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Chromatographic Identification and the Effect of the Alkaloidal Extract of *Buccholzia coriaceae* Seeds on the Body Weights and Relative Liver Weights of Mice

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Abstract: *Buccholzia coriaceae* seed has been known to posses pharmacological activities. The extract of the seed is used as a remedy for headache, stomach ache, fever and inflammation. Alkaloids are alkali – like compounds. They constitute a large family of nitrogen – containing compounds of plant origin which are physiologically active when administered to humans. Alkaloids have been shown to possess both pharmacological and toxicological activities. The aim of this research was to extract and analyze the alkaloidal extracts of *Buccholzia coriaceae* seed as well as test the effects of the extract on the relative liver and body weights of mice. Different solvent systems were used in the chromatographic analysis of the alkaloidal extracts. Different alkaloidal fractions were identified using the references from a similar study. The alkaloidal extracts were shown to cause changes in body weights as well as the relative liver weights which were used as an index of hepatoxicity. The phenolic alkaloidal extract caused increases in body weights after four days of administration of extracts was compared with the control. The non-phenolic alkaloidal extract caused decreases in body weights after four days of administration of extracts when compared with the control. Also, the phenolic alkaloidal extract caused a significant increase in relative liver weights when compared to the control. The results of this study suggest possible toxic potential for the alkaloidal extract of the seed of *Buccholzia coriaceae*. The mechanism of this toxicity awaits further studies.

Key words: Buccholzia Coriaceae · Alkaloidal Extracts · Phenolic Fraction · Relative Liver Weights and Body Weights

INTRODUCTION

Over the years, the use of plant materials in the treatment of diseases has been well known. Different parts of the plant like the leaves, seeds, bark, stem and roots are used.

Buccholzia coriacea is one of the thirty six species of the *Capparidaceae* family [1, 2]. It is a tree which grows to about 20m in height. It is commonly known as the musk tree or wonderful cola and is found mainly in Guinea, Cameroon and Gabon [3].

Coriacea seed extracts have been exploited, its use as an antibacterial and antimalarial agent has made it imperative to evaluate its effects on reproductive functions [4]. *Buccholzia coriacea* is a perennial plant which grows as a tree. It belongs to the family *Capparaceaae* and its local names include Uworo (Yoruba), Owi (Edo) and Uke (Ibo). The plant parts commonly eaten are the seeds which are either cooked or eaten raw [5,6].

The term alkaloid means 'alkali – like' [7,8]. The term alkaloid is not a chemical notation but rather a name traditionally and conventionally accepted for a group of nitrogen – containing basic substances from plants with rather widely different chemical constituents.

The alkaloids are large group of many compounds, which differ greatly. This means the classification of alkaloids is very difficult. However, attempts have been made to classify alkaloids based on the parent compound [9] and also based on the parent amino acid [10-12].

Alkaloid bearing plants have been found in almost every habitat in which vascular plants grow, so no researcher should be short of plant material because of geographical constraints [13-15]. There are however, no taxonomic characteristics by which a plant may arbitrarily be assigned to a group suitable for alkaloid study.

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It has been well established that alkaloids have been found to occur in some thirty eight-plant families and it may be safely said that the remaining families will provide only an occasional alkaloid bearing plants [16,17].

In view of the pharmacological importance of *Buccholzia coriaceae* seed and the possible dependence of this property on its alkaloid content, this investigation is targeted at discovering the possible toxicological effects of the alkaloidal contents of *B. coriaceae* seed.

MATERIAL AND METHODS

Material

Plant Materials: The seeds of *Buccholzia coriaceae* were bought from the local Nsukka market, Enugu State, Nigeria. Identification of the plant material was done by Mr. Ozioko F. C. of the department of Botany, University of Nigeria, Nsukka.

Animals: Twenty adult albino mice were bought from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka.

Chemicals: The following chemicals which were of analytical grade were used for the experiment. 95% Ethanol, Hydrochloric acid, Ammonium Hydroxide, Chloroform M and B, Ethylacetate M and B, Sodium hydroxide, Anhydrous sodium sulphate UCB Chemical, Distilled Water, Hexane M and B, Acetic Acid, Iodine Crystals Merck, Olive oil and Methylated spirit.

Apparatus: Cages, Insulin syringes, Beam balance, Dissecting set, Dissecting board, Detergent, Capillary tube, Metre rule, Chromatogram Tank, TLC Plates (20 X 10CM), Silica gel G, Ultraviolet Lamp, Oven, Vaseline, Office pin, Measuring cylinders, Beakers, Separating funnel, Glass funnel, Filter paper, Volumetric flask, Glass slab, Weighing balance, Mettler H8, Retort stand, pH water and Instruments 8417.

Methods

Preparation of Reagents:

- 2% HCl- This was prepared by diluting 2ml of conc. HCl with 100ml of distilled water.
- 5% NaOH- This was prepared by dissolving 5g of NaOH in 100ml of distilled water.
- 0. IN NaOH- This was prepared by dissolving 0.2g of NaOH in 100ml of distilled water.

Extraction: 360g of the ground dry *Buccholzia coriaceae* seeds were soaked in 1000ml of 95% ethanol for 18 hours in an airtight volumetric flask. The mixture was then concentrated in vacuo and mixed with 2% HCl (300ml) and the mixture was filtered.

The filtrate was made alkaline with ammonium hydroxide solution and extracted with chloroform (160ml). The chloroform extract was evaporated in vacuo. The residue was then dissolved in ethylacelate to subdivide into ethyl acetate soluble fraction and ethyl acetate insoluble fraction.

The ethyl acetate- soluble fraction was further subdivided into phenolic and non-phenolic fraction by re-dissolving the material in chloroform and extracting with a 5% NaOH solution. The NaOH extractive was brought to pH 6 filtered and then made alkaline with NH_4OH solution and extracted with chloroform. The chloroform was dried with anhydrous sodium sulfate and evaporated to dryness in vacuo to give 30mg of a phenolic alkaloid fraction.

The chloroform mother- liquor remaining after the NaOH extraction was washed with distilled water, dried over anhydrous sodium sulphate, filtered and then evaporated to dryness in vacuo to give 500mg of a non-phenolic alkaloid fraction.

Thin Layer Chromatography (TLC) Assay: Thin layer chromatographic plates (20 x 10cm) were coated with silica gel, G. A slurry of the gel was made by mixing the gel with O.IN NaOH in the ratio of 1g of gel to 2ml of NaOH. The slurry was spread over the plates to a thickness of 0.25mm using a Shandam spreader. The plates were then left to dry in air overnight.

Before use, the plates were activated by heating in the oven for one hour at a temperature of 110°C. Solutions of the separated extracts were spotted on the thin layer plates, air dried and developed in a pre-equilibrated chromate tanks.

The developing solvent used was methanol using silica gel mixed with O.IN NaOH [13]. The solvent was allowed to equilibrate for one hour before use, in an airtight chromatographic tank.

Drops of both phenolic and non-phenolic extracts were applied at the origin of the thin layer plates. A development time of 45mins was allowed. This was the time required for the solvent front to move 10cm on the gel. The plates were removed and air-dried.

The dried plates were viewed under long wave UV light and the colour of the spots noted. The spots were developed in an iodine tank and the positions marked.

Furthermore, other solvent systems were used. They include: chloroform/methanol (30:1), ethanol, ethanol/ethyl acetate (3:1), chloroform/ethanol (20:1), methanol, hexane/acetic acid (3:1) and hexane/ethylacetate (20:1). The plates were prepared as described above. However, the silica gel G was mixed with distilled water and the thickness was 0.5mm.

Treatment of Animals with the Extract: Concentrations of the extracts were determined by weighing the extracts using a sensitive balance and dissolving in known amount of olive oil (solvent).

The dosage to be administered to each rat was calculated using the dosage control formula

$$\frac{Mg\ of\ drug\ per\ body\ weight}{1000} \times \frac{Weight\ of\ animals\ (g)}{cone\ of\ soln\ in\ mg/ml}$$

The optimum doses of 400mg/kg (non-phenolic alkaloid extract) and 50mg/kg (phenolic alkaloid extract) were used.

Altogether, three groups of mice were used. The control mice were injected with olive oil while the test animals were injected with the plant extracts consecutively for 3 days. The weight of each mouse was determined each day for four days.

The mice were sacrificed afterwards and the liver dissected and weighed.

RESULTS

Results of Thin Layer Chromatographic Analysis: The results above showed the different resolutions of the akaloidal extracts using different solvent systems on silica gel G (o.5mm thickness). The number of spots, their Rf values and their colour under long wave ultraviolet (U.V) light.

Table 2 shows the results of the thin layer chromatographic analysis of the alkaloidal extracts using methanol and basic silica get G prepared with O.IN NaOH. The experimental Rf values and reference Rf values were shown. The suggested alkaloid classes were based on the result of similar work done by Lynda Allouche *et al.[*13].

Table 1: Results of thin layer chromatographic analysis 1

Solvent System	No. of Spots	Rf Values	Colour Under Longwave UV.
Chloroform/methanol (30:1)	1	0.00	Violet blue
Phenolic extract	1	0.00	Violet blue
Non-phenolic extract			
Ethanol (absolute)	2	0.00	Violet blue
Phenolic extract	2	0.96	Yellow green
Non-phenolic extract		0.00	Violet blue
		0.94	Violet blue
Ethanol/ethyl/acetate (3:1)	1	0.00	Violet blue
Phenolic extract	1	0.00	Violet blue
Non-phenolic extract			
Methanol (absolute)	1	0.00	Violet blue
Phenolic extract	1	0.00	Violet blue
Non-phenolic extract			
Chloroform/ethanol (20:1)	1	0.00	Violet blue
Phenolic extract	1	0.00	Violet blue
Non-phenolic extract			
Hexane/acetic acid (3:1)	3	0.94	Yellow green
Phenolic extract		0.50	Violet blue
Non-phenolic extract		0.44	Violet blue
		0.94	Yellow green
		0.45	Violet blue
		0.43	Violet blue

Table 2: Result of thin layer chromatography II

Solvent System	No. of Spots	Experimental Rf Values	Reference Rf Values	Colour Under Long Wave U.V	Suggested Alkaloid Classes
Methanol	2	0.00	0.00	Violet blue	Narceine,
phenolic extract		0.97	0.72	Yellow green	Sarpagine
Non-phenolic	2	0.00	0.00	Violet Blue	Narcotine
extract		0.94	0.72	Yellow Green	Dihydroerg-otamine Narcotine

Table 3: Mean changes in body weights (g)

Group	1st Day	Last Day	Change in Result
Phenolic fraction			
Mice 1	28.7	28.9	0.2
Mice 2	3.8	39.6	0.8
Mice 3	25.9	35.9	0.0
Mice 4	27.4	28.2	0.8
		Mean change in WT = 0.6 ±0.346	
Non-phenolic fraction			
Mice 1	37.4	35.2	-2.2
Mice 2	39.6	37.9	-1.7
Mice 3	36.2	36.7	-0.5
Mice 4	33.2	33.1	-0.1
		Mean change in WT = -1.13±0.988	
Control			
Mice 1	27.2	26.9	-0.3
Mice 2	28.5	28.9	0.4
Mice 3	35.4	36.5	1.1
Mice 4	29.7	29.1	-0.6
		Mean change in WT = 0.15±0.759	

Table 4: Effect of the extracts on the relative liver weights

Sample	Mean Liver WT (G)/100G Body WT
Non-phenolic extract	3.51±0.547
Phenolic extract	4.12±0.296
Control	3.58±0.439

Results of Effect of Intraperitoneal Administration on the Phenolic and Non-phenolic Fractions of the Akaloidal Extract of *B.coriacea* on the Body Weights of Mice after 4 Days of Treatment with the Extract

The result above indicates that the mean change in body weights for the mice treated with the phenolic alkaloid extract was high while the mean change in body weight for those treated with the non-phenolic alkaloid extract decreased in relation to the control animals. The mean body weight per day increased in phenolic alkaloid group while the mean body weight per day decreased in non-phenolic alkaloid group in relation to the control animals.

Result of Effect of the Extracts on Relative Liver Weights of the Mice The mean liver weights per 100g body weights were calculated for each of the three groups and were shown in table 4.

The result above shows that the optimum dose of the non-phenolic alkaloids extract did not cause any significant change in liver weight/100g body weight whereas the phenolic alkaloid extract did when compared with the control.

DISCUSSION

The results presented in tables 1 and 2 shows that hexane/acetic acid (3:1) solvent system separated the alkaloidal extracts better than the other solvent systems. However, the different alkaloidal fractions could not be identified due to absence of standard reference Rf values for this solvent system.

The results observed in figure 1 and presented in table 2 show that the solvent system used (methanol) as suggested by Lynda Allouche *et al*. and Randerhath

[13, 18] gave some separation. In addition, when compared with standard Rf values, the following alkaloids were suggested: Phenolic alkaloidal extract, Narceine, Sarpagine and Narcotine. Non-phenolic alkaloidal extract, Narcotine and dihydroergotamine.

The results of the effect of extracts on body weights showed that there was a marked difference between the body weights of the experimental animals treated with phenolic fraction as well as those treated with the non-phenolic fraction and the control animals. The mean difference in body weights show that those treated with phenolic extract gained weight while the animals treated with the non-phenolic extract lost weight.

It was observed that the relative liver weights of the animals treated with the phenolic alkaloid extract increased significantly compared to the relative liver weights of the controls. However, the relative liver weights of the animals treated weight non-phenolic extract showed no significant deviation from the control animals.

CONCLUSION

From the observations made with reference to the above results, it may suggest that there is interference of the extracts with normal growth and development of the animals. This corresponds with the result of treatment of animals with ethanolic extract of *Bucholzia coriaceae* [14] which gave rise to changes in body weights of the experimental animals.

Furthermore, one could infer that the ethanol extract component that gave rise to the observed changes exerted similar action to those of the alkaloids under study.

In addition, the increase in relative liver weights caused by the treatment of animals with the phenolic extract could serve as an index of hepatotoxicity. Change in relative liver weight caused by treatment of animals with drugs have been used in several studies as an index of hepatotoxicity [14,15].

Also, the methanolic extract of *Bucholzia coriaceae* caused an increase in the serum glutamate oxaloacetate transminase activity which was attributed to damage in the liver of experimental animals [13-18].

In view of these evidences, the change or difference in the relative liver weights of animals treated with the phenolic alkaloidal extract could be attributed to hepatotoxicity which may have been induced by the alkaloidal extract of the plant.

However, further investigation should be carried out to ascertain the individual alkaloid (s) responsible for this toxicological effect as well as elucidate their mechanism of action and subsequent modifications of the alkaloid(s) to reduce toxicity.

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