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Cutaneous Wound Healing Activity of Herbal Ointment containing *Tetrapleura tetraptera* Fruit Extract

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ABSTRACT

Phytochemicals are known to possess many wounds healing properties. Phytochemicals are known to be used to protect humans against similar threat to haemostatis. To confirm this statement, wound excision measuring about 170mm was created on the back of each healthy male Albino wistar rat of same age group. They were 30 rats in all divided into 5 groups of six rats each (n=6). Three different concentrations of *Tetrapleura tetraptera* fruit aqueous extract were prepared (2.5g/2qg, 5.0g/20 and 10.0g/20g) of white petroleum jelly as base were used as test drugs, while gentamycin 1% ointment was used as standard drug, while the control group receive no medication. Skin irritation was determined. The ointment was applied topically on wounded areas which was measured every two days interval until full epithelialization and complete closure. Topical application of the aqueous extract of *Tetrapleura tetraptera* fruit ointment showed significant wound healing rate ($p<0.05$) and reduced epithelialization period which as also increased in dose dependent manner. Application of ointment batch containing *T. tetraptera* fruits extract 10.0g/20g ointment showed highest rate of wound closure with reduction in epithalization period within 14 days. *Tetrapleura tetraptera* fruit extract wound dressing material showed great promise and potential of the plant in treating wounds that have defied all surgical and medical interventions.

Keywords: *Tetrapleura tetraptera, phytochemicals, incisions, aqueous extract and wound healing*.

INTRODUCTION

In the present world, many patients would readily accept inclusion of herbal or botanical medicine as part of their medical intervention based on the recognition that herbal medicine is considered to be natural and that the practice have been part of our culture for thousand of years. In this regard, the use of plants products to treat a specific disease condition appears to have been part of medical care observed for thousands of years. Although the use of plant products is no longer a major aspect of medical care as practiced in western world, it is still extremely popular in large number of world's populace particularly Asia and Europe (Griggs, 1981). However, for medicine as practiced in the western countries, one observation that appears to be forgotten is that many of the pharmaceutical agents currently prescribed appear to have been derived from natural compounds found in traditional medicinal plants (Trease and Evans,1999). Herbal therapy for skin disorders have been used for thousand of years. Specified herbs and their uses developed regionally, based on locally available plants and through trade in ethnobotanical remedies. Systems of herbal use developed regionally in Europe, the Middle East (Ghazanfer, 1994), Africa, India (Behi and Srivastava, 2002), China, Japan, Australia and Americas. Two well-known systems still in use are the Ayurvedic herbs in India (Kapoor , 1990) and herbs combinations and developed as part of traditional Chinese medicine (TCM) in China (Xu, 2004). In Europe and the United States, use of herbs declined as purified extract and synthetic chemical drugs became available. In recent years, there has been resurgence of the use of herbs due to the following reasons. The side effects of chemical drugs became apparent, there was a call to return to nature, natural remedies became a part of the green revolution and there was a return to organic produce. Herbal remedies including those of skin disorders are currently gaining popularity among patients and to a lesser degree among physicians (Bulmenthal *et al.*, 1998). Wound care is consistently evolving with advance in medicine. Search for the ideal dressing material still continues as wound care professionals are faced with several challenges. Due to the emergence of multi-resistant organisms and decrease in new anti-biotics, wound care professionals have revisited the ancient healing methods by using traditional and alternative medicine in wound management.

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People's perception towards traditional medicine has also changed and is very encouraging. The concept of moist wound healing has been well accepted and traditional medicine has also incorporated the method to fasten the healing process. Several studies using herbal and traditional medicine from different continents have been documented in wound care management. Recent scientific evidences and clinical trials conducted using traditional and alternative medicine in wound therapy holds promise in the future. Medicinal plants have generated much interest in recent years for treating skin ailment as they are affordable and purportedly safe due to less hypersensitivity (Raina, *et al* 2008). Tetrapleura tetraptera (family, mimosaceat) is a single stemmed, robust, perennial tree with dark green leaves and thick woody base spreading branches. Various preparations of the plants are known to be used in folklore medicine for treating human ailments including cardiovascular disorders such as hypertension, asthma, diabetes mellitus, epilepsy and schistosomiasis (Aladesanma 2006). The plant is also frequently used in Tropical African traditional medicine for the management and/or of several women's diseases such as breast and uterine cancers as well as inflammatory conditions (Ojewole and Adesina 1983). The fruit extract of the plant have been shown to possess hypocholesterolemic effects in rats (Ajayi *et al*., 2011) as well as alteration of various parameters in rabbits (Adesanmi et al 2011). Cardiovascular and neuro-muscular actions of scopeletin isolated from *T. tetraptera* were also described. The pods and/or fruit have been shown to have anti-bacterial effect against *Bacillus species, Entercoccus faecalis, Eschorichia coli, Klebsiella pneumonia, Staphyloccoccus aureus, Salmonella typhi, Pseudomonas auriginosa and Shigella* (Ekwerenye and Okorie, 2010; Awofisayo *et al.,* 2010; Abaoba *et al.,* 2011). The documented biological and pharmalogical activities are found to be molluscicidal, cardiovascular, neuromuscular, hypotensive, anticonvulsive, trypanosomicidal, schistosomiasis control, antiulcerative, cytotoxic, anti-inflammatory, hypoglycaemic, antimicrobial, emulsifying, important at birth control, of food value and the control of intestinal parasites (Adetungi, 2007). Elizzi *et al*., (1990) reported that the stem bark of *T. Tetraptera* showed an inhibitory effect on luteinizing hormone released by pituitary cells. *T. Tetraptera* has been shown to cause elevation in serum. Aspartate amino transferase (AST) and alteration of various metabolites and did not induce any marked pathological lesion in the liver (Odesanmi *et al* 2009). The sedative, anticonvulsant and analgesic effects of *T. Tetraptera* in mice have been reported (Aderibigbe *et al.,* 2007). The aqueous extract of *T. tetraptera* fruits have been shown to possess anti-inflamatory and hypoglycaemic properties (Ojewole and Adewunmi 2004). The fruit is used to prepare food for mothers from the day of birth to prevent post partum contraction (Nwanu and Akah 1986). The fruit is also used as a dietary supplement rich in vitamins in southern and eastern Nigeria (Okwu, 2003). The allelopathic potential of *T. tetraptera* has led ot its integration into agroforestry (Amoo *et al*., 2008). Many researchers have reported the use of *T. tetraptera* aqueous stem-back on treatment of wounds (David, 2014) but very few researchers have ever reported the use of *T. tetraptera* fruit aqueous extract on wound dressing. This may be due to thick and deep brown nature of the crushed boiled (decoction) which may need special technique to separate the thick mixture. *T. tetraptera* have immense potential for the management and treatment of wounds. Large number of plant are used by tribal and folklore in many countries for treatment of wounds and burns. These natural agents induce healing and regeneration of the lost issue by multiple mechanisms. These phytomedicines are not only cheap and affordable but are also safe. The presence of various life sustaining constituent in plants has urged scientists to examine these plants with a view to determine potential wound healing properties. Many phytopharmaceutical laboratories are now concentrating their efforts to identify the active constituents and mode of action of various medicinal plants. These medicinal values of those plants lie in bioactive phyto-chemical constituents that produce definite physiological action on human body. These constituents include various chemical families like alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins and phenolic compounds. The screening of herbal extract has been of great interest in the scientific world for the discovery of new effective drugs. A number of reports concerning the antibacterial, anti-inflammatory and wound healing activity of various plants have appeared in the literature, but the fast majority has yet to be explored. Various pharmacological reports are available on plants employed in different wound healing models and their underlying molecular mechanism for the validation of their traditional claims and effectiveness are globally accepted (Shuid, 2005; Nayak *et al.,* 2006; Bhat *et al.,* 2007; Asif ,2007;

Gurung and Skalko-Basnet, 2009). Therefore this research becomes quite imperative in order to harness the potential of *T. tetraptera* in wound healing.

MATERIALS AND METHODS

Collection ,processing and extraction of Plant Material: Ripe fruits of *T. tetraptera* were bought in Akpan Andem market in Uyo, the capital city of Akwa Ibom State. The fruits were authenticated by a Botanist in the Department of Botany, University of Uyo. A voucher of the specimen Number 32(F) was deposited in Herbarium of the Faculty of Pharmacy, University of Uyo. The fruits were watered under tap running water and thereafter rinse with distilled water. They were shade dried for 1 week. The fleshy portion of the dried fruits were carefully chopped off from the central hard wooden part and were ground into very tiny bits almost like powder using grinding machine. About 3kg of the ground fruits was measured and mixed with about 5 litres of distilled water. The mixture was boiled for about 1 hour in order to reduce the quantity of the water and thereby increase the concentration of the extract. The extract was allowed to cool and then squeezed using fine nylon cloth and finally subjected to filtration and ultrafiltration using various sizes of microfilters Phytochemical analysis of the extract was carried out and the following phytochemical components were identified; alkaloids, tannins, flavonoids and saponin. Animals: Animals used in this study were obtained from College of Medicine, University of Calabar, Cross River State, Nigeria after due consultation for approval of the study from Faculty of Pharmacy University of Uyo ethical Committee. Healthy male Albino wistar rats of same age group weighing (140-200g) were used in this study. All animals were housed in a standard laboratory conditions of 12 hours day light and 12 hours dark cycles and fed with standard animal pellets and water ad libitum. The animals were housed individually in a sterile polyvinyl/propylene metal cages containing paper cuttings as bedding. The animals used in this study were 30 in number. They were divided into 5 group of six animals in each group (n=6) for the study. Wound Creation: All the surgical intervention were carried out under sterile conditions using ether as general anaesthesia. The predetermined area for wound infliction at the back of the animals were prepared for surgery by removing hair with shaving machine. They were anaesthesized using ether by open mask method. The area of surgery induced by local anaesthetic using lignocaine solution to render the area painless. Wound size of about 170mm was created on the dorsum of each animal and haemostasis was secured before returning the animals to their cage for recovery. They were housed individually and monitored for respiration, colour and temperature. They were maintained under standard husbandry condition and on uniform diet and managed throughout the experimental period. They were closely observed for any sign of infection. They were periodically weighed before and after the experiment.

Preparation of Ointment: The following substances were used in preparing herbal ointment of *Tetrapleura tetraptera*. Stearyl alcohol, white petroleum, lauryl sulphate, methyl and propyl paraben. In preparation of hydrophilic ointment of *T. tetraptera* stearyl alcohol and whilte petroleum were melted at 75^0C. extract of *T. tetraptera* treat was added while stirring until the mixture cogealed. Sodium lauryl sulphate was added as emulsifying agent. Finally methyl and propyl paraben were added as preservatives. Acute Skin Irritation: This test was performed on Albino Wistar Rats weighing 150-200g. the animals were divided into five groups of six animals in each group. Dorsal hair on the back of the animals were removed one day before the ommencement of the study. They were kept individually in each cage to avoid contact with each other. Two groups were used as control and standard irritant while the other groups were used as test. 50 mg of each formulation of different concentrations were applied over one square centimeter area of whole abraded skin of each animal.

RESULTS

This study showed how herbal extract of *T. tetraptera* fruit caused increased rate of wound contraction and decreased period of epithelialization in the test group as compared to the control group which received no treatment ($p<0.05$). The study also indicated that the healing effect of *T. tetraptera* depended on its concentration. The highest concentration of *T. tetraptera* fruit extract (10g/10g ointment) produced faster rate of healing of wound with epithelialization period of 14 days when compared to the blank or control with no treatment which had epithelialization period of 21 days and standard treatment of 18 days. The result is our shown in table 1 and figure 1 below:

Table 1. **The Effect of *Tetrapleuratetraptera* based ointment on excision wound healing in rats**

	TreatmentGroups				
Wound Area in mm2 (percentage wound contraction in parenthesis)	Tetrapleuratetraptera 2.5g/10g of ointment base	Tetrapleuratetraptera 5.0g/10g of ointment base	Tetrapleuratetraptera 10g/10g of ointment base	Gentamycin Ointment (1%)	Blank without Treatment
Day 2	164.52±1.01*	164.52±1.00*	161.17±0.53**	164.64±1.12*	169.27±0.85
Day 4	154.60±0.68**	152.13±0.60**	149.42±0.61**	154.22±0.53**	164.19±0.81
Day 6	143.43±0.54*	132.88±0.87**	120.65±0.60**	140.42±0.82**	150.43±0.67
Day 8	121.45±0.60**	112.50±0.74**	83.68±1.00**	112.23±0.84**	132.13±0.54
Day 10	91.75±0.70**	82.80±0.56**	22.50±0.57**	82.13±0.49**	112.10±0.64
Day 12	52.13±0.48*	42.77±0.54**	2.40±0.17**	51.13±0.43*	91.10±0.42
Day 14	33.83±0.54**	15.73±0.43**	0.62±0.20**	28.67±0.34**	70.45±0.63
Day 16	10.05±0.50**	1.03±0.12**	0.00±0.00**	2.62±0.21**	54.47±1.62
Day 18	5.18±0.12**	0.00±0.00**	0.00±0.00**	0.00±0.00**	30.45±0.49
Day 20	0.70±0.11**	0.00±0.00**	0.00±0.00**	0.00±0.00**	10.20±0.62

The values are presented as Mean±SEM (n=6). The asterisk denotes the significance levels in comparison with the negative control: *p<0.01, **p<0.001.

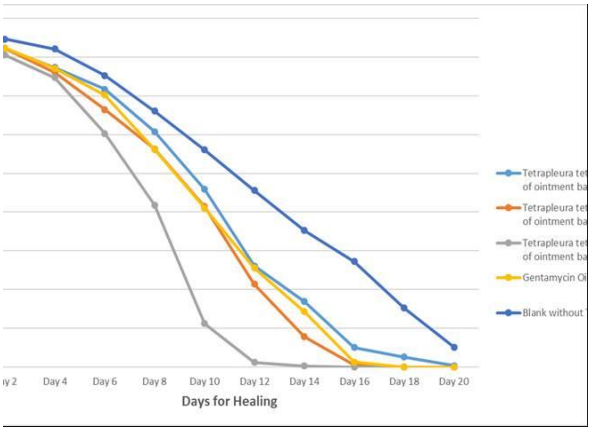

Figure 1. Showing Comparative Treatment of Standard Drugs and Tetrapleura tetraptera (2.5g, 5.0g and 10g/10g of Ointment Base)
The asterisk denotes the significance levels in comparison with the negative control: *p<0.01, **p<0.001.

DISCUSSION OF RESULT

Phytochemicals are known to possess many wound healing properties. Plants produce phytochemicals to protect themselves against environment, disease causing microorganisms. Researches demonstrate that phytochemicals products may be useful to protect humans against similar threat to homeostatis (Salcido, 2014). While current therapeutic agents for treatment of wounds have generally inadequate efficacy, the medicinal plants have been used in medicine since ancient times are well known for their ability to promote wound healing and prevent infection without grave side effects. Thus herbal therapy may be alternative strategy for treatment of wounds (Budovsky *et al*., 2015). There is an increasing interest in finding herbal extracts with wound healing efficacy although the use of extract for treating wounds is a common practice in traditional medicine. Herbal extracts are known to arrest bleeding effectively from fresh wounds, inhbited microbial growth and accelerated wound healing (Jalalpure *et al,* 2008). The enhanced wound healing potency of the phytoconstituents present in the extract and the quicker process of wound healing could be a function of either the individual or synergestic effectives of bioactive molecules. These active constituents promote the process of wound healing

by increasing the viability of collagen fibrils, by increasing the strength of collagen fibres either by increasing the circulation or by preventing the cell damage or by promoting the DNA synthesis (Patwardhan *et al* 2007). Pharmaceutically and biologically phytochemicals are known to promote fast wound healing in different studies. The process of wound healing is promoted by several phytochemical compounds like alkaloids, flavonoids, saponins, tannins, phenols etc. Tannins are known to act as free radical scavengers, triterpenoids and flavonoids promote healing due to their astringent and antimicrobial property while saponins are well demonstrated to possess antioxidant activity (Govindarajan *et al.,* 2004; majumdar *et al.,* 2007). Alkaloids content of some herbal root, such as *Cassia alata* have been reported to exhibit marked dose-dependent pharmacological effect on gastrointestinal smooth muscles (Tologbonse *et al*.,2015). Flavonoids are well documented to possess antioxidant and free radical scavenging effects enhancing the level of antioxidant effects enhancing the level of antioxidant enzymes in the body tissues. Saponins are well known to possess ability to regenerate epithelial tissues (Krishnaveni *et al* 2009). Herbal extracts with antioxidant potentials are known to be good therapeutic agents for accelerating wound healing process. Phytochemicals are known to interfere with wound healing by restoring the blood flow through scavenging of free radicals and reduction in damage due to oxidative stress (Krishnaveni *et al* 2009) and thereby being able to regenerate epithelial cells. Thus, the antioxidants present in the herbal extract could be expected to promote epithelialization by controlling oxidative stress. Botanicals with antioxidant or free radical-scavenging activity thus can play a significant role in healing of wounds (Baravka *et al* 2008). In the light of this significant role plants play in the treatment of wounds and other human diseases various part of *T. tetraptera* have been documented to possess clinical efficacy in treating various kinds of human diseases including wounds but never a time has any part of it been processed in any form of clinical use. Therefore this study significantly creates opportunity and possibility of processing *T. tetraptera* fruits in whatever forms ointment, cream or solution in treating different kinds of wounds that have plagued humanity over the centuries

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