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should

be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to elucidate the possible mechanism of action of new constituents in the extract.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"of Delta State University, Abraka.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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