Volume 2 | Issue 1 | Page 5 - 9

Ewemen Journal of Natural Product Research
ISSN: 2504-8643

Available online at http://ewemen.com/category/ejnpr/

Original Research

ASSESSMENT OF SOME PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF AN 'INSOLUBLE' EXTRACT FROM THE STEM OF *CHENOPODIUM AMBROSIOIDES* LINN

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Received 4 July, 2017
Revised on the 12 July, 2017
Accepted 17 July, 2017

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ABSTRACT

This work aimed to determine some physical properties, test for possible bioactive compounds and antimicrobial activity of an extractive which had been obtained as a by-product of another research work which involved cold acid extraction of the stem of *Chenopodium ambrosioides* Linn with 0.1M HCl. The extract was obtained by cold acid extraction of the stem of *Chenopodium ambrosioides* Linn with 0.1M HCl. Solubility and melting point were determined and colour observed. Presence of unsaturation and functional group, as well as phytochemicals in the extractive were determined. Antimicrobial activity was determined using *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosae* and *Staphylococcus aureus*. The results showed that the extractive was insoluble in petroleum ether, ethyl acetate, acetone, carbontetrachloride, dichloromethane, benzene, n-hexane, tetrachloromethane, n-butanol, acetic acid and cyclohexane; while it was sparingly soluble in water, methanol, ethanol and chloroform. Melting point range was 185-190°C. Phytochemical screening revealed that steroids/terpenoids, flavonoids, unsaturated compounds and alcohols were present, while alkaloids and tannins were absent. The extract inhibited the growth of all the test microorganisms. The antimicrobial activity of the 'insoluble' extract could be attributed the presence of the bioactive compounds mentioned above.

Keywords: Physicochemical properties, Antimicrobial, 'Insoluble' Extract

INTRODUCTION

Much has been reported on the phytochemicals and some pharmacological activities of the crude extract and some compounds isolated from *Chenopodium ambrosioides* Linn. These include its use as an antihelmintic drug (Ketzis and Brown, 1998); its usefulness in curing anorexia, cough, dysentery, diarrhea, oedema and piles (Bakshi *et al.,* 1999). Lohdip *et al.,* (2009 and 2008a,b) have reported the effect of the crude extract as well as sugar extracted from *C. ambrosioides* Linn on cat blood pressure; as well as anti-venom activities, antibacterial, anti-inflammatory, analgesic and antipyretic activities of the plant.

Chenopodium ambrosioides Linn in the family *Chenopodiacea* is a multi-branched, reddish stemmed herb, covered with small sharply toothed leaves. The leaves, numerous small yellow flowers that are in clusters, as well as a distinctive ordour are its major characteristics. It produces thousands of tiny black seeds in small fruit cluster and is easily spread and re-grown from the numerous seeds it produces. It is believed to be native to Mexico and the Tropical regions of central and South America, but has now been widely naturalized throughout the world, including Nigeria (Lohdip *et al*., 2009 and 2008a,b; Taylor, 2004; Burkill, 1985). Its pharmacological activities have been reported over the years.

This paper is aimed at reporting the analysis of an extractive which was obtained as a by-product of another research work which involved cold acid extraction of the stem of *Chenopodium ambrosioides* Linn with 0.1M HCl (Harborne, 2003). The unexpected and extractive was to be discarded as but the study team decided to screen it for the presence of bioactive compounds and possible antimicrobial activity, with a view of possible application as a raw material for drug production in the future.

MATERIALS AND METHODS

Materials

All reagents used were of analar grade and procured from Yokel International Company, No. 6 Niger Avenue, Jos, Nigeria.

Plant Collection and Preparation

The plant (whole) was collected from Jos North Local Government of Plateau State, Nigeria, in June. After collection, the plant was identified at the herbarium in the Federal College of Forestry, Bauchi Road, Jos. This corroborated an earlier authentication done at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where 1921 was given as the Voucher specimen number. Various parts of the plant (leaves, stems and roots) were separated manually and the stems were washed thoroughly with water and dried under shade for at least two weeks. The dried stem sample was pulverized using a mortar and pestle and the powder stored in a black air-tight polythene bag for further use.

Preparation of the Extract

The extract was obtained as a by-product during a cold acid extraction with 0.1M HCl during another experiment. The stem powder (150 g) was extracted in 0.1M HCl ($1000cm^3$). The mixture was allowed to stand for about 18 hours after which the supernatant liquid was decanted and filtered with a Whatman filter paper. The filtrate was concentrated to about one quarter of the volume using a rotary evaporator. It was basified by dropwise addition of concentrated ammonia solution, checked with the help of a limus paper. During the basification, a precipitate (which we call the 'insoluble extract) was formed. This precipitate was filtered and dried in an oven at about 50°C for about 1 hour. This was stored in a desiccator for further use.

Test for Solubility

The solubility of the extract in petroleum ether, ethyl acetate, acetone, carbontetrachloride, dichloromethane, benzene, n-hexane, tetrachloromethane, n-butanol, acetic acid, cyclohexane, water, methanol, ethanol and chloroform was determined at room temperature (30°C) by adding about 0.1 mg of the sample to 1 ml of each solvent and shaking for at least 1 minute (Online Lab Manual Home, 2017). The value of the solubility product constant, K_{sp}, for the extractive was not known, therefore, it was not possible to determine the actual solubility values in the various solvents used. However, the clarity of the solvents after shaking with the extractive (1 mg/ml at 30°C) was taking as evidence of solubility.

Melting point

The melting temperature range of the extract was determined following as described by John and Chris (2010).

Phytochemical screening

Phytochemical screening was performed on the extract for the presence of alkaloids, tannins, flavonoids, steroids/terpenoids, saponins, carbohydrates and alcohols as described in literature (Harborne, 2003; Evans, 1989).

Antimicrobial Test

Test for antimicrobial activity of the insoluble' extract was done using four bacteria: *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosae, Staphylococcus aureus*. The Muiller Hinton Agar Diffusion method was employed as in literature (Falkow *et al*., 1987; Cheesebrough, 1984; Novick *et al*., 1979).

The extract was only sparingly soluble in water, so it was made soluble by adding and mixing with a drop of 'Tween 80'. The various concentrations of the extract (60, 30, 15 and 7.5 mg/ml) and the standard, gentamycin (20 µg/ml)

were used for the test. The experiment was observed after 24 hours and zones of inhibition (ZI) were taken in millimeters (mm) by measuring the diameter horizontally and vertically and the mean determined. The plates were further incubated for another 24 hours and zones of inhibition were again taken. The mean zone of inhibition for the two days was determined for each concentration.

The ZI values were expressed as $\bar{x} \pm SE_{\bar{x}}$ where $\bar{x}$ is the Arithemetic Mean and $SE_{\bar{x}}$ is the Standard Error of the Mean. $SE_{\bar{x}}$ was calculated using the formula in equation 1 below, where s = Standard Deviation. The standard deviation was calculated using equation 2 (Probability and statistics, 2017).

$$SE_{\bar{x}} = \frac{S}{\sqrt{N}} \text{ ----------1}$$

$$s = \sqrt{\frac{1}{N}\sum_{I=1}^{N}(x_i - \bar{x})^2} \text{ ----------2}$$

RESULTS AND DISCUSSION

Solubility

The results for solubility showed that the extractive was insoluble in most of the solvents used, which included: petroleum ether, ethyl acetate, acetone, carbontetrachloride, dichloromethane, benzene, n-hexane, tetrachloromethane, n-butanol, acetic acid, cyclohexane, etc; while it was sparingly soluble in water, methanol, ethanol and chloroform. It is important to note that the extractive was termed 'insoluble' because it was insoluble in most of the solvents and only sparingly soluble in only a few.

Melting point

Melting point range of 185-190°C with decomposition. This high melting temperature (range) implies that the extract contains highly polar bonds that leads to hydrogen bond and also that the possible compound(s) is/are of high molecular weight (Finar, 1977).

Phytochemical screening

The phyto-constituents present in the 'insoluble' extract from the stem of *Chenopodium ambrosioides* Linn are summarized in Table 1. The result of the phytochemical screening revealed that steroids/ terpenoids, flavonoids, unsaturated compounds and alcohols were present, while alkaloids and tannins were absent.

Table 1: Results of phytochemical screening of 'insoluble' extract.

Phytochemical parameter	Inference
Alkaloids	-
Saponins	+
Tannins	-
Flavonoids	+
Steroids/Terpenoids	+
Unsaturated compounds	+
Carbohydrates	+
Alcohols	+

Key: + = Present - = Absent

The presence of these compounds may account for some of the antibacterial activities reported in this work. Some saponins have been reported to show antimicrobial activity against selected Gram-positive and Gram-negative organism (Cheesebrough, 1984; Novick *et al*., 1979). Literature has also reported that a saponin extracted *Euphorbia hirta* Linn was active on *P. aeruginosae* and *S. auerus*; also that some flavonoids (condensed tannins) were active on *E. coli, P. aeruginosae, S.aureus* and *Salmonella typhi* (Oyewale *et al*., 2002). Also, the presence of these compounds implies that the 'insoluble' extract could possess some other pharmacological activities such as anti-inflammatory activity due to saponins and flavonoids (Evans, 1989).

Antimicrobial Activity

The results for test for antimicrobial activities of the 'insoluble' extract from the stem of *Chenopodium ambrosioides* are summarized on Table 2.

For the antimicrobial test, different concentrations of the 'insoluble' extract were used (60 mg/ml, 30 mg/ml, 15 mg/ml and 7.5 mg/ml). The extract gave considerable inhibition against *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Bacillus subtilis* with the concentration 60 mg/ml. The activities of the test organisms could be attributed to the presence of some natural products as shown in Table 1.

Table 2: Result of antimicrobial test of the 'insoluble' extract

Bacteria	Concentrations of test extract (mg/ml)				Gentamycin (μg/ml)
	60.00	30.00	15.00	7.50	20.00
	Zones of inhibition (mm)				
E. coli (G-ve)	22.25±0.17	0.00±0.00	0.00±0.00	0.00±0.00	24.50±0.20
S. aureus (G+ve)	30.75±0.16	26.25±0.12	23.00±0.35	21.50±0.35	35.75±0.17
P. aeruginosae (G-ve)	27.50±0.00	25.75±0.11	17.75±0.06	16.50±0.00	28.25±0.12
B. subtilis (G+ve)	27.50±0.17	18.75±0.35	9.25±0.12	0.00±0.00	33.25±0.17

Key: 0.00 = no inhibition, ZI given in mean±SEM

From the zones of inhibition (ZIs) in the result, all the test organisms could be said to be sensitive to the extract because any extract with ZI (diameter) up to 6 mm (0.6 cm) or more is considered active (Boda, 1997); any with ZI between 6-13 mm (0.6-1.3 cm) is intermediate, while any above 13 mm (1.3 cm) is appreciable (Sofowora, 2008).

From the result obtained the degree of inhibition by the 60 mg/ml concentration, especially on *Escherichia coli* and *Pseudomonas aeruginosa,* was very high compared to that of standard drug (gentamycin) used. This work agrees with the report on antimicrobial activity of aqueous extract of *Chenopodium ambrosioides* (Lohdip *et al.*, 2008a,b), with reported that the basic extract was more active than the crude aqueous extract.

The fact that the extract showed activities on the test micro-organims implies it has some medicinal potential which include the possible use to treat or manage sores, swellings and abscesses which could be infected by the micro-organisms used in this experiment. *E. coli, P. aeruginosae, S. aureus* and *P. mirabilis* are known to infect wounds. The antimicrobial activities of the extract also imply that it could be useful in treating or managing certain conditions and diseases caused by these microorganisms. For instance, *S. aureus* is reported to cause necrosis and toxic shock syndrome (TSS) (Novick *et al*., 1979). *E. coli* causes urinary tract infections, diarrhea, dysentery, etc. *P. aeruginosae* infects the respiratory tract. B*. subtilis*, even though recognized by some medical practitioners as being safe, can cause opportunistic infections (Brisson, 2017). This result also implies that the extract could be useful in managing HIV (Human Immuno-deficiency Virus) patients since *S. aureus and P. aeruginosae* are reported to have been involved in secondary infections in HIV patients, such as superficial wound and burn infections (Novick *et al*., 1979; Falkow, 1987; Cheesebrough, 1984).

It is worth noting that the 'insoluble' extract was active on both Gram positive and Gram Negative bacteria. *S. aureus* and B. *subtilis* are G*+ve* (Gonzalez *et al*., 2011) while *E. coli* and *P. aeruginosae* are G-ve (Wikipedia 2017a-c; *Pseudomonas aeruginosae* Infections, 2017; Obritsch *et al.*, 2004). The G-*ve* bacteria are known to be generally resistant to antibiotics (Gram-Negative Bacteria Infections in Healthcare Settings, 2017; Nutanbala *et al*., 2011; Obritsch *et al*., 2004). Even though B*. subtilis* is G*+ve*, when it causes opportunistic infections it resists antibiotics used to treat it (Brisson, 2017; Gonzalez *et al.*, 2011). This, therefore, suggests that this 'insoluble' extract could be a leeway to solving the problem of the resistance of these organisms to antibiotics, if the extract is purified and compounded into an antibiotic putting into consideration the issue of toxicity.

CONCLUSION

A comparison of the result of phytochemical screening of the 'insoluble' extract to that of the crude aqueous extract of *Chenopodium ambrosioides* Linn (Lohdip, 2012) showed that apart from alkaloids and tannins which were absent in the former, it contains almost all the classes of the pharmacological compounds found in the crude extract.

The 'insoluble' extract was found to inhibit the growth of *E. Coli, P. aeroginosae* as well as *S. aureus* as had earlier been reported for the crude extract. In addition, the former was also found to be active on *B. subtilis*. The above assertions imply that this 'insoluble' extract 'which hitherto was to be thrown away in the course of another work' is not a waste, after all. It promises to be a very useful raw material for developing a novel antibacterial drug. Infact, it could bring a solution to the problem of resistance of the microorganisms to antibiotics since it was active on the resistant G-*ve* bacteria as well as *B. subtilis* which is resistant drugs even though it is G*+ve*.

ACKNOWLEDGEMENT

The authors appreciate the assistance of Rev. D. Ayewah, Mr. S. Atsen and Mr. S. Dasam of the Department of Chemistry, University of Jos, Nigeria for their assistance in provision of some of the materials used for the lab works. Mr. Y. Thomos of the Department Pharmacognosy, University of Jos, Nigeria is also acknowledges for his assistance during the phytochemical screening. Mr. P.O. Adah of the Department of Pharmaceutical Technology, University of Jos, Nigeria is acknowledged for his assistance with the antimicrobial screening.

CONFLICT OF INTEREST

None declared.

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Article Citation:

Lohdip AM, Uduah OV, Ogunga OO and Akingbemisihi TS (2017). Assessment of some physicochemical properties and antimicrobial activity of an 'insoluble' extract from the stem of *Chenopodium ambrosioides* Linn. Ew J Nat Prod Res 2(1): 5 - 9.