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In-vitro Antioxidant and Wound Healing Properties of *Combretum dolichopetalum* Engl. & Diels (Combretaceae)

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ABSTRACT

Combretum dolichopetalum Engl. & Diels (Combretaceae) is a medicinal plant used in the Kpando Traditional Area for treating wounds. However, it has received little attention by the scientific world. The study was designed to evaluate the wound healing potential and antioxidant activities of the plant to confirm its folkloric use. Excision wound model was used with Penicillin as the standard reference drug. Sensitivity test was carried out on the extract against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* using disc diffusion method. The antioxidant activity was measured by three different spectrophotometric assays. The content of total phenolics and total flavonoids were also determined. The extract significantly caused wound healing and reduced microbial load. Similarly, the methanol extract significantly inhibited the growth of *S. aureus* and *K. pneumonia* at higher doses. Almost all results from the antioxidant activity models were concentration dependent with no significant difference from the standard Ascorbic acid except at very low concentrations. Tannins and flavonoids were present whose synergistic effects may be responsible for the high antioxidant activity. Correlation analysis between the values of DPPH and TAC (r^2=0.7790) indicates the viability of the two models for evaluating antioxidants from medicinal plants.

INTRODUCTION

Medicinal, herbal and aromatic plants constitute a large segment of the flora, which provide raw materials for use by pharmaceutical, cosmetic, fragrance and flavour industries (Padmaja *et al.,* 2011). They have been used in the country for a long time for their medicinal properties. Medicinal plants are commonly used in treating or preventing specific ailments or diseases and are considered to play a beneficial role in health care. Plants are able to do this because of the presence of bioactive compounds which possess antimicrobial and antioxidant properties. Such plants contain chemical constituents that fight microbial infections and thereby cause wound healing and fight other ailments. The antioxidant compounds in plants, notably phenolics, protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (Dasgupta and Bratati, 2007). Phenolics have been known to possess a capacity to scavenge free radicals. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as hydrogen donors hence reducing agents. Phenolics are common in leaves, flowering tissues and woody parts, such as stems and barks. Studies have shown that apart from contributing to wound healing, phenolic compounds play an important preventive role in the development of cancer, heart diseases and ageing related diseases (Larson, 1988). Therefore, the study of plants as a resource of medicine has become more important in the fight of many infectious diseases and many oxidative stress related diseases like cancer, diabetes, neurodegenerative disorders and cardiovascular diseases which occur as a result of accumulation of free radicals in the body (Sahoo *et al.,* 2012). These diseases are found to be one of the major causes of health hazards (Padmaja *et al.,* 2011).

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Many natural products chemists or scientists continue to explore the biodiversity in search for plant medicines to overcome the above mentioned diseases that are caused by the accumulation of free radicals. Consequently, plant sources of antioxidants are vigorously sought for. The medicinal use of plants of the family Combretaceae is widely described in the scientific literature. Phytochemical studies carried out in the genus *Combretum* have demonstrated the occurrence of many classes of constituents, including triterpenes, flavonoids, lignans and non-protein amino acids, among others. The leaves and roots are extensively used in ethnomedical practices of many cultures.

In Nigeria, the plant is used in the treatment of burns and gastrointestinal disorders (Asuzu and Njoku, 1992). In Ghana, an infusion of the leaves, roots and stem is used in the management of a condition of "stomach stagger" in cattle (Uzor *et al.*, 2014). The antiulcer, antihepatotoxic, trypanocidal, antiinflammatory, antidiabetic, gastric antisecretory, smooth muscle relaxant and antispasmolytic activities of *C. dolichopetalum* have been reported by previous workers (Udem *et al.*, 1997; Asuzu and Adimorah 1998). The study conducted by Onoja et al 2015 to validate the ethnomedicinal uses of *C. dolichopetalum* also showed that the hydromethanolic extract of the leaves possess antidiarrheal properties. Scientific data supporting the ethnomedicinal uses of C. dolichopetalum as wound healing agent and a potential source of antioxidants is somehow lacking.

The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from diseases compelled us to undertake this study to identify the potential usefulness of *C. dolichopetalum* in wound healing as well as determining its potentials as an antioxidant source.

EXPERIMENTAL

General instruments, Reagents and chemicals

All the Solvents used were Analar BDH (PROLABO): ethanol (99.8%), methanol (99.8 %), chloroform (99.2 %), ethyl acetate (99.8 %), pet-ether (80-100 ^{o}C) and butanol. They were purchased from MES Equipment Limited. Nutrient agar media, sterile yeast and Mould extract agar were also obtained from Department of Laboratory Technology & Medical Laboratory Central Store. UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, UK) was used to measure all absorbances. Evaporation was conducted using an EYELA rotary evaporator system (Japan) in vacuo.

Plant Material and Extraction

The fresh leaves of *Combretum dolichopetalum* were collected from Jukwa near Cape Coast in the Central Region in the month of December 2013. The sample was authenticated by Mr. Agyarkwa and Mr. Otoo, the curators of the herbarium, Department of Environmental sciences, School of Biological Sciences, with the UCC voucher specimen no. UCC/H/308 dated 15-02-1981. The plant sample was air dried at room temperature and powdered.

Preparation of Methanol and Aqueous Extract

A mass of 100 g of the powdered sample was cold macerated with 500 ml of methanol. The cold macerate was filtered (Whatman filter paper, No 1). The filtrate was concentrated to remove the solvent under reduced pressure at 35-45°C using rotavapor. The resultant semi dried extract was placed in a dessicator for three days to further dry the sample. The dried extract was weighed which gave a percentage yield of 20.5 %. The same procedure was followed to obtain the aqueous extract (10.2%) using distilled water as the extracting solvent. The aqueous extract was used for the wound healing experiment to agree with the folkloric usage of this plant since the poultice form is traditionally used in treatment of wounds.

Successive solvent extraction

Part of the methanol extract (32.5 g) was re-dissolved in distilled water (160 ml) and filtered with Whatmann no. 3 filter paper. The water soluble portion was successively partitioned into petrloleum ether (80-100 ^{o}C; (4 x 50 ml)), Chloroform (4 x 50 ml), ethyl acetate (4 x 50 ml) and n-butanol (4 x 50 ml), in increasing order of polarity to yield (1.52 g) petroleum ether fraction, (0.14 g) chloroform fraction, (0.53 g) ethyl acetate fraction and (0.21 g) n-butanol fraction. Ethyl acetate, methanol and n-butanol that tested positive for polyphenolic compounds were used for the antioxidant activity tests.

Antimicrobial activity assay

Microbial cultures of three different strain bacteria were used for antibacterial activity. The three standard bacterial strains viz. *Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922)* and *Klebsiella pneumonia (ATCC4352)* were obtained from Korle-Bu Teaching Hospital Central Laboratory, Accra (Ghana).Well diffusion method using Müeller-Hinton agar plates were used to demonstrate the antimicrobial properties of the Methanol crude extracts (Forbes et al, 1990). A suspension of the bacteria compared to 0.5 Macfarland standard was seeded on the Mueller-Hinton agar plates. Wells of 6mm in diameter and 2 cm apart were punctured in the culture media using sterile cork borers. 80 µl of the crude extracts was administered to fullness in each well and the plates were incubated overnight at 37 °C. Growth was determined by measuring the diameter of the zone of inhibition. The solvents were used as the negative controls whiles 10 µg ampicillin disc (Oxoid) was used as the positive control. The control zones of the solvents were deducted from the zones of inhibition created by the crude extracts. The experiments were carried out in triplicates and results were calculated as mean ± SD.

Wound healing activity

Experimental animals

Albino Wistar rats of either sex weighing 150-300 mg were used for the study. The animals were maintained under hygienic conditions and they were provided with commercial food pellets and tap water. Cleaning and sanitation work were done on

alternate days. The cages were maintained clean and all experiments were conducted between the hours of 9 am to 5 pm.

Grouping of animals

The animal weights (150-300 mg) were recorded. The animal groupings were stratified according to weights, so that the average weights of all groups were comparable. Seven groups of the animals with four rats each were used.

GROUP 1- rats treated with 5% w/w of powdered plant ointment (5 gm of powdered plant
material mixed with 100 g of shea butter).
GROUP 2- rats treated with 10.0% w/w of powdered plant ointment (10gm of powdered plant
material mixed with 100 g of shea butter).
GROUP 3- rats treated with 30 mg/ml of aqueous extract.
GROUP 4- rats treated with 100 mg/ml of aqueous extract.
GROUP 5- rats treated with standard drug (penicillin ointment).
GROUP 6- rats treated with Shea butter ointment.
GROUP 7-rats left untreated

Creation of excision wound

An excision wound model was used for studying the wound healing activity (Nalesh and Sanjay, 2009). Fresh 50 mg/ml ketamine chloride solution was prepared for anesthesia and a single-use syringe for injection. For the IV injection, the mouse was held at its neck directly behind the ears and the tail was grasped while holding the rats with its head down. The rats were placed back into the cage, so that rats will not become agitated. This type of anesthesia prevents any movement of the animals at least for 2 hours after the administration of the anesthetic solution so that they were left without being restrained. Hair was removed by shaving the dorsal of all rats. A full thickness of the excision wound of approximately 490 mm^2 and 2 mm depth was created along the markings using toothed forceps and pointed scissors. The back of the anesthetized rats were shaved using the razor blade and the hair was carefully removed from the back of the animal. The anesthetized and shaved rats were placed on a paper towel. The shaved back of the animal was wiped with a sufficient amount of 70% alcohol. The mouse was held at its neck directly behind the ears and the tail of the rats was held down. The back of the skin was lifted using forceps. The skin was incised first and carefully cut using the scissors. Lifting up the skin ensured that the incision will move through the panniculus Carnosus. After completion of excision wounding, the wound was left undressed to the open environment and no local or systemic anti-microbial agents were used before the animals were transferred into cages.

Determination of microbial load on the wound

Swabs were taken from the excision wound each on day 5, 10, and 15. The collected swabs were immediately sent to the laboratory for testing. In the quantitative count study, each swab stick was added to 2 ml of peptone water. The sample was mixed thoroughly and a 5-fold serial dilution was performed. A volume of 0.1 ml of each sample dilution was spread onto MacConkey and blood agar plates. They were incubated at 37 °C for 24 hours. The colonies were counted and the results were recorded (Demetriou *et al.*, 2013).

Determination of Antioxidant activity

Determination of flavonoid contents

The aluminum chloride colorimetric method was used to measure the flavonoid content of all plant extracts (Chang et al, 2002). Each plant extract of 0.5 ml was added to 1.5 ml methanol, 0.1 ml 1M sodium acetate and 2.8 ml distilled water after which 0.15ml of 10% aluminium chloride was added. The mixture was allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm with a UV/VIS spectrophotometer. Quercetin was used as the standard for the calibration curve. Flavonoid contents were expressed as mg quercetin equivalent /g dry weight (D.W.) using the regression equation $y = 0.0012x + 0.0101$, $R^2 = 0.9499$).

Determination of total phenolic content

Total phenol content was estimated using Folin-Ciocalteu reagent based assay as previously described (McDonald *et al.*, 2001). To an aliquot from each plant extract, 10 ml of water, 1.5 ml of Folin-Ciocalteu reagent (Sigma-Aldrich, Switzerland) were added. The mixture was kept for 5 minutes at room temperature and then 4 ml of 20% Na_2CO_3 was added and the volume brought to 25 ml with double-distilled water. The mixture was allowed to stand at room temperature for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. Gallic acid was used as standard for calibration curve. Total phenol value was obtained from the regression equation: $y = 0.0581x + 0.0116$; $R^2 = 0.9995$ and expressed as mg/g gallic acid equivalent using the formula, $C = cV/M$; where C = total content of phenolic compounds in mg/g GAE, c = the concentration of gallic acid (mg/ml) established from the calibration curve, V = volume of extract in ml and m = the weight of pure plant methanolic extract in g (diluted ten times).

Ferric Reducing Antioxidant Power Assay

The reducing antioxidant power of plant methanolic extracts was determined by the method of Oyaizu (Oyaizu 1986). Different concentrations of plant extracts (50 – 200 ppm) in 1 ml of distilled water were mixed with phosphate buffer (3.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50 ^oC for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%).

The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicates increase in reducing power. Ascorbic acid was used as standard.

Scavenging activity against 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH)

The crude methanol extract, ethyl acetate and n-butanol fractions of *C. dolichopetalum*, were screened for 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical Scavenging activity. DPPH radical scavenging activity was measured according to standard methods in literature (Braca *et al.*, 2003; Rajeswara *et al.*, 2012). An aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of plant extract at various concentrations were mixed and incubated at 37 °C for 30 min. and absorbance of the test mixture was read at 517 nm. All experiments were performed in triplicate and the results were averaged. The results were compared with that of ascorbic acid as standard. The percentage inhibition/scavenging activity of DPPH-radical was calculated using the formula:

% scavenging activity of DPPH-radical = (Abs control – Abs sample)/ (Abs control)*100

Determination of Total Antioxidant Activity (TAC)

The total antioxidant capacity of the methanol extract was evaluated by the phosphomolybdenum method (Prieto *et al.*, 1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid ρH. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated for 60 minutes at 95 ^{o}C. The absorbance of the green phosphomolybdenum complex was measured at 695 nm using a spectrophotometer (Jenway 6025) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity was expressed as the number of gram equivalent of ascorbic acid. Different concentrations of ascorbic acid (0.02-0.20 mg/ml) were prepared in methanol and used to obtain the calibration curve.

RESULTS AND DISCUSSION

Antioxidant activity

The DPPH radical has been used widely to test for the potential of compounds as free radical scavengers and as hydrogen donors to investigate the antioxidant activity of plant extracts. The DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by extract either by transfer of hydrogen or of an electron (Knezevic *et al.*, 2011; Sahoo et al, 2013).The results from this study showed that all the different solvent extracts possessed free radical scavenging properties, but to varying degrees, ranging from 89.64 ± 1.03 to 93.92 ± 0.05% at 800 ppm. Similarly, the extracts displayed varying degrees of Total antioxidant activity ranging from 55.83 ± 0.41 to 65.86 ± 2.84. Table 1 presents the scavenging abilities and the total antioxidant capacities of the various solvent extracts. Ferric reducing antioxidant power (Figure 1) was no exception. The values increased with increasing extract concentration with ethyl acetate exhibiting the highest reducing potentials followed by butanol in the similar pattern with results from the DPPH scavenging ability shown in Table 1. The results proved the abilities of the various extracts to either donate hydrogen atoms to scavenge radicals or cause the transfer of electrons to reduce metal ions that catalyse oxidative processes. These varying degrees in abilities of the plant extract are good indications of antioxidant properties of the plant extracts.

Table 1: Scavenging abilities (%) and the total antioxidant capacities of the various solvent at different concentrations extracts.

Extract	Concentration (ppm)	Inhibition (*I*%)	TAC
Methanol	200	49.46 ± 3.24	19.97 ± 0.75
	400	86.18 ± 2.10	35.40 ± 3.01
	600	89.72 ± 0.73	43.96 ± 0.90
	800	89.64 ± 1.03	65.38 ± 0.45
Ethyl acetate	200	52.72 ± 5.90	17.40 ± 0.52
	400	82.65 ± 2.69	33.13 ± 3.63
	600	92.78 ± 0.37	52.84 ± 7.94
	800	93.92 ± 0.05	65.86 ± 2.84
Butanol	200	50.29 ± 2.03	19.86 ± 4.78
	400	81.52 ± 2.17	31.35 ± 2.34
	600	91.02 ± 0.03	44.04 ± 2.65
	800	93.00 ± 1.10	55.83 ± 0.41
Ascorbic acid	200	94.61± 2.52	19.57 ± 0.20
	400	99.10 ± 0.52	39.51 ± 1.66
	600	99.64 ± 0.40	63.17 ± 4.77
	800	99.94 ± 0.08	78.66 ± 2.27

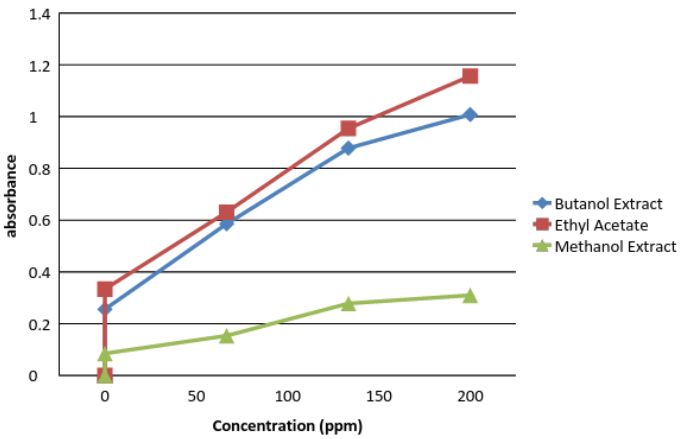

Fig. 1: Ferric reducing power of the different solvent plant extracts.

These findings revealed that extracts of *C. dolichopetalum* have rich antioxidant properties which might contribute to the medicinal uses of this plant in traditional medicine. Correlation analysis between the values obtained from the DPPH and TAC is presented in Figures 3, 4 and 5. The two models showed a good high positively linear correlation for all the solvent extracts (methanol (R^2=0.6135), n-butanol (R^2 = 0.8013) and ethyl acetate (R^2 = 0.75). The existence of this high positive correlation did not only confirm the presence of antioxidants but indicated the viability of the two models for evaluating antioxidants from medicinal plants. The results obtained from these two methods of estimation antioxidants presented the solvents used (methanol, n-butanol and ethyl acetate) as very good potential solvents for the extraction of antioxidant compounds. However, the results showed differential abilities among the solvents to extract the antioxidant compounds. It was our

expectation that the two most polar solvents, methanol and butanol would have given a higher antioxidant results as reported (Sineiro *et al.*, 2008) that Solvent polarity might determine the quantity and perhaps the quality of antioxidant compounds.

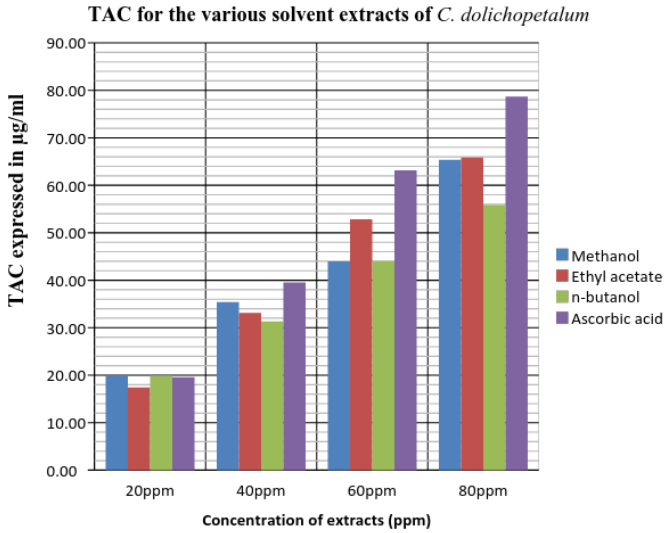

Fig. 2: TAC for various solvent extracts of *C. dolichopetalum*

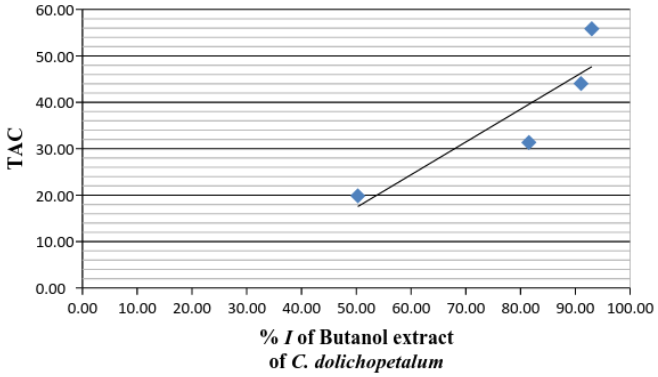

Fig. 3: Correlation between DPPH and TAC in butanol extract

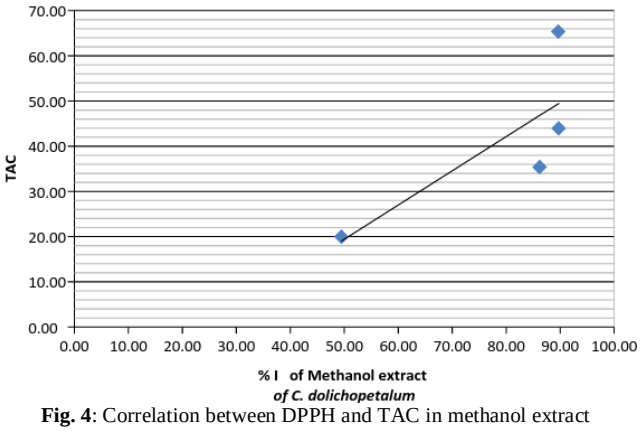

Fig. 4: Correlation between DPPH and TAC in methanol extract

From that study, methanol and ethanol have been found to have higher yields of polyphenolic compounds extractable from plants material. However, ethyl acetate extract generally, recorded the highest results in all the three antioxidant models used as shown in Figure 1, 2 and Table 1. The result can be compared to similar results found in literature showing the significant impacts extraction solvents had on antioxidant activity estimation, as well as different extraction capacity and selectivity for free phenolic compounds (Zhou & Yu, 2004; Zhao *et al.*, 2006). By this result, ethyl acetate can be said to be most suitable solvent for the extraction of antioxidant compounds from *C. dolichopetalum* even though ethyl acetate recorded a lower Total Phenolic Content (173.89 ± 0.11) than that of methanol (219.15 ± 29.02). This may be due to the fact that phenolic compounds are often extracted in higher amounts in more polar solvents (Sultana *et al.*, 2009).

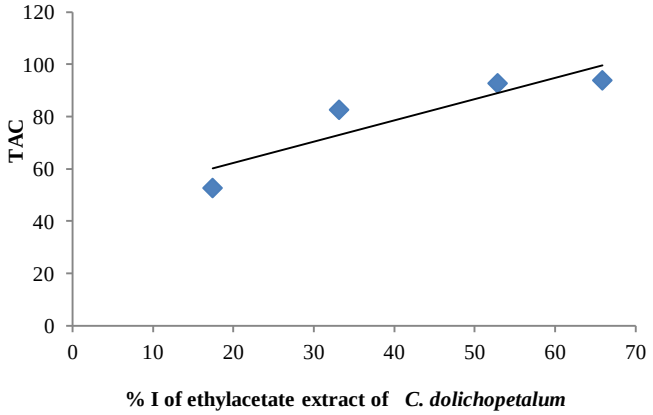

Fig. 5: Correlation between DPPH and TAC in ethyl acetate extract.

Table 2: Results on Total phenolics content, Flavonoid content and some phytochemicals determined in the various solvent extracts.

Extracts	Flavonoids	Tannins	Phenolic content (mg/g)	Flavonoid content (mg/g)
methanol	+	+	219.15±29.02	574.88± 16.6
Ethyl acetate	+	+	173.89 ± 0.11	925.42±6.63
n-butanol	+	+	89.21 ± 0.11	785.28 ± 6.3
chloroform	-	-	4.1 ± 0.00	75.0 ± 3.88

+= present, - = absent.

Wound healing activity of *C. dolichopetalum*

A significant promotion of wound-healing activity was observed in the various extracts of *C. dolichopetalum* in the excision wound model. The diameter of the wound was measured in order to determine the rate of wound closure and to determine the wound healing activity of the plant. The mean percentage wound closure was calculated on 0, 3, 6, 9, 12, 15 and 18 post-wounding days. Table 3 presented the mean percentage wound closure recorded on the plant. Result from the wound contraction indicated that both the aqueous extract and the powder of *C. dolichopetalum* leaf have significant wound healing activity. Almost all animals treated with the plant extracts, healed completely on the 15 post-wounding days. However, the aqueous extracts performed better than the ointment preparations of the leaf powder. Figure 5 shows the total bacterial counts from granulation tissue on different days for the various treatments. The estimation of microbial load on the surface of the wounds had a similar trend just like the wound healing ability with the aqueous extract again performing better than the ointment preparation of the plant powder. Topical application of the various plant extracts resulted

Table 3: Wound diameter (mm) and Mean percentage wound closure on different days of topical application of *Combretum dolichopetalum* leaf ointment on excisional wound.

Treatment	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Penicillin ointment	719.32±22.6	433.99±10.6 (33.67)**	306.54±7.6 (57.38)***	113.50±7.7 (84.22)***	27.31±6.6 (96.20)***	0.60 ± 0.2 (99.20)***
Shea butter	683.77±13.3	627.42±21.0 (8.24)	482.10±24.1 (29.49)	332.27±30.2 (51.41)	133.93±15.0 (80.41)	76.39±13.5 (88.83)
5% w/w	707.34±19.2	511.75±25.8 (27.65)	381.36±23.8 (46.09%)	269.82±18.7 (61.85)	116.06±37.5 (83.90)	21.60±19.0 (96.95)**
10% w/w	695.76±22.5	462.46±18.0 (33.60)*	346.80±13.5 (50.16)**	182.83 ± 6.1 (73.72)***	53.02 ± 12.5 (92.38)**	0.98 ± 0.7 (99.86%)***
30 mg/ml	684.79±29.9	491.49±16.0 (28.23)	338.86±15.7 (50.52)**	171.10 ± 5.7 (75.01)***	87.22 ± 8.6 (87.26)	11.22±1.4 (98.36)**
100 mg/ml	731.15±13.8	330.41 ± 9.3 (54.81)***	159.71 ± 5.7 (78.16)***	67.81 ± 6.8 (90.73)***	11.22 ± 1.4 (98.47)***	0.00 ± 0.0 (100.00)***
Untreated (control)	695.76±22.53	573.81±29.44 (17.53)	453.63±26.11 (34.80)	322.64±15.08 (53.63)	171.83±14.23 (75.30)	78.94±6.4 (88.65)

Values are expressed as Mean ± SEM from four animals in each group. Numbers in parenthesis indicates % wound closure; *P< 0.05, **P< 0.01, ***P <0.001 (compared with control).

Table 4: Zone of inhibition (mm) measured for each bacterium at different concentrations of methanol extract of *C. dolichopetalum.*

Microorganism	Zone of inhibition in mm				
	0.025 mg/ml	0.05 mg/ml	1.0 mg/ml	1.50mg/ml	10.0 µg ampicillin
E. coli	-	-	-	-	-
K. pneumonia	-	-	-	-	-
S. aureus	-	-	23.0 ± 0.05	26.0± 0.08	30.0 ± 0.1

in diminishing total bacterial counts in the infected wound from days 5, 10 and 15. There was significant decrease in the number of microbes in the wounds treated with the plant extract preparations and that the treatment was also dose dependent.

The topical application of the treatments efficiently destroyed microbial populations and this indeed led to enhanced wound healing activity.

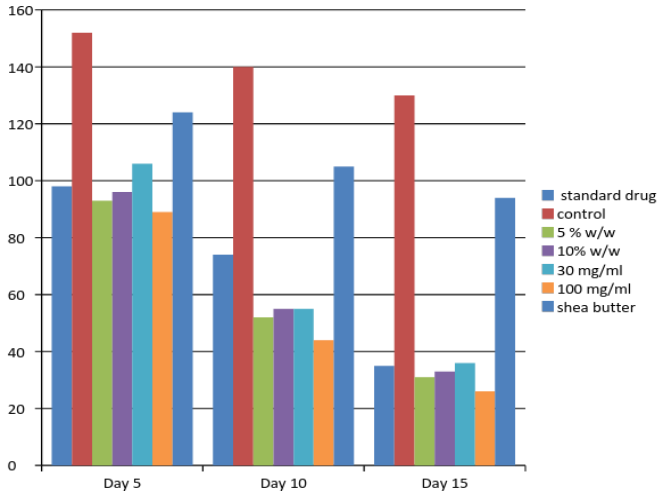

Fig. 5: Comparison of the microbial load of the various rat groups with different treatments *C. dolichopetalum* extract. Each bar indicates the microbial load of different treated wound on the given days.

Some experts consider the microbial density to be critical in predicting wound healing and infection, while others consider the types of microorganisms to be of greater importance (Bowler et al, 2001). It is in this vein that we found our work very interesting and useful. Microbial density decreases from day to day with the application of the extracts. In addition, *S. aureus* one of the most commonly microorganisms that causes wound infection (Collier, 2004) has been inhibited by the plant under study.

All the plant extracts indicated the presence of flavonoids and tannins in addition to the high level of phenolic and flavonoid content measured (Table 2). Tannins are known to have antimicrobial and astringent properties which are responsible for wound contraction (Getie *et al.*, 2002). Tannins are also known to have protein coagulatory properties that aid in wound healing together with the contribution of other secondary metabolites (Agyare *et al.*, 2014). Flavonoids, on the other hand, are noted to possess antioxidant and anti-inflammatory properties (Akuodor *et al.*, 2010).These properties of both tannins and flavonoids synergistically, could be responsible for the closure and acceleration of wound healing properties of the extract. Ability of the plant extract to inhibit the growth of this microorganism contributes tremendously to wound healing. The study agrees with work done by researchers (Okoli *et al.*, 2007) who stated that plant extracts inhibit microbial growth and accelerate wound healing. The primary objective of wound care is to prevent or minimize infection and promote healing. The ability of the plant to decrease the bacteria load and to inhibit the growth of bacteria indicated the wound healing potential of the plant. The presence of tannins in the extract may be attributed to the antimicrobial activity.

Polyphenols are phytochemicals that acts as antioxidant to fight free radicals that have the potential to damage biological tissues by disrupting cell membranes. These free radicals are destructive to molecules in the membrane that contain carbon-carbon double bonds (C=C) for instance, polyunsaturated fatty acids, proteins and DNA through oxidation. When this happens the ability of the cell to transport substances across the membrane is hindered or affected leading to its associated diseases such as cardiovascular diseases and poor wound healing process. The

antioxidant properties exhibited by the plant explained the presence of phenolic compounds that showed such marked wound healing potentials. These natural agents which are not only cheap and affordable but also safe, induce healing and regeneration of the lost tissue by multiple mechanisms. Various herbal products have been used in management and treatment of wounds over the years. Wound healing properties appeared to be associated with antioxidant properties (Suntar et al, 2012) and that wound healing plants are good sources of antioxidants. The plants under study can therefore be applied not only in wound healing but also to either prevent oxidative stress or treat cardiovascular diseases and some types of cancers.

CONCLUSION

C. dolichopetalum has shown to possess not only antimicrobial and wound healing properties but potential sources of antioxidants. The study has also shown the differential abilities of various solvents in antioxidant extraction and that this ability sometimes depends on the plant in use. To reduce the impact of ROS there is the need to provide the body with a constant supply of antioxidants through dietary supplementation. This plant proves to be worthy of dependence in future research for the prevention of oxidative stress or treat cardiovascular diseases and some types of cancers associated with ROS. The study also supported and justified the traditional use of this plant for wound healing.

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