Available online through

www.jbsoweb.com

Research Article

EVALUATION OF ANTIMICROBIAL PROPERTIES OF HERBAL OINTMENTS FORMULATED WITH ETHANOLIC EXTRACT OF *ACALYPHA WILKESIANA*

Azubuike Chukwuemeka P*, Igbokwe Nwamaka H, Essien Godwin S, Elendu Ngozi J

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria

***Correspondence**

Azubuike Chukwuemeka P

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria

DOI: 10.7897/2321-6328.01201

Article Received on: 02/04/13

Accepted on: 10/06/13

Abstract

The antimicrobial activity of ethanolic extract of dried fresh leaves of *Acalypha wilkesiana* and also those of the ointments formulated with the extract was evaluated. The preliminary *in vitro* antimicrobial activity of the extract at concentrations of 2.5 mg/ml, 5 mg/ml and 10 mg/ml and those of the ointments was determined against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus flavus* and *Penicillium notatum* using the agar cup plate method. Their Minimal Inhibitory Concentrations (MIC) were also determined by agar dilution method. Herbal ointments formulated by incorporating the ethanolic extract of *Acalypha wilkesiana* (10 % w/w) into emulsifying ointment and simple ointment bases respectively and a commercial brand (Funbact A® cream) were evaluated for their *in vitro* antimicrobial efficacy and some physical properties. The zones of inhibition (mm) of the extract on the growth of the microorganisms were *Escherichia coli* (25.2 ± 1.0), *Staphylococcus aureus (*28.1 ± 0.5), *Pseudomonas aeruginosa* (32.4 ± 0.4) and *Candida albicans (*22.4 ± 1.3) but there were no zone of inhibition for *Aspergillus flavus* and *Penicillium notatum.* The MIC values of the extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans* were 5.00, 1.25, 10.00 and 5.00 mg/ml respectively. The formulation containing *Acalypha wilkesiana* extract in emulsifying ointment showed better antibacterial activity than the simple ointment. The herbal ointment also compared favourably with a commercial brand of Funbact A® cream. This study shows that ethanolic extract of *Acalypha wilkesiana* has both antibacterial and anti-candidal activity and also has high potential as antimicrobial agent when formulated as ointment for topical use.

Keywords: *Acalypha wilkesiana*, antimicrobial properties, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Candida albicans*, herbal ointments.

INTRODUCTION

The issue of resistance of dermatological infections to some medicaments available in the market has sparked up interest in the research of the antimicrobial properties of drugs from natural sources which are active against the common causative agents to skin infections[1]. The renewed interest in the use of medicinal plants may be attributed to cheapness, availability and accessibility by the local populace, high incidence of side effects of synthetic medicines and environmental friendliness of plant extracts. One of the ways to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Apart from the problem of resistance, environmental degradation, cost and pollution associated with irrational use of orthodox medicines have necessitated renewed interest in nature as a source of effective and safer alternatives in the management of human infections[2]. Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine[3]. *Acalypha wilkesiana* Muell Arg. (syn. *Acalypha amentacea*; *Acalypha tricolor*) belongs to the family Euphorbiaceae (spurge family). It grows as a spreading evergreen shrub with upright branches that tend to originate near the base. It is a native of tropical Asia that has been introduced to many tropical countries[4]. Investigations have been carried out with respect to the phytochemical and medicinal uses of *Acalypha wilkesiana* [4-9]. Consequently, the plant has been reported to have antimicrobial properties[8,10]. Some of these studies have confirmed that the nature of the solvent and extraction process employed plays very crucial roles in the phytochemicals present in an extract, hence their medicinal properties. There are still limited studies on the formulation of ethanolic extract of dried fresh leaves of *Acalypha wilkesiana* as solid dosage preparations. Secondly the type of ointment base and formulation process affects the antimicrobial potency of the preparations. The aim of this study is to evaluate the antimicrobial properties of ethanolic extract of dried fresh leaves of *Acalypha wilkesiana* and to formulate an effective antimicrobial ointment using the ethanolic extract.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Acalypha wilkesiana* (Euphorbiaceae) were bought from Mushin market in Lagos State, Nigeria. They were collected in February, 2012 and were identified and authenticated at the Herbarium of the Department of Botany and Microbiology, University of Lagos. Voucher specimen (PCGH LAB 40) for *Acalypha wilkesiana* was deposited for future reference.

Test microorganisms

The microorganisms used for the study were clinical isolates of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa Candida albicans, Aspergillus niger* and *Penicillium notatum* obtained from Lagos University Teaching Hospital Lagos, (LUTH) Nigeria.

Preparation of extracts and phytochemical screening

The sun-dried leaves of *Acalypha wilkesiana* were pulverized using a laboratory mill (Christy and Norris Ltd, Chelmsford, England). 160 g of milled leaves of *Acalypha wilkesiana* was extracted with 1.6 litres of ethanol using a Sohxlet apparatus for 72 hours. The extract was filtered and concentrated using a Buchi V-801 rotary evaporator at 37°C. The phytochemical screening for glycosides, alkaloids, tannins, flavonoids and saponins were carried out using standard procedures as described by Sofowora[11].

Evaluation of the antimicrobial activity of the extract

The antimicrobial activities of the ethanolic extract of the leaves of *Acalypha wilkesiana* at concentrations of 2.5 mg/ml, 5 mg/ml and 10 mg/ml were determined using the cup plate method employed in an earlier study[12]. The following microorganisms were used: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* and *Penicillium notatum.*

The Minimal Inhibitory Concentrations (MIC) were determined by agar dilution method[12]. Commercial brand of antimicrobial cream, Funbact A® (a combination of Clotrimazole USP 1.0 %w/w and 0.05 %w/w, Neomycin Sulphate 0.5 %w/w, manufactured by Gopaldas Visram and Co. Ltd A-590/591, TTC Industrial Area, M.I.D.C. Mahape, Navi Mumbai, India) was used as positive control.

Preparation and evaluation of ointment

Two topical ointment bases of varying degrees of aqueous/anhydrous character namely: simple ointment BP and emulsifying ointment BP were prepared by fusion method (Table 1). The constituents of the base were placed together in a melting pan and allowed to melt at 70°C. After melting, the ingredients were stirred gently at 70°C for 5 minutes and then cooled with continuous stirring. Incorporation of 10 g of the ethanolic extract of *Acalypha wilkesiana* into the various bases was achieved by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments. The prepared herbal ointments were put in ointment jars, labelled and were stored at 25°C. The two formulated ointments and the standard, Funbact A® cream were evaluated for the following parameters: appearance, odour, texture, spread ability, homogeneity and irritant effect using the method employed by Alalor *et al.*[12].

In vitro antimicrobial efficacy of formulated ointments was carried out using the cup-plate method[12].

Table 1: Preparation of medicated formulations with ethanolic extract of *Acalypha wilkesiana*

Formulations	Ingredients	Concentration (%w/w)
Formulation 1	Extract	10
	Wool fat	4.5
	Cetostearyl alcohol	4.5
	Hard paraffin	4.5
	White soft paraffin	76.5
Formulation 2	Extract	10
	Liquid paraffin	18
	Emulsifying wax	27
	White soft paraffin	45

Formulation 1 =*Acalypha wilkesiana* 10 %w/w in simple ointment B.P, Formulation 2 =*Acalypha wilkesiana* in emulsifying ointment B.P

Table 2: Zones of inhibition (mm) of leaf ethanolic extract of *Acalypha wilkesiana* on microorganisms

Organisms	Extract 2.5 mg/ml	Extract 5 mg/ml	Extract 10 mg/ml
Escherichia coli	20.0 ± 0.4	25.2 ± 1.0	30.2 ± 1.1
Staphylococcus aureus	-	28.1 ± 0.5	30.4 ± 0.3
Pseudomonas aeruginosa	-	32.4 ± 0.4	35.0 ± 0.8
Candida albicans	-	22.4 ± 1.3	22.8 ± 1.6
Aspergillus flavus	-	-	-
Penicillium notatum	-	-	-

Values are the means of triplicate treatments with standard deviations. - = No zone of inhibition. Extract = leaf extract of *Acalypha wilkesiana*

Table 3: The MIC of the ethanolic leaf extracts of *Acalypha wilkesiana* and Funbact A® cream on microorganisms

Organisms	MIC (mg/ml) of ethanolic extract of *Acalypha wilkesiana*	MIC (mg/ml) of Funbact A® cream
Escherichia coli	5.00	5.00
Staphylococcus aureus	1.25	5.00
Pseudomonas aeruginosa	10.00	2.50
Candida albicans	5.00	2.50

Table 4: Zones of inhibition (mm) of the ethanolic extract of *Acalypha wilkesiana* -based herbal ointments and Funbact A® cream

Micro-organisms	Formulation 1	Formulation 2	Funbact A® cream
E. coli	20.9 ± 0.5	18.4 ± 0.6	25.7 ± 1.0
Staph aureus	25.6 ± 0.9	22.5 ± 0.9	33.7 ± 0.6
Pseudomonas aeruginosa	22.7 ± 0.3	21.6 ± 0.8	27.7 ± 0.5
Candida albicans	25.9 ± 0.3	23.5 ± 0.9	30.9 ± 1.1

Values are the means of triplicate treatments with standard deviations, Formulation 1 = *Acalypha wilkesiana* 10 %w/w in simple ointment B.P, Formulation 2 =*Acalypha wilkesiana* in emulsifying ointment B.P

Statistical Analysis
Data obtained was expressed as mean ± SD (standard deviation). The ANOVA test was used to assess if there were any difference in the data obtained. p-values less than 0.05 were considered statistically significant.

RESULTS

Preparation of extract and phytochemical screening
The percentage yield of the ethanolic extract was 34.12 %. The phytochemical screening of the ethanolic extract of the leaves of *Acalypha wilkensiana* revealed the presence of glycosides, alkaloids, tannins, saponins and flavonoids.

Antimicrobial activity of the extract
The preliminary *in vitro* antibacterial activity of the ethanolic extract showed activity against *Escherichia coli*, *Staphylococcus aureus and Pseudomonas aeruginosa* while the *in vitro* antifungal activity of the ethanolic extract showed activity against *Candida albicans* but no activity against *Aspergillus flavus* and *Penicillium notatum* as shown in Table 2. The MIC values of the ethanolic leaf extracts of *Acalypha wilkesiana* and Funbact A® cream on the test microorganisms are presented in Table 3.

Evaluation of the ointments
The formulated ointments appeared to be uniformly mixed, without any lumps. No pungent or irritating smell was observed. The ointments readily spread when applied on the skin topically and rubbed gently. The *in vitro* antimicrobial activity of the ethanolic extract of *Acalypha wilkesiana* based herbal ointments exhibited antimicrobial activity in both ointment bases, although the emulsifying base containing ointment showed a slightly wider zone of inhibition (Table 4).

DISCUSSION

Preparation of extracts and phytochemical screening
In the development of a new drug, extraction is one of the preliminary stages of research and large scale production and quality control of materials are important considerations. It must be noted however, extract yields from plants are influenced by plant strain, geographical location, extraction medium and procedure, among other factors[13]. The antimicrobial activities observed in this study may be attributed to the presence of these phytochemicals in the leaf.

Antimicrobial activity of the extract
The result of antimicrobial activity of the extract implies that the plant extract possesses both antibacterial and anti-candidal properties. The activity of the extract was concentration-dependent as revealed by the zones of inhibition. The MIC value (1.25 mg/ml) was remarkable for the ethanolic extract for *Staphylococcus aureus* compared to MIC value (5.00 mg/ml) for the Funbact A® cream however, for *Pseudomonas aeruginosa* and *Candida albicans,* Funbact A® cream had lower MIC values. This shows that this plant extract may be better bactericidal agent in the cases arising from *Staphylococcus aureus* than Funbact A® cream.

Evaluation of the ointments
The *Acalypha wilkesiana* based herbal ointments demonstrated good antimicrobial activity. The antimicrobial activity of *Acalypha wilkesiana* in Emulsifying ointment B.P base was higher than that of Simple ointment B.P. The results also revealed that the extracts incorporated into the ointment bases showed less activity than that of the crude extract of *Acalypha wilkesiana*. This may be attributed to the choice of ointment base and formulation process of the herbal ointments. The activity against *Staphylococcus aureus* is of significant interest because it is commonly found on the hands, face and in deep layers of the skin and is perhaps the most widely encountered and very undesirable. *Staphylococcus aureus* is not easily eliminated especially in the deeper skin layers, sweat gland, sebaceous gland and the hair-follicles by routine washing and scrubbing even with some antiseptic soap[14]. The formulation containing the emulsifying ointment base was comparable with Funbact A® cream for its antimicrobial activity against *E. coli*, *S. aureus, Kleb sp* and *C. albicans;* although Funbact A® had a higher activity (Table 4). The prepared herbal ointments showed a smooth appearance, had an agreeable odour and possessed good spreadability which was comparable to that of Funbact A® cream.

CONCLUSION

This study shows that the ethanolic extract of the leaves of *Acalypha wilkesiana* has antimicrobial activity and has high potential as antibacterial agent when formulated as ointment for topical use. The potency of the ethanolic extract of *Acalypha wilkesiana* was higher against bacteria than fungi. The ethanolic extract of *Acalypha wilkesiana* formulation containing the emulsifying ointment base (hydrophobic ointments) showed superior antibacterial and anti-candidal activity against tested microorganisms than simple ointment based formulation, however, Funbact A® cream had higher antibacterial and anti-candidal activity than emulsifying ointment base formulation.

REFERENCES

1. Latha PS, Kannabiran K. Antimicrobial activity and phytochemicals of *Solanum trilobatum* Linn. African Journal of Biotechnology 2006; 5: 2402-2404.
2. Chah KF, Eze CA, Emuelosi CE, Esimone CO. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. Journal of Ethnopharmacology 2006; 104: 164-167. http://dx.doi.org/10.1016/j.jep.2005.08.070 PMid:16226414
3. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turkish Journal of Biology 2005; 29: 41-47.
4. Adesina SK, Idowu O, Ogundaini A, Oladimeji H, Olugbade TA, Onawunmi GO, Pais M. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytotherapy Research 2000; 14: 371–374. http://dx.doi.org/10.1002/1099-1573(200008)14:5<371::AID-PTR625>3.3.CO;2-6
5. Akinyemi KO, Oluwa OK, Omomigbehin EO. Antimicrobial Activity of crude extracts of three medicinal plants used in South-Western Nigerian folk medicine on some food borne bacterial pathogens. African Journal of Traditional, Complimentary and Alternative Medicines 2006; 3: 13–22. http://dx.doi.org/10.4314/ajtcam.v3i4.31173
6. Oladunmoye MK. Comparative evaluation of antimicrobial activities and phytochemical screening of two varieties of *Acalypha wilkesiana.* Trends Applied Sci. Res 2006; 1: 538-541. http://dx.doi.org/10.3923/tasr.2006.538.541
7. Ogbo EM, Oyibo AE. Effects of three plants extract (*Ocimum gratissimum*, *Acalypha wilkesiana* and *Acalypha macrostachya*) on post harvest pathogen of *Persea Americana.* J. Med. Plants Res 2008; 2: 311-314.
8. Ezekiel CN, Anokwuru E, Nsofor E, Odusanya OA, Adebanjo O. Antimicrobial activity of the methanolic and crude alkaloid extracts of *Acalypha wilkesiana cv. macafeeana* Copper leaf Research Journal of Microbiology 2009; 4(7): 269-277.

9. Udobang JA, Nwafor PA, Okokon JE. Analgesic and antimalarial activities of crude leaf extract and fractions of *Acalypha wilkensiana* Journal of Ethnopharmacology 2010; 127: 373–378. http://dx.doi.org/10.1016/j.jep.2009.10.028 PMid:19892007
10. Alade PI, Irobi ON. Antimicrobial activities of crude extracts of *Acalypha wilkesiana*, J. Ethnopharmacol 1993; 39: 171-171. http://dx.doi.org/10.1016/0378-8741(93)90033-2
11. Sofowora A. Medicinal Plants and Traditional Medicines in Africa. Ibadan: Spectrum Books Ltd; 1993. p. 289
12. Alalor CA, Igwilo CI, Azubuike CP. Evaluation of the Antibacterial activity of Herbal ointments formulated with Methanolic extract of *Cassia alata.* Asian Journal of Biomedical and Pharmaceutical Sciences 2012; 2(13): 15-19.
13. Rates K. Plants as source of drugs. Laboratory of Pharmacognosy, Department of Production of Raw Material, School of Pharmacy, Federal University of Rio Grande do Sul, Brazil. Elsevier limited; 2000. p. 607
14. Denyer SP, Hodges N, Gorman SP, Gilmore BF. Hugo and Russel's Pharmaceutical Microbiology. 8thed Chichester, West Sussex, UK: Wiley-Blackwell A John Wiley and Sons Publication; 2011. p. 243

Cite this article as:
Azubuike Chukwuemeka P, Igbokwe Nwamaka H, Essien Godwin S, Elendu Ngozi J. Evaluation of antimicrobial properties of herbal ointments formulated with ethanolic extract of *Acalypha wilkesiana*. J Biol Sci Opin 2013; 1(2): 41-44 http://dx.doi.org/10.7897/2321–6328.01201

Source of support: Nil; Conflict of interest: None Declared