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Original Research Article

Comparative antimicrobial activity of A*calypha wilkesiana* extracts and cream formulations

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Abstract

Purpose: This study was carried out to compare the antimicrobial activities of leaf extracts of *Acalypha wilkesiana* Muell Arg. (Euphorbiaceae) and cream formulations.
Methods: Extracts of *Acalypha wilkesiana* was obtained using four approaches; maceration of the powdered leaves with methanol, or water (hot and cold) and boiling the leaves and kept overnight before filtration. Agar diffusion method was used to determine the antimicrobial activity of the various extracts against test organisms and diameter of zones of inhibition measured. The extract with the best activity was formulated into creams and evaluated microbiologically against a commercial antimicrobial cream with plain creams acting as controls.
Results: The percentage yield of the extraction process was 1.7 % with methanol, 1.9 % with water, 4.04 % with boiled water and 2.72 % with the boiled and left overnight method. Diameter of zones of inhibition produced against gram-positive bacterium (*Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and *Candida albicans* showed that the boiled aqueous extract was more potent in its antimicrobial activity. *Pseudomonas aeruginosa* was not inhibited by all the extracts.
Conclusion: The cream formulations of the superior extract was stable and had significant antibacterial and antifungal activity against the test pathogenic organisms.

Keywords: *Acalypha wilkesiana*, extraction methods, zones of inhibition, antimicrobial.

Indexing: Index Copernicus, African Index Medicus

Introduction

Medicines derived from plants are the oldest health care products [1]. Their importance is still growing although it varies depending on the ethnological, medical and historical background of each country. These days, with the increase in industrialization, most medicinal plants are being formulated into pharmaceutical dosage forms like tablets, creams, ointments, syrups, and lotions. Pharmaceutical industries have come to consider traditional medicines as a source of bioactive agents that can be used in the preparation of medicines. Many of the pharmacologically interesting medicinal plant species in use around the world are employed in more than one community, and often in more than one country for multiple uses [2].

Acalypha wilkesiana Muell Arg. (Euphorbiaceae) is a tropical and sub-tropical evergreen shrub of Fijian origin. It grows 3 m high and spread 2 m across. The leaves are bronze red to muted red colour splashed with rose-pink, crimson, pink or brown often bordered with various colours. They are 4-8 inch long and heart shaped [3]. It is used as an ornamental plant in parks and gardens as it is a popular outdoor plant that provides colour throughout the year. The leaf decoction of this plant is popularly used in *Yoruba* traditional medicine in Nigeria for the treatment of gastro-intestinal disorders and fungal skin infections [4,5]

Many studies have been carried out and reported on *A. wilkesiana* leaf extracts especially of its antimicrobial activities [5-7]. This study is aimed at comparing the antimicrobial activity of *A. wilkesiana* extracts from different extraction approaches against

gram-positive organisms (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*) and a fungus (*Candida albican*) and formulating the extract with the best activity into topical creams for antimicrobial activity evaluation.

Experimental

Materials

Methanol and chlorocresol (BDH Chemicals Ltd, Poole, England), Cetomacrogol emulsifying wax, white soft paraffin and liquid paraffin were obtained from Halewood Chemicals Ltd, England, U.K, Mueller Hinton and Sabouraud Agar (HiMedia Laboratories Ltd). A commercial cream containing clotrimazole 1.0 %w/w and neomycin sulphate 0.5 %w/w was purchased locally at a pharmacy in Benin City. The fresh leaves of *Acalypha wilkesiana* were collected from the premises of Faculty of Pharmacy, University of Benin, Benin City, Edo State. An authenticated sample specimen was deposited in the local herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin Benin City, Edo State. Standard strains of the micro-organisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Edo State.

Extraction of Acalypha wilkesiana

The fresh leaves of *A. wilkesiana* were collected, washed and sun-dried over a period of 7 days and then blended into powder. Fifty grams of the powder was mixed with 500 ml of methanol and 0.33 % sodium metabisulphite solution and macerated over a period of 48 h with intermittent stirring every 4 h for 12 h. Sodium metabisulphite was added to prevent oxidation and discolouration. The mixture was filtered through a filter paper No. 1 (Whatman, UK) and the filtrate concentrated to dryness using a hot water bath. The same procedure was repeated for the aqueous extract using distilled water as the solvent.

About 500 g of fresh leaves of *A. wilkesiana* were divided into two parts of 250 g each. Each part was placed in a separate beaker containing 500 ml of water and boiled. The boiled content of one beaker was filtered immediately while the other was left to stay overnight before filtration. The filtrates were concentrated to dryness using a hot water bath. The percentage yield of the extracts obtained using these four different approaches was computed.

Antimicrobial activity of the extracts

The agar diffusion seeded plate method was employed to assess the antimicrobial activity of *Acalypha wilkesiana* extracts. Mueller-Hinton agar was used for the bacteria organisms and Saboraud agar used for the fungi. Five (5) sterile petri dishes were prepared and labelled for each extract. Agar bottles containing 20 ml freshly prepared Muller Hinton or Saboraud agar were cooled to 45 ºC and inoculated aseptically with 0.3 ml of the test organisms. The agar bottles were rotated and swirled to allow for mixing. The bottle contents were poured into the corresponding labelled petri dishes and allowed to set.

A sterile cork borer was used to bore holes (wells) into the solidified seeded agar in the plates. The bottom of each hole was sealed with a drop of molten agar. A sterile syringe was used to introduce 0.4 ml of 4 %w/v solution of extracts into the wells. The plates were left for 40 min to allow for diffusion of the extract into the agar and incubated at 37 ºC for 48 h. Three plates was used per extract and the diameters of zone of inhibition produced by the extracts were measured and their mean values reported [8].

Preparation of cetomacrogol ointment BP

Cetomacrogol emulsifying wax (30 g), white soft paraffin (50 g) and liquid paraffin (20 g) were melted together in a porcelain dish over a hot water bath and allowed to cool [9].

Preparation of *Acalypha wilkesiana* extracts cream

The boiled aqueous extract (BAE) with the best antimicrobial activity was formulated into creams of different concentrations. Cetomacrogol ointment BP (9 g) was melted over a hot water bath in a porcelain dish. A mixture containing 0.5 g of the extract and 3 ml of chlorocresol (equivalent to 0.03 %) added to 19 ml of distilled water was brought to the same temperature (60 ºC) as the melted ointment over a hot water bath and mixed. The mixture was transferred to a cold water bath and stirred continuously until a cream was formed to give a 1.67 %w/w cream [10]. This procedure was repeated using 1 and 2 g of the extract to give 3.33 and 6.67% w/w creams respectively. Plain cream (without extract) containing chlorocresol was formulated to act as control. A commercial cream labelled to contain 1.0 %w/w clotrimazole and 0.5 %w/w neomycin sulphate was purchased to be tested as a comparative sample.

Physical properties of formulated creams

The creams were observed physically for stability, changes in colour, odour and texture. The pH was determined using a pH meter by dissolving 1 g of the creams in 10 ml of purified water (Hanna Instruments).

Antimicrobial activity of formulated creams

The antimicrobial activity of the formulated creams was tested using the same procedures employed in testing the extracts. The procedure was repeated after 2 and 4 weeks with the creams stored at ambient temperature conditions.

Results

The percentage yield of the extraction process was 1.7 % with methanol, 1.9 % with water, 4.04 % with boiling water and 2.72 % with the boiled and left overnight method. The methanol extract was greenish-black in colour with a pungent odour while the extract from the other methods was reddish-brown with a characteristic odour. All the extracts were soluble in water.

Antimicrobial activities of the extracts measured as diameters of zone of inhibition are shown in Table 1. The mean diameters of zone of inhibition produced against gram-positive bacterium (*Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and *Candida albicans* showed that the boiled aqueous extract (BAE) was more potent in its antimicrobial activity than the others. At the extract concentration used, there was no antimicrobial effect against *Pseudomonas aeruginosa* for all the extracts.

Table 1: Zones of inhibition produced at 4 %w/v of the crude extracts (n=3)

Organisms	Diameters of zone of inhibition (mm)			
	ME	AE	BAE	BOE
Staphylococcus aureus	8	12	16	12
Escherichia coli	8	12	12	8
Klebsiella pneumonia	4	4	6	4
Pseudomonas aeruginosa	-	-	-	-
Candida albicans	12	12	16	12

ME = methanol extract, AE = aqueous extract, BAE boiled aqueous extract, BOE = boiled and left over-night extract.

The creams formulated were dark green in colour except the plain creams that were white in colour. All the creams had a smooth texture when applied on a surface. The pH of 20 % solution of the 1.67, 3.33 and 6.67 %w/w cream were 5.8, 5.6 and 4.95, respectively, while those of the plain cream (control) and commercial cream were 4.6, and 5.71, respectively. This means that the creams are suitable for skin application which has an average pH of 5.27 [11].

The antimicrobial activities of the creams measured as diameters of zone of inhibition are shown in Table 2. There was a reduction in the diameters of zone of inhibition produced against the test organisms at 2 and 4 weeks. The commercial antimicrobial cream was most effective against all the organisms with no noticeable reduction in diameters of zone of inhibition. The plain creams showed no antimicrobial effect on all test organisms.

Discussion

A comparative antibacterial and antifungal activity of *A. wilkesiana* extracts from different methods of extractions have been evaluated in this study. Results from the study agree with previous works done on the leaf where methanol or water was used as the solvent of extraction [12-14].

The percentage yield of the extracts from the four extraction approaches were low when compared with those of other studies. These low values could be attributed to the high efficiency of the filtration process of the extraction. The crude extracts irrespective of method of extraction had activity against virtually all the test organisms except *Pseudomonas aeruginosa*. A possible explanation for this lack of activity could be that the concentration of the crude extract was not high enough to cause any activity. This is in line with the works of Enwa [15] and Gotep, *et al*., [16] where they increased their extract concentration for activity against *P. aeruginosa*. The extract from the boiled leaves gave the highest activity and this supports the traditional method of extraction by West African local inhabitants for treating skin problems where the leaves are boiled with water, filtered and used immediately [17]. It would appear that heated water is vital in extracting the leaf components responsible for its antimicrobial activities. This being the case, the

Table 2: Zones of inhibition produced by the cream formulations

Conc. (%w/w)	Diameters of zone of inhibition (mm)											
	24 h				2 weeks				4 weeks			
	SA	*EC*	*KP*	*CA*	*SA*	*EC*	*KP*	*CA*	*SA*	*EC*	*KP*	*CA*
6.67	18	16	10	20	12	10	8	16	12	10	6	16
3.33	12	8	8	14	10	10	6	12	10	8	4	14
1.67	12	8	4	10	8	8	4	10	8	6	4	12
Control	-	-	-	-	-	-	-	-	-	-	-	-
CC	30	12	16	25	30	12	16	25	27	12	16	25

CC = Commercial cream, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, KP = *Klebsiella pneumonia*, CA = *Candida albicans*

extract from the boiled leaves that was left overnight before filtration would be expected to have as much activity as the other that was boiled and filtered immediately. Results showed a reduced activity for this extract which could mean that the antimicrobial components of the extract may have undergone precipitation and degradation. Therefore, it would be advisable that a fresh extract be used since the solubility of the active principles tends to decrease on storage.

The 6.67 %w/w cream formulation of the boiled extract had the highest antimicrobial effect on the test organisms although not comparable with the commercial cream. *C. albicans* was the most sensitive to the cream, followed by *S. aureus*, then *E. coli* and *Klebsiella spp.* This supports previous research, where it was observed that the leaf extract possessed a broad spectrum of activity on both fungi and bacteria with Gram negative bacteria being more resistant than Gram positive bacteria [6]. The reduction in the diameters of zone of inhibition of the extract with time may have resulted from the decreased antimicrobial activity possibly due to aging since it is a natural product. The least antimicrobial activity with *Klebsiella pneumonia* was not surprising as earlier observed by Alade and Irobi [12] working with an aqueous leaf extract reported no inhibitory effect on this organism while Enwa [15] reported some activity with methanol extract.

The plain cream (negative control) did not show any activity against any of the test organisms. The plain cream containing chlorocresol solution was so formulated to determine if chlorocresol will have activity against the micro-organisms. This indicates that chlorocresol solution did not have any activity at 0.03 % used.

Conclusion

A comparison of the antimicrobial activities of the extracts from the four extraction approaches employed in the study reveals that the extract obtained from boiling the leaves and immediately filtering exhibited a superior activity hence this approach is suggested as the best method for aqueous extraction. The cream formulation of the extract was also stable and retained its antimicrobial activity in this dosage form.

Declarations

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. JOE conceived and designed the study, SOE supervised the laboratory works, collection, analysis of data and write-up, MIA co-supervised the laboratory works and reviewed the write-up while AOO carried out the laboratory work.

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