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Evaluation of the Wound Healing Activity of a Polyherbal Remedy

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ABSTRACT

The wound healing activity of a popular Nigeria traditional Polyherbal (A. conyzoids, C. scandens and M. villosus) remedy was investigated. The ethanol leaves extracts were formulated in form of an ointment using palm kernel oil as base. Two, 4 and 6 g/ml of the extracts and their combination were prepared and applied topically on the wounds daily for 20 days. Cicatrine powder (neomycin-bacitracin) served as the standard while the control group received only palm kernel oil. Daily wound contraction and epithelization time was recorded for each group. Agar well diffusion method was used for the determination of antimicrobial activity of the extracts. Phytochemical analysis of the plant extracts revealed the presence of alkaloids, saponins, flavonoids and tannins. Ageratum conyzoids and C. scandens showed activity against all the tested microorganisms while M. villosus inhibited S. aureus, B. subtilis and P. aeruginosa. All the extract and their combination showed significant ($p < 0.05$) wound contraction activity compared with the control. No significant ($p > 0.05$) different was observed in the wound contraction activity of A. conyzoids and C. scandens compared with the standard from the 12^{th} to 20^{th} day. Also no significant ($p > 0.05$) difference was observed between A. conyzoids, C. scandens and extracts combination at 2 and 4 g/ml. Significant epithelization time was also observed for all the extracts compared with control. Based on our findings there is no justification for the traditional combination of the 3 plant extracts as equal effect as combination was observed especially in C. scandens and A. conyzoids.

Key words: Wound healing, Polyherbal remedy, antimicrobial activity

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INTRODUCTION

Wounds may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue [1]. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production, letter the epithelial tissue is regenerated [2]. The cellular and biochemical complex cascade involved in wound healing is geared towards restoration of structural and functional integrity with regain of strength of injured tissue [3]. Wound contracture involving migration of the fibroblast to the injured tissue followed by shrinkage of the wound area play a significant part of wound healing process. Though wound healing is a natural process and have the ability to heal on its own, for rapid healing of wound, there is need to provide better conditions that can regenerate the damaged tissue [4].

Plants and their extracts have immense potentials for the management and treatment of wounds, not only that they are readily available, cheap and affordable but also are generally considered safe as hyper-sensitivity reactions are rarely encountered with the use of these agents [1].

Mitracarpus villosus have antifungal activity [5] and is used traditionally in treating skin infections like eczema. Culcasia scandens possess anti-inflammatory, analgesic and antimicrobial activities [6] while Ageratum conyzoides have analgesic, antimicrobial activity and is used to treat wounds and burns [7]. Traditionally, use of polyherbal formulations are greatly practiced owing to the additive and in most cases synergistic effect of these combinations. In South Eastern Nigeria, polyherbal decoction of the leaf extracts of *Mitracarpus villosus, Culcasia scandens* and *Ageratum conyzoides* is employed in wound healing treatment. However, no study has been done to evaluate the wound healing effects of this polyherbal remedy. This study evaluates the wound healing activities of these plant extracts individually and as a polyherbal decoction.

MATERIALS AND METHODS

Plant Materials:
The fresh leaves of the plants were each collected during the dry season from Agulu, Anambra State of Nigeria and were identified and authenticated by Mr. Paulinus Ugwuozor in the Department of Botany, Nnamdi Azikiwe University, Awka.

Preparation of the Extracts: The leaves were air dried under room temperature for seven days and pulverized. The dried powdered plant materials were cold macerated separately using 70% v/v ethanol (Sigma Aldrich) for seven days. The resulting solutions were filtered using Waltman No.1 filter paper, and the filtrates obtained were concentrated to dryness at 40^0C in vacuo using rotary evaporator.

Animals: Seventy healthy and wound free male rabbits weighting 800-1000g were obtained from animal house of Pharmacology & Toxicology Department, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria. The animals were acclimatized for 2 weeks, with free access to food and water *ad libitum* and were placed under standard housing conditions in compliance with NIH Guide for Care and Use of Laboratory Animals.

Phytochemical Test
The phytochemical analysis of *Ageratum conyzoides*, *Culcasia scandens* and *Mitracarpus villosus* extracts were carried out using standard protocols [8].

Formulation:
The herbal extracts were formulated in the form of ointment and administered topically. The ointment base (palm kernel oil) was used, and 2g, 4g and 6g of the extracts were incorporated into the base using the trituration method of preparing medicated ointments. The required quantity of the ointment base was measured in grams separately and melted at a temperature of about 70°C in a hot water bath.

The designated quantity of each extract was added to the melted base at 40°C, mixed and swirled gently and continuously until a homogenous dispersion was obtained.

Antimicrobial Activity:
Using the agar well diffusion method, sterilized Muller Hinton agar plates were seeded with 1 $\times 10^6$cfu/mL concentration of 5 test organisms. Using a sterile cork borer (6mm diameter), wells were bored on the surface of agar and the serial diluted concentrations of the herbal extracts (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562mg/ml) were introduced into appropriate wells in the nutrient agar plates.

Control disks contained the vehicle (palm kernel oil), whereas Cicatrin powder (Glaxowellcome, Nigeria) was used as the standard. The plates were kept undisturbed at room temperature for prediffusion period of 30mins and incubated for 24hrs at 37◦C.

After incubation, the diameter of the inhibition zone for each well was measured and the mean obtained

Wound Induction:
For each plant extract and combination, 3 groups of 5 animals each was used, while for the standard (Cicatrin® (neomycin-bacitracin), a standard antibiotic combination used in wound healing in Nigeria) and the control (palm kernel oil treated) groups, 5 animals each was used. The animals were anesthetized prior to and during creation

of the wounds with chloroform using open mask method. The rabbits were inflicted with excision wounds as described by Esimone *et al*. [9].

The wounds on each animal of various groups were treated topically daily with the vehicle (palm kernel oil), Cicatrin powder and the prepared formulations.

The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind.

% contraction = $(W_{AO} - W_{AT} / W_{AO}) X 100$

W_{AO} = % wound contraction on day 1
W_{AT} = % wound contraction on day T (i.e. day 2-20)

Statistical Analysis
Data obtained from the percentage wound contraction were subjected to one way ANOVA using SPSS 16.0 and Student-Newman-Keuls test was applied for mean comparison. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The phytochemical analysis of the extracts with their yields is as presented in table 1. Alkaloids, saponins, flavonoids and tannins were all present in all the plant extracts. There was no considerable change in color, odor and stability of all the formulations and no phase separation was observed in the course of the study. No mortality was recorded in all the treated groups.

Ageratum Conyzoids and *C. scandens* extracts showed activity against all the microorganisms tested while *M. villosus* inhibited only *S. aureus*, *B. subtilis* and *P. aeruginosa* (Table 2). *A. conyzoids* gave very low MIC values against E. coli (6.25 mg/ml), *B. subtilis* (12.5 mg/ml) and *P. aeruginosa* (12.5 mg/ml). Of all the test organisms, *B. subtilis* was the most sensitive with the lowest MIC for all the extracts. Wounds are known to be easy portals for infections and provides suitable medium for the proliferation of microbial organisms [10]. Bowler et al. [11] identified wound infection as one of the most important factors that delay wound repair processes and outcome. The wound healing effect exhibited by these plant extracts may have been contributed by their antimicrobial activities.

All the extracts and their combination produce significant ($p < 0.05$) wound contraction compared with the vehicle treated group (Table 3). No significant ($p < 0.05$) difference was observed in the wound contractile effect of *C. scandens* compared with standard for all the doses from 12^{th} to 20^{th} day and between *A. conyzoides* and standard, extracts combination and standard from the 8^{th} to 20^{th} day. The percentage wound contraction exhibited by the various doses of *C. scandens* does not differ significantly ($p < 0.05$) from the 12^{th} to 20^{th} day while the doses of *A. conyzoids* and the extract combination does not differ significantly ($p < 0.05$) from the 8^{th} to 20^{th} day. At higher concentration (6g/ml) *M. villosus* exhibited significant activity ($p < 0.05$) compared with other doses (2 and 4 g/ml) from the 12^{th} to the 20^{th} day.

The percentage wound contraction effect exhibited by the extract combination was only significantly ($p < 0.05$) better than the individual extracts at 6 g/ml on the 4^{th} to 8^{th} day but no significant ($p < 0.05$) activity was recorded compared with *A. conyzoids* and *C. scandens* at this dose from the 12^{th} to the 20^{th} day and at all day at 4 and 2 g/ml (Table 3, fig 1 - 3). *M. villosus* has the least percentage wound contraction as it is significantly different at all doses and days from the effect produced by the combination and from the standard from the 4^{th} to the 16^{th} day at the tested doses.
The epithelization time (time at which complete scar formation occur) suggest that both the standard and the extract treated groups were found to be significant ($p < 0.05$) compared with the control (Table 4). *M. villosus* had the longest epithelization time.

Both the antimicrobial and wound contraction activities exhibited by the extracts could be attributed to their phytocompounds. Several phytoconstituents such as alkaloids, saponins, flavonoid have been shown to promote wound healing process due to their antimicrobial property [12-14].

Table 1: Extraction and phytochemical analysis

plants	Initial weight of powder (g)	Yield (%)	Phytochemical Compounds
A. conyzoids	154.92	56.61	Alkaloids, Saponins, Flavonoids, Tannins
M. villosus	148.45	65.25	Alkaloids, Saponins, Flavonoids, Tannins
C. scandens	256.50	57.20	Alkaloids, Saponins, Flavonoids, Tannins

Table 2: Minimum Inhibition Concentration (MIC) of Extracts

	MIC mg/ml				
Extracts	**E. coli**	**S. aureus**	**B. subtilis**	**P. aeruginosa**	**S. typhi**
Cicatrin	10	8	14	4	10
A. conyzoids	6.25	25	12.5	12.5	100
C. scandens	100	25	6.25	100	12.5
M. villosus	-	50	3.125	50	-

Table 3: Percentage wound contraction activity of extracts

Plants/ Concentrations	**% of wound contraction**				
	4 days	**8 days**	**12 days**	**16 days**	**20 days**
C. scandens					
Control	13.76±0.29**A***	20.13±2.09**A***	21.24±1.56**A***	29.13±1.79**A***	34.29±1.12**A***
Cicatrin	63.19±1.77**C**	71.39±1.46**D**	91.74±1.65**B**	98.05±1.05**B**	99.88±0.06**B**
2 g/ml	36.09±3.16**B**	57.05±2.69**B**	88.79±2.43**B**	98.67±0.71**B**	99.91±0.01**B**
4 g/ml	43.48±5.17**B**	62.43±2.77**BC**	84.55±2.28**B**	97.21±1.94**B**	99.89±0.12**B**
6 g/ml	35.30±2.25**B***	65.82±3.73**CD***	89.37±4.05**B**	97.93±1.35**B**	99.25±0.77**B**
Conyzoides					
Control	13.76±0.29**A***	20.13±2.09**A***	21.24±1.56**A***	29.13±1.79**A***	34.29±1.12**A***
Cicatrin	63.19±1.77**C**	71.39±1.46**B**	91.74±1.65**B**	98.05±1.05**B**	99.88±0.06**B**
2g/ml	38.59±4.34**B**	66.04±9.06**B**	84.68±8.77**B**	94.10±5.26**B**	98.52±1.08**B**
4 g/ml	34.32±7.11**B**	69.72±2.69**B**	84.36±4.33**B**	91.92±4.77**B**	96.95±2.33**B**
6 g/ml	35.82±12.25**B***	63.81±7.53**B***	90.02±2.06**B**	97.17±0.84**B**	99.35±0.76**B**
M. villosus					
Control	13.76±0.29**A***	20.13±2.09**A***	21.24±1.56**A***	29.13±1.79**A***	34.29±1.12**A***
Cicatrin	63.19±1.77**C**	71.39±1.46**C**	91.74±1.65**C**	98.05±1.05**C**	99.88±0.06**C**
2g/ml	18.53±2.51**A***	35.16±0.93**B***	41.87±1.87**B***	59.29±3.31**B***	85.31±1.69**B***
4 g/ml	29.28±2.14**B***	34.91±2.42**B***	46.44±2.00**B***	62.12±2.32**B***	83.18±1.42**B***
6 g/ml	31.77±1.82**B***	42.24±1.61**B***	73.00±2.29**D***	90.31±1.52**D***	97.49±1.16**C***
Combination					
Control	13.76±0.29**A**	20.13±2.09**A**	21.24±1.56**A**	29.13±1.79**A**	34.29±1.12**A**
Cicatrin	63.19±1.77**C**	71.39±1.46**B**	91.74±1.65**B**	98.05±1.05**B**	99.88±0.06**B**
2g/ml	48.59±9.89**B**	72.69±12.26**B**	89.90±7.36**B**	96.67±5.58**B**	99.45±0.95**B**
4 g/ml	43.46±3.60**B**	66.19±5.82**B**	84.37±7.65**B**	95.56±4.32**B**	99.08±1.46**B**
6 g/ml	53.37±3.85**C**	76.69±5.36**B**	88.87±6.31**B**	94.80±5.06**B**	99.77±0.34**B**

*Mean ±SD in the same column for the same category of percentage wound contraction followed by the same letter do not differ significantly at p = 0.05 (Student-Newman-Keuls test). Each datum represents the mean of 5 replicates. * indicates significant difference (p < 0.05) compared with extract combination*

Table 4: Epithelization Time for the extract treated groups

	Mean epithelization period (days)			
Extracts	**2 g/ml**	**4 g/ml**	**6 g/ml**	
A. conyzoids	21.2 ± 0.05*	21.0 ± 0.02*	20.6 ± 0.05*	
C. scandens	21.4 ± 0.04*	21.4 ± 0.04*	20.8 ± 0.08*	
M. villosus	23.5 ± 0.10*	23.2 ± 0.09*	23.0 ± 0.06*	
Combination	21.2 ± 0.04*	21.4 ± 0.06*	20.8 ± 0.04*	
control				30.2 ± 0.2
cicatrin				21.3+0.08*

*= $p < 0.05$

Fig 1: Comparison of extracts with their combination at 2 g/ml

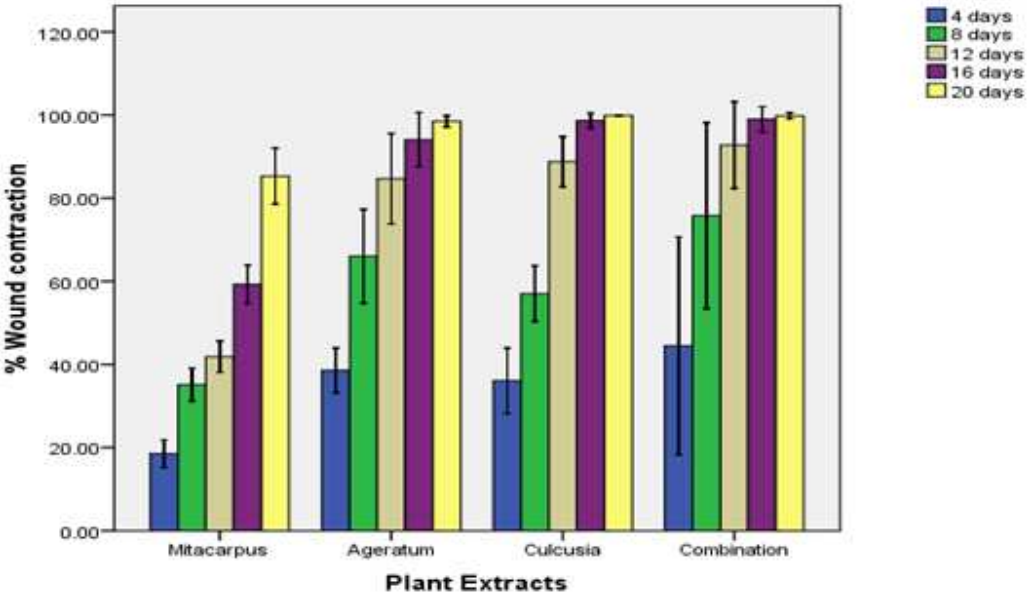


Fig 2: Comparison of extracts with their combination at 4 g/ml

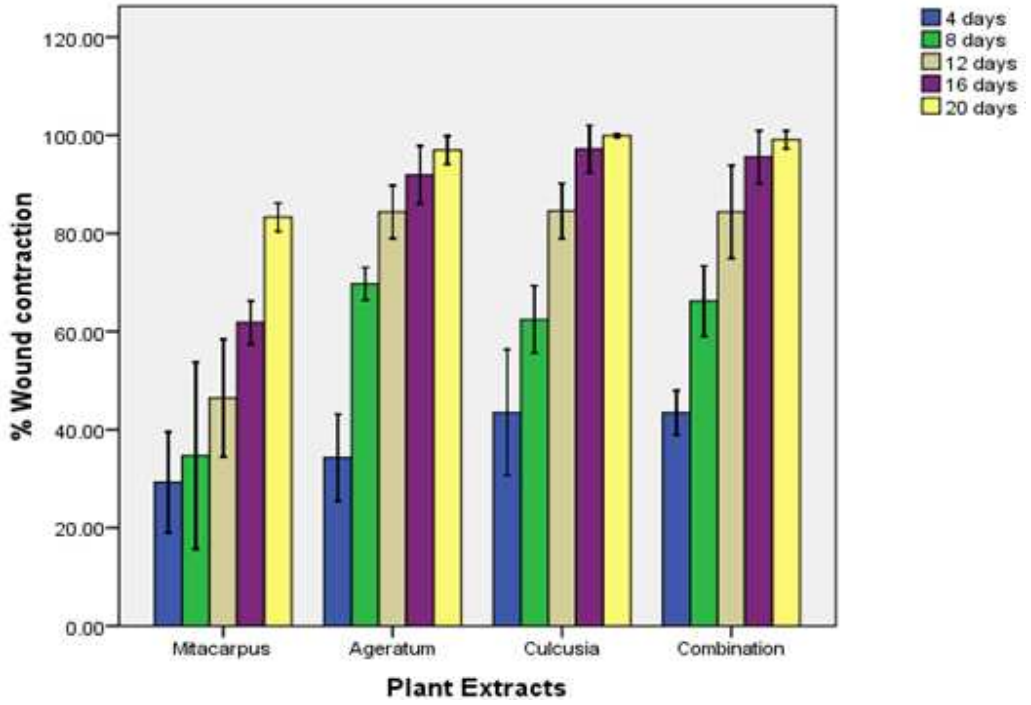
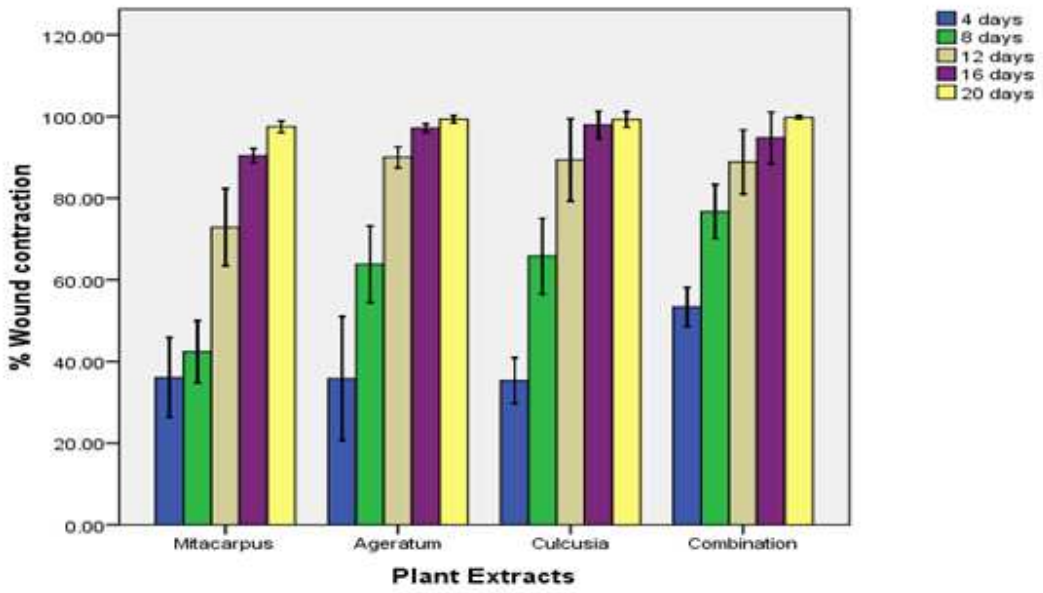


Fig 3: Comparison of extracts with their combination at 6 g/ml



CONCLUSION

All the plant extracts showed antimicrobial and wound healing activities. *A. conyzoids* has the highest activity followed by *C. scandens* and *M. villosus* respectively. From these studies, there is no justification for the traditional combination of the 3 plant extracts as no additive or synergistic effect was observed.

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