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RESEARCH ARTICLE

Antibacterial and Wound Healing Properties of Methanolic extract of dried fresh *Gossypium barbadense* Leaves

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ABSTRACT

This study was conducted to evaluate the antibacterial and wound healing properties of methanolic extract of dried fresh leaves of *Gossypium barbadense*. The antibacterial properties of the extract were studied against five wound isolates *(Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, and Shigella sonnei)* using the Well diffusion method. The wound healing properties were carried out using the excision wound model on healthy albino rats.

The results showed that methanolic extract of dried fresh *Gossypium barbadense* leaves had a dose dependent activity against all the test organisms except *Escherichia coli*. The extract solvent, propylene glycol, used as a negative control, had no activity against any of the test organisms. Dettol® antiseptic, and Cicatrin® powder used for the positive control also had a dose dependent activity against all the test organisms except *Escherichia coli* and *Pseudomonas aeruginosa*. The concentrated Dettol® (i.e. the undiluted solution) however inhibited growth of all test organisms. Comparing all test substances, it was observed that after ten days of treatment of the rats that when the extract was applied at a concentration of 20mg/ml, there were about 91% healing of wound on the rats whereas about 80 % healing of wound on the rats was noticed for Cicatrin® powder. The distilled water used as a negative control however produced only about 36 % healing of wound on the rats. The distilled water treated group percentage healing of wounds was significantly different ($p < 0.05$) from those of extract and antibacterial-treated groups.

KEYWORDS: Antibacterial, Wound Healing, Methanolic extract, Medicinal plants, *Gossypium barbadense* leaves.

1. INTRODUCTION

The incidence of resistance of microorganisms to antibiotics and chemical agents, environmental degradation and pollution associated with irrational use of orthodox medicines, therapeutic failure of orthodox medicine for some ailments and incurability of certain ailments with orthodox medicines have spurred a renewed interest in researchers to explore natural products (natural medicines) for possible solutions to the above problems [1]. Antibacterial substances e.g. ciprofloxacin, pefloxacin, metronidazole, sulphamethoxazole, trimethroprim etc are used to combat bacterial infection in hospitals but their attendant side effects and allergic reactions on different patients limits their wide usage among different groups of the populace. Allergic reaction to certain antibacterial substance for instance, Stevens–Johnson syndrome limits the use of sulpher group drugs like Sulphamethoxazole/trimethroprim in patient allergic to

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these drugs [2]. Reconstitution of antibacterial substances before being administered either as a suspension for oral usage or for intramuscular/intravenous usage is also another source of concern. Moreover, the antibacterial drugs that are presented in form of granules/powders are to be reconstituted prior to administration, when left in the hands of the patients or patients' relation, they are either wrongly reconstituted or inappropriate water are used for its reconstitution. This will pave way for bacterial entry to the antibacterial drug. When these inappropriately reconstituted antibacterial agents are used, they worsen the health conditions of the patients. The use of natural medicine however is not associated with these shortcomings and has served as one of the drive for the exploration of natural medicine for cure of man's ailment.

Wound defined simply as the disruption of the cellular and anatomic continuity of a tissue may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue [3]. This insult to the tissue is the portal of entry for many bacterial or microbial organisms and there is therefore need to administer substance that would heal wound rapidly to prevent the ingress of bacterial in tissues through wound openings. Some of the orthodox wound healing substances are either inaccessible or expensive. Even when the available ones provides would healing it has not been rapidly and this may serve as the needed window for bacterial infection during wound dressing or exposure by patients.

Nature has provided us with a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [4]. However, many more potential modern drugs abound in these traditional medicines which are yet to be explored. Most of the research strategy is based on inventory and identification of the plants used, demonstration of therapeutic activity of an extract of the plant, bioassay-guided fractionation, isolation and characterization of the active compounds and studies of structure activity relationship [5].

Gossypium barbadense Linn (family Malvaceae) is a perennial under shrub 1 - 3 m high, native to South America and now distributed from Senegal to Nigeria and widely cultivated in tropics. *Gossypium barbadense* is a plant well known for the cotton it produces. The cottonseed provides raw materials for local spinning and weaving industry. It also has some medicinal applications in emetics, veneral diseases, tumours, paralysis, epilepsy, convulsions, spasm, and cutaneous and subcutaneous parasitic infection [6]. It has antifungal properties and also sometimes used as a male anti-fertility drug [7, 8]. The leaves of *Gossypium barbadense* are used to treat hypertension and delayed or irregular menstruation [9]. An infusion of the leaf is taken as an antidote for colds and bronchitis and the young shoots pulped for palpitations and as dressings for wounds and in the treatment of systematic diarrhoeas [10]. The aqueous leaf extract of *Gossypium barbadense*, when used alone as monotherapy, has a slight suppressive antimalarial effect [11].

A number of bioactive triterpenoid and sesquiterpenoid aldehydes compounds have been isolated and characterized from *G. barbadense* [12]. Many of these compounds have exhibited several biological potentials such as antimicrobial, insecticidal and cytotoxic properties. The chemical composition of the Nigerian-grown cotton leaf essential oil, *Gossypium barbadense* revealed the presence of nineteen components, accounting for 92.6% of the total oil fraction. The oil displayed moderate antimicrobial potentials to some tested organisms [12].

As part of the on-going efforts to fully utilize the medicinal properties of natural products, in this study, the antibacterial activity, wound healing property and irritation potentials of methanolic extract of the dried fresh powdered leaves of *Gossypium barbadense* plant have been evaluated.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents and Chemicals

Dettol® liquid (antiseptic liquid preparation containing Chloroxylenol 4.8%w/v BP manufactured by Reckitt Benckiser Nigeria) and Cicatrin® powder (antibacterial preparation containing Neomycin Sulphate 3,300 units/g and Bacitracin zinc 250 units/g manufactured by GlaxoSmithKline UK) were purchased from a registered pharmacy premises in Lagos.

2.1.2 Plant materials

Fresh leaves of *Gossypium barbadense* were collected from Ayetoro market in Ikire, Irewole Local Government, Osun State. The plant sample was identified and authenticated by Mr. Odewo T.K. of the Department of Botany, University of Lagos, Nigeria. A specimen of the plant was preserved in the herbarium.

2.1.3 Laboratory Animals

Seventeen healthy adult albino mice (male and female), average weight 25±5g, were obtained from the Laboratory Animal Centre of College of Medicine, University of Lagos, Nigeria and used for both the wound healing and local irritation potentials of the plant extract. Fifteen rats were used for evaluation of the wound healing properties while two rats were used to determine local irritation properties of the extract. They were maintained in accordance with the recommendation in the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication N. 85-23, revised

1996) for studies involving experimental animals. They had free access to feed and clean drinking water during the two weeks acclimatization period and throughout the experimental period. Ethical permission for use of animals for this research was obtained from the College of Medicine University of Lagos research ethics committee.

2.2 Preparation of methanolic extract of *Gossypium barbadense* leaves

The plant materials were washed with distilled water, cut into small pieces and dried in the oven at 40 to 60˚C for 5 days. The dried materials were reduced to coarse powder of about mesh size 18 using a pestle and mortar and the pulverized plant material was extracted with methanol at room temperature for 48 hours. The extract was concentrated using the rotary evaporator (Rotavopor R-215) and refrigerated for 5 days. The required quantity was cut from the bulk and used for the preparation of the extract and the balance was kept refrigerated for the whole period of the study.

2.3 Evaluation of extract for antibacterial activities.

The antibacterial activities of the extract were determined using agar Well diffusion techniques [13, 14]. In this study, multi – drug resistant wound isolates bacteria from hospital patients at the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria were used. The isolates consisted of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus mirabilis* and *Shigella sonnei.* Mueller-Hilton agar was prepared in 20 ml amounts, sterilized by autoclaving at 121º C for 15 minutes and allowed to cool to a temperature of 45˚C. 0.1 ml of each of the 3 hour Broth culture suspension was dispensed into the warm molten Mueller-Hinton agar in universal bottle, mixed gently and poured into the Petri dish. It was allowed to solidify and cork borer, 10mm in diameter was used to drill four wells 4mm deep in each of the Petri dishes. Using a 1 ml needle and syringe, 0.2 ml of the extract in concentrations of 10mg/ml, 20mg/ml and 30mg/ml were dispensed into the three wells and the fourth well had only propylene glycol and served as the negative control. The positive control Petri dishes had the four wells dispensed with 0.2 ml of the four working concentration of the Dettol® and Cicatrin® components (i.e. Neomycin/Bacitracin powder) solutions respectively. Five separate Petri dishes were used to evaluate the undiluted (i.e. the concentrated) solution of the Dettol® against the test organisms. The extract, propylene glycol and the test standards in the Petri dishes were allowed to stand for 4 hours for diffusion to take place. The plates were incubated, lid up position, at 37˚C for 24 hours. After incubation, inhibition zone diameter (IZD) was measured and recorded to the nearest whole millimetre.

2.4 Evaluation of extract for wound healing properties.

Wound sites were made as described by Dash *et al.* [5]. Procedures involving animals were conducted in compliance with US guidelines as contained in the NIH Guide for the care and use of laboratory animals (NIH Publication No. 18-23, 1995). Each rat was anaesthetized with diethyl ether and the hair on the back was scrapped off with a pair of scissors. This area was disinfected with 70% ethanol. A circular incision of about 20 mm in diameter was made on the disinfected area of the skin surface and the skin carefully dissected out. The wound area was measured immediately by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was then placed on a 1 mm² graph sheet and traced out. The squares were counted and the area recorded [1].

Treatment started shortly after the wound excision on the rats and involved applying 0.2 ml of 10 mg/ml of the extract to group I animals, 0.2 ml of 20 mg/ml of the extract to group II animals and 0.2 ml of 40mg/ml of the extract to group III animals. Group IV animals, which served as the positive control, were treated by applying Cicatrin® powder lightly over the wound while sterile distilled water were applied on the wound of group V animals .This was the negative control group.

Application mode of all treatment substances was topically, once daily for 10 days and was done after cleaning the wound with dilute solution of Dettol®. The wound area of each animal was measured on the 1st, 4th, 7th, 10th, 14th and 17th day post surgery. The percentage wound healing on these days was determined.

2.5 Evaluation of extract for local skin irritation potential.

The skin of the remaining two albino rats were shaved individually at three different positions on the dorsal side, each about 25mm^2. The 1st area was kept as control, to which the extract vehicle (propylene glycol) was applied; the 2nd area was applied with 20mg/ml extract and the 3rd area with 40mg/ml extract. After four hours, the skin was observed for signs of inflammation.

2.6 Statistical analysis

The data on percentage wound healing was statistically analyzed using one-way analysis of variance (ANOVA) and significant means were separated using the Turkey's multiple comparison test. Differences were considered significant at $P < 0.05$.

3. RESULTS

The results of the antibacterial activities of different concentrations of the methanolic extract of *Gossypium barbadense* leaves, Dettol® liquid and Cicatrin® powder against the test organisms are presented in tables 1, 2 and 3 respectively. The data on percentage wound healing properties of the methanolic extract of *Gossypium barbadense* leaves and Cicatrin® powder on the rats is presented in table 1 and figure 1. After four hours of

applying the extract and the extract solvent on the skin of the rats, the result showed that there was no inflammation observed on the skin of the rats.

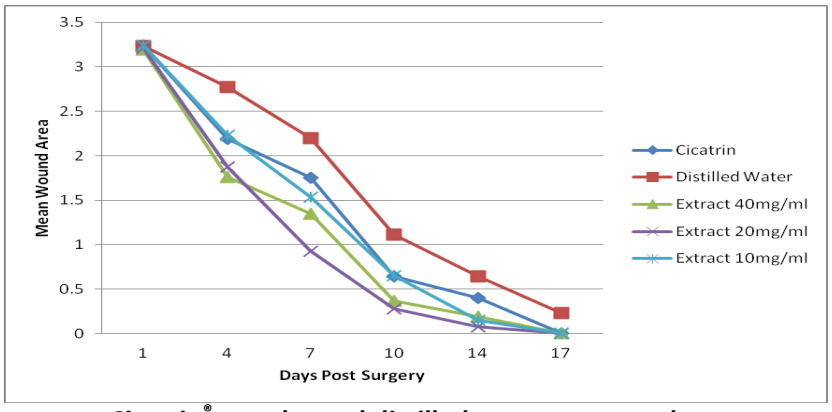

Fig 1. Effects of the plant extract, Cicatrin® powder and distilled water on wound area.

ay organisms	Mean inhibition zone diameter (mm) produced by extract and propylene glycol			
	10mg/ml	**20mg/ml**	**30mg/ml**	**PG(Control)**
Staphylococcus aureus	13	14	16	–
Escherichia coli	–	–	–	–
Pseudomonas aeruginosa	12	14	16	–
Proteus mirabilis	–	13	15	–
Shigella sonnei	–	15	17	–

Table 1: Antibacterial activities of methanolic extract of *Gossypium barbadense* leaves/Propylene Glycol (PG).

Assay organisms	**Mean inhibition zone diameter (mm) of Dettol at different concentration**				
	0.125% v/v	0.25% v/v	0.5% v/v	1% v/v	Undiluted Dettol®
Staphylococcus aureus	–	–	12	14	25
Escherichia coli	–	–	–	–	20
Pseudomonas aeruginosa	–	–	–	–	13
Proteus mirabilis	–	–	–	12	19
Shigella sonnei	–	–	–	12	12

Table 2: Antibacterial activities of Dettol®.

Assay organisms	**Mean inhibition zone diameter (mm) of Cicatrin® at different concentration**			
	75/100 (µg/ml)	**150/200 (µg/ml)**	**300/400 (µg/ml)**	**600/800 (µg/ml)**
Staphylococcus aureus	17	18	20	23
Escherichia coli	–	–	–	–
Pseudomonas aeruginosa	–	–	–	–
Proteus mirabilis	12	14	16	18
Shigella sonnei	12	12	16	18

Table 3: Antibacterial activities of Cicatrin®

Group	Test substance	Percentage wound healing (mean ± S.E.M) on day post surgery 1	4	7	10	14	17
I	10mg/ml	0.0	31.17± 0.19	52.78± 0.21	79.94± 0.25	95.37± 0.04	100.0± 0.00
II	20mg/ml	0.0	70.87±0.08	71.03± 0.27	91.28± 0.06	97.51± 0.00	100.0± 0.00
III	40mg/ml	0.0	44.83±0.27	57.68± 0.22	88.40± 0.04	94.04± 0.03	100.0± 0.00
IV	Cicatrin® powder	0.0	32.41± 0.15	43.00± 0.28	80.25± 0.08	92.59± 0.07	100.0± 0.00
V	Distilled water	0.0	14.24± 0.25	31.89± 0.22	36.63± 0.23	80.19± 0.08	94.4± 0.07

Table 4: Effects of plant extracts, Cicatrin® and distilled water on wound healing.
Note: SEM=Standard Error of Mean

4. DISCUSSION

The results of the antibacterial activity of the methanolic extracts of dried fresh *Gossypium barbadense* presented in table 1 shows that the extract inhibited the growth of all the test organisms except *Escherichia coli* and had mean inhibition zone diameter (IZD) ranging from 12 to 17mm. At concentration of 10mg/ml, the extract had no activity against *Proteus mirabilis* and *Shigella sonnei* but was able to inhibit the growth of these two organisms at 20mg/ml and showed a dose related increase in IZD when the 30mg/ml extract was used. The propylene glycol, which served as the negative control and also as extract solvent had no activity against any of the test organisms. This observation may lend credence to the antimicrobial properties of the extracts. The antimicrobial properties of this extract might be attributed to the essential oil component of the extract. Essien *et al.* in a study reported the presence of nineteen components in the leaves of air-dried pulverized *Gossypium barbadense* plant including α-pinene which accounted for 12.8 % [12]. It has been reported that the essential oil of *Eucalyptus* torreliana [15] and *Taxodium distichum* [16] rich in α-pinene exhibited antimicrobial activities. As shown in tables 2 and 3, Dettol® and Cicatrin® exhibited antimicrobial activities against some of the test organisms and displayed a dose correlated activity against all the test organisms except *Escherichia coli* and *Pseudomonas aeruginosa*, While increase in concentration of the Dettol® at 0.5% showed activity against *Staphylococcus aureus,* activity was recorded for *Proteus mirabilis* and *Shigella sonnei* at 1%v/v and none of these concentrations inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa* except when the undiluted Dettol® was used. It did show that these organisms are resistant to the positive control test substances, while the extract had only *Escherichia coli* resistant to it at all test concentrations. The results displayed a better antibacterial activity of the *Gossypium barbadense* extract than the conventional antibacterial and antiseptic used.

The results of the wound healing property of the methanolic extracts of dried fresh *Gossypium barbadense* and Cicatrin® powder are presented in table 4 and figure 1. As shown in table 4, there was a progressive daily decrease in the wound area throughout the experimental period in all the groups. In comparison however, the distilled water treated group (negative control), decrease in wound area was not as drastic as the extract and antibacterial treated groups. In the 20mg/ml treated group, a sharp remarkable decrease in wound area was observed between 1st and 4th day, having 71% wound healing and comparatively showing the best wound healing activity than the other extract concentrations and Cicatrin® powder. As the number of the days increased, wound healing was observed for all the treated groups but only from day 14 was a significant wound healing observed for distilled water treated group (recording 80%). This was probably due to natural response to wound as the body immune system and blood clot factors set to play and bring about wound healing without the aid of any wound healing agent. At day 10 post surgery over 91% wound healing had been recorded in the extract and 80 % in the Cicatrin® treated groups. Although 100% healing was observed in the extract and Cicatrin® powder treated groups by day 17, it was not significantly different ($P > 0.05$) for both groups. The percentage healing in the distilled water treated group throughout the experimental period was however significantly lower ($P < 0.05$) than those of the extract and antibacterial-treated groups.

Furthermore, after four hours of applying the extract and the extract solvent on the skin of the rats, there was no inflammation observed on the skin of the rats indicating that the extract is unlikely to cause local skin irritation potential.

5. CONCLUSION

The results of this study have shown that methanolic extract of dried fresh *Gossypium barbadense* leaves have antibacterial and wound healing potentials with evidence of not causing skin irritation. Compared with the results obtained for Cicatrin® powder and Dettol® antiseptic, the extract showed superior antimicrobial properties against *Pseudomonas aeruginosa*. However, further study of the toxicological profile of the extract is required.

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Conflict of Interest: None Declared

