

Review

Review of twelve West Africa medicinal plants: Active phytochemical combinations in direct biochemically wound healing process

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Received 28 May, 2015; Accepted 29 July, 2015

The aim of wound care is to promote wound healing in the shortest time possible, and plants are a great source of medicines, especially in traditional medicine. Traditionally, medicinal plants have been used for many years as topical and internal preparations to promote wound repair. This work will give an overview of twelve West Africa medicinal plant studies and their active phytochemicals in direct wound healing. The process of wound healing is promoted by several herbal extracts, which are composed of active agents like triterpenes, alkaloids, flavonoids, tannins, saponins, anthraquinones, and other biomolecules. 55% of overviewed plants contain tannins and we found that 45% of them contain terpenoids. Saponins are present at a level of 36% in these overviewed plants. Finally, we remarked that 80% of plants studied here contain flavonoids. The antioxidant and anti-inflammatory activities of flavonoids were believed to be one of the important mechanisms in wound healing and in the presence of tannin it improved the regeneration and organization of the new tissue and hastened the wound healing process. Sterols and polyphenols are also responsible for wound healing due to free radical-scavenging and antioxidant activity, which are known to reduce lipid peroxidation. Cactus extract up-regulates VEGF expression in wounded skin, stimulating in return angiogenesis for supplying nutrients and oxygen for skin regrowth. The expression of MMPs revealed the enhanced healing pattern in the *E. guineensis* leaf extract treated rats group. In conclusion, biochemically wound healing process is promoted by active agents in herbal extracts and those components act synergistically.

Key words: Medicinal plants, phytochemicals, biochemically wound healing.

INTRODUCTION

Wound healing processes are well organized biochemical and cellular events leading to the growth and

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regeneration of wounded tissue in a special manner: the restoration to normal condition of the injured skin or tissue (Clark, 1991). The aim of wound care is to promote wound healing shortly, with minimal pain, discomfort, and scarring to the patient and must occur in a physiologic environment (Odimegwu et al., 2008). Burns and trauma wounds are common and at the same time they constitute a major problem in developing countries because financial resources are limited (Gur et al., 2013). In these countries, one important aspect in treatment of burns and wounds is the use of local herbs. Plants are a great source of medicines, especially in traditional medicine. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO, 2002).

A medicinal plant is any plant which contains substances that can be used for therapeutic purposes or substances which are precursors for the synthesis of useful drugs, according to the World Health Organization (WHO, 1977). Therefore, a plant becomes a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically established (Elujoba, 1997). Traditionally, medicinal plants have been used for many years as topical and internal preparations to promote wound repair. With the development of scientific research methods, the significant successes reported have led to investigation into medicinal plants with a view to confirming these acclaimed properties (Farahpour and Mavaddati, 2012). Then, the formulation which claims to possess wound healing activity shall be studied with the features like tensile strength of newly formed tissue, biochemical parameters like serum and tissue levels of hydroxyproline, lysyl oxidase, methionine, ascorbic acid etc. which contribute to the all-round healing of wound (Ganju and Pathak, 2013). An example to illustrate tensile strength is one of fibrin network. In fact fibrin network is the first scaffold that a cell encounters as it performs its role in healing wounds due to trauma or other insults to normal tissue (Janmey et al., 2009). *In vivo*, transglutaminase activity contributes to the mechanical properties of the blood clot, which needs to have the right degree of stiffness and plasticity to stem the flow of blood and yet be digestible by lytic enzymes (Akpalo and Larreta-Garde, 2010).

More, in another work, we demonstrate that the storage moduli of fibrin interpenetrating networks increase from 1300 Pa to 2800 Pa (Akpalo et al., 2011) and this variation is correlated with the cellular growth increasing. This difference, related to mechanical properties, has previously been observed for various stem cells and has been demonstrated to influence cell differentiation (Engler et al., 2006). The aspect of wound healing activities of medicinal plants we want to focus is that of some which owe their effects to direct effect on the

wound healing processes. But, a combination of direct effect, anti-inflammatory and anti-microbial properties are also possible in some of the medicinal plants used in wound care. This work will give an overview of twelve West Africa medicinal plant studies and their active phytochemicals in direct wound healing. It is important to note that we do not focus on researches study of the antimicrobial and inflammatory properties but on biochemically direct wound healing parameters.

METHODOLOGY

Plants and their healing actions

Ageratum conyzoides Linn. (Asteraceae)

Ageratum conyzoides Linn. (Family Asteraceae, Tribe Eupatorieae) is an annual herb with a long history of traditional medicinal use in the tropical and sub-tropical region of the world: Africa, Asia and South America (Singh et al., 2013).

Traditional uses: Traditionally, *A. conyzoides* has been used for wound dressing, curing various skin diseases, ophthalmic, colic, ulcers treatment, as purgative and febrifuge. The common medicinal use of the plant in West Africa is for healing wounds, especially burns. For this treatment the juice of the bruised leaves is squeezed into the wound, which is then covered by a bruised but intact leaf (Oliver-Bever, 1986).

Phytochemical substances: Monoterpenes and sesquiterpenes, benzofuran, chromene, chromone and coumarin, flavonoids and alkaloids, triterpenes and steroids. The whole plant produces volatile strong smelled oil which also possesses various biological activities.

Mode of extraction: *A. conyzoides* methanolic extract for wound healing and hydro alcoholic extract were studied (Tailor and Goyal, 2012).

Wound models: Excision wound models were used to evaluate the wound healing activity (Chah et al., 2006).

Mode of administration: Wounds prepared in excised areas of the skin were packed with gauze soaked by the extracts and were determined histologically after 10 days. A group of rats were orally treated with 250 or 500 mg/kg extract (Tailor and Goyal, 2012).

Wound healing: The *Ageratum* sections showed fewer inflammatory cells and more fibrosis than the controls. In a study, it was found that wounds treated with aqueous leaves extract in combination with honey and with solcosery ointment significantly accelerate wound healing process and the rates of wounds sterility compared to wounds treated with honey alone (Mustafa et al., 2005). A group of rats were orally treated with 250 mg/kg extract for anti-inflammatory and was found to have a 38.7% reduction in the cotton pellet-induced granuloma ($p < 0.05$) (Tailor and Goyal, 2012). The development of chronically induced paw edema was also reduced significantly ($p < 0.05$) and serum glutamic pyruvic transaminase (SGPT) activity in the blood of rats treated with 500 mg/kg was reduced to 30.2% ($p < 0.05$) which confirm the anti-inflammatory properties of *A. conyzoides* (Moura et al., 2005).

Bidens pilosa

This plant is common to the tribes of Uganda, in East Africa (Hassan et al., 2011).

Traditional uses: *Bidens pilosa* (Asteraceae) is used in traditional medicine for treatment of wounds. In West Africa, the leaves are used. The plant acts on wounds, ulcers, reacts with DNA and kills human fibroblast cells at 10 ppm (Oliver-Bever, 1986).

Phytochemical substances: *A. pilosa* has previously been found to contain alkaloids, flavonoids, essential oils and polyacetylenes (Geissberger and Sequin, 1991; Deba et al., 2008).

Mode of extraction: The powder (250 g) was exhaustively extracted by cold maceration in 90% ethanol (750 ml) for 72 h.

Wound models: Excision wounds were made on each rat. The mid-dorsal region of each rat was shaved and disinfected using 70% alcohol. A circular piece of full thickness was cut off from predetermined area on the back of the rat using a surgical blade and scissors. Bleeding was arrested using cotton wool and initial diameters (a = transverse diameter and b = longitudinal diameters) were measured after 12 h from excision time.

Mode of administration: The animals were treated as follows: Group I: distilled water; group II: neomycin cream; group III: *B. pilosa* extract. The extracts were applied on the wounds in the amounts sufficient to cover the entire area and this was done twice a day till complete healing of the wounds.

Wound healing: Extract of *B. pilosa* significantly increased the rate of wound closure compared to negative control (distilled water). Epithelialization time and total healing time were also significantly reduced. In the histological sections, *B. pilosa* treated rats showed collagen organization. Wounds treated with distilled water were poorly remodeled, with infiltration of tissue by macrophages and lymphocytes which were indicative of delayed healing. Alkaloids and flavonoids have been demonstrated to play key role in promoting wound healing, therefore we hypothesize that they could be responsible for the wound healing activity of the *B. pilosa* extracts. The plant extracts left no prominent scar at the wound sites while neomycin sulfate treated groups had prominent scars.

Dissotis theifolia

D. theifolia popularly called "aziza ohia" is used locally by communities in Nsukka area of south eastern Nigeria (Odimegwu et al., 2008).

Traditional uses: *D. theifolia* is used to treat inflammations, wounds, and sores. The stem of the plants are often converted to chewing stick to treat gum infections.

Phytochemical substances: Phytochemical studies show that crude *D. theifolia* stem contains saponins, tannins, glycosides, flavonoids, terpenoids, carbohydrates, alkaloids and steroids.

Mode of extraction: About 200 g of pulverised stem was extracted with methanol by maceration for 72 h. The methanol extract did not show positive reaction. Simple ointment containing the methanol extract of *D. theifolia* was prepared by trituration method in a ceramic mortar and pestle using soft paraffin ointment base. Three

batches of the ointment containing 60, 90 and 120 mg/g of the extract were prepared for the studies. Another batch of commercial gentamicin ointment, Gentalek cream® (Taylek Nigeria Limited.) containing 1% gentamicin was used as a positive control treatment.

Wound models: Excision wound was inflicted on the rats. Full skin thickness was excised from the marked area to get a wound measuring about 314 mm².

Mode of administration: Topical application: Group 1 was treated with blank soft paraffin base (negative control); group 2 was treated with 1 mg/g gentamicin ointment (standard treatment); groups 3, 4, and 5 were treated with 60, 90 and 120 mg/g of the *D. theifolia* ointment.

Wound healing: Incorporation of *D. theifolia* extract (60, 90, and 120 mg/g) into the applied ointment enhanced the rate of wound closure and reduced the epithelialization period from 14.98 ± 0.46 days for the control treated with blank ointment to 8.8 ± 0.2 days for the group treated with 120 mg/g of *D. theifolia* ointment. Some of the phyto constituents extracted are known to have anti-inflammatory, anti-infective and pro-wound healing activities which could be responsible for the results of these studies.

Echium amaenum

Borage (*Echium amaenum*) is a member of Boraginaceae family. It grows in most of Europe, in the Mediterranean region, and also in northern parts of Iran. The borage found in Iran is *Echium amoenum*, which is different from the borage grown in Europe, *Borago officialis* L. (Boraginaceae) (Farahpour and Mavaddati, 2012).

Traditional uses: The flowers and the leaves of borage are used medicinally in France as an anti-febrile, anti-depressive, for the treatment of stress and of circulatory heart diseases, for pulmonary complaints, as a poultice for inflammatory swellings (Kapoor and Klimaszewski, 1999), as a diuretic (due to potassium nitrate), as a laxative, emollient and demulcent (due to the mucilage), and as a possible protective factor against cancer (Gonzalez et al., 1993).

Phytochemical substances: Phytochemical studies reveal the presence of tannins, resins, ascorbic acid, beta-carotene, niacin, riboflavin, thiamine, silicic acid, choline arabinose, unsaturated pyrrolizidines alkaloids including amabiline, lycopsamine and supinidine, polyphenolics including phenolic acid, vanillic acid, p-coumaric acid, p-hydroxy benzoic acid, gentisic acid, caffeic acid, rosmarinic acid and chlorogenic acid, scopoletin and flavonoids (Gudej and Tomczyk, 1996). The plant constituents also include gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), delta 6 fatty acid denaturase, delta 8 sphingolipid desaturase (Coupland, 2008).

Mode of extraction: Cold aqueous extract (pH 5.8) of dried *E. amaenum* flowers (5%, w/v) was used in all the experiments.

Wound models: Two circle shapes, full thickness surgical wounds with 7 mm diameters in both side of the backbone, 1 cm away from backbone and 5 cm away from each other were made with biopsy punch with 7 mm.

Mode of administration: In group 1, ointment with 1.5% borage extract was administered. Group 2 as control did not receive any administration and group 3 as placebo were administrated with

eucerin and vaseline.

Wound healing: The wound area measurement showed the wound size of the test groups were reduced early as compared with control group. On the 14th day, clinical observation showed a smaller wound area characterized by the proximity of the edges (contraction) with irregular outlines and better presence of granulation tissue, like that seen in the study with linoleic acid in sheep (Marques et al., 2004). Borage oil extract contains therapeutics that gamma-3, 6 fatty acids are one. The gamma-3 fatty acids exhibit anti-inflammatory properties by inhibiting the production of eicosanoids and other mediators, such as platelet-activating factor, interleukin-1, and tumor necrosis factor alpha (Kremer et al., 1987; Simopoulos, 1991; Albina et al., 1993).

The major constituent of BO which have been reported to exert anti-proliferative and anti-inflammatory effects *in vitro* is believed to be oxidative metabolites of essential fatty acids (EFA), gamma-linolenic acid (GLA) prostaglandin E1 (PGE1), and 15-hydroxyeicosatrienoic acid 15HETrE), (Miller et al., 1988; Chung et al., 2002). It is possible that in this study, the presence of linoleic acid in the test preparation contributed to a similar event. A study carried out with polyunsaturated fatty acids showed a tendency for the wound area to diminish in the first ten days of treatment, and demonstrated overall that Polyunsaturated fatty acids (PUFAs) may play an important role in wound healing (Cardoso et al., 2004).

Elaeis guineensis

Malaysia is the largest producer of oil palm (*Elaeis guineensis* Jacq.) in the world and producing more than 7 Million tons of crude palm oil (Sreenivasan et al., 2012).

Traditional uses: In traditional medicine, the leaf of *E. guineensis* is squeezed and the juice that is obtained is placed on wounds to promote healing (Irvin, 1985). Soap prepared with ash from fruit-husk is used for the preparation of a soap used for skin infections. In West Africa, studies of this plant focalized on sex hormones and thyroid hormones. The oestrone content of certain varieties could be confirmed (Bep, 1986). It is used orally in Togo to treat epilepsy (Tchacondo et al., 2012).

Phytochemical substances: Our previous phytochemical analysis results revealed the presence of tannins, alkaloids, reducing sugars, steroids, saponins, terpenoid, and flavonoids in the methanolic extract.

Mode of extraction: Approximately 100 g of dried sample was added to 300 ml of methanol and soaked for 4 days at room temperature ($30 \pm 2^\circ\text{C}$).

Wound models: Excision wound: full thickness wound (1.5×1.5 cm) was made on a shaved dorsal area.

Mode of administration: The wounds were treated topically with 10% formulated crude extract, while the control rats were treated only with yellow soft paraffin for 16 days.

Wound healing: The animals treated with methanol extract exhibited a significant increase in collagen deposition with fewer macrophages and more fibroblasts. A significant increase in collagen content was observed during the wound healing process in the treated group resulted due to enhanced migration of fibroblasts and epithelial cells to the wound site. Moreover, as shown by

previous studies, oil palm contains ascorbic acid, which acts as a cofactor for the synthesis of collagen as well as elastin fibers (Sauermann et al., 2004). The constituents of the oil palm leaf extract, such as terpenoids and alkaloids, may play a major role in the wound healing process.

In this study, we also have clearly observed an enhanced wound contraction induced by the *E. guineensis* leaf extract. This could be attributed to the enhanced contractile property of myofibroblast resulting in the increase of epithelialization. Early dermal and epidermal regeneration in the treated group confirmed that the ointment containing *E. guineensis* extract had a positive effect toward cellular proliferation, granulation tissue formation, and epithelialization. Treated rats have also shown new blood vessel formation.

Euphorbia hirta

Different species of *Euphorbia* from Euphorbiaceae family are found in West Africa (Ganju and Pathak, 2013).

Traditional uses: In West Africa, particularly in Togo, this plant is used to treat female infertility and intestinal parasites (Tchacondo et al., 2012).

Phytochemical substances: *Euphorbia hirta* contains leucocyanidol, quercitol, camphol, quercitin and quercitol derivatives containing rhamnose and chlorophenolic acid (Rastogi and Mehrotra, 1994).

Mode of extraction: Dried ethanolic extract of the dried rhizome of *Curcuma longa* and dried whole plants of *T. procumbens*, *Eclipta alba*, *E. hirta* was taken and gel of *Aloe barbadensis* was collected. Two ointments were prepared by fusion method that is, one containing ethanolic extract of whole plant of *Euphorbia hirta* (10% w/w), and other containing equal proportions (2 g each) of ethanolic extract of whole plant of *T. procumbens*, ethanolic extract of whole plant of *E. alba*, ethanolic extract of whole plant of *E. hirta*, ethanolic extract of dried rhizomes of *Curcuma longa* and gel of *Aloe barbadensis* (10% w/w) in hydrophilic ointment USP base.

Two suspensions were formulated that is, one containing ethanolic extract of whole plant of *E. hirta* (10% w/v) and other containing equal proportions (2.5 g each) of ethanolic extract of whole plant of *T. procumbens*, ethanolic extract of whole plant of *E. alba*, ethanolic extract of whole plant of *E. hirta* and ethanolic extract of dried rhizomes of *C. longa* to make 10% w/v of suspensions.

Wound models: Excision wound model: a wound of about 1 cm^2 area was made on the depilated dorsal thoracic region of rats. Incision wound model: paravertebral incisions of 3 cm were made on the depilated skin of the rats.

Mode of administration: The modes of administration used were topical application (ointment) and oral administration (suspension).

Wound healing: The increase in the biochemical parameters of the topical and oral *E. hirta* formulations was found to be significant. The period of epithelialization was found to be least in the topical poly-herbal formulation and that of the oral poly-herbal was found to be better than *E. hirta* topical formulation. It was found that the tensile strength of new tissue was more in the treated groups with least significant signs of infection. The strength of the tissues was statistically significant in the poly-herbal topical group followed by

poly-herbal oral group. Similarly, the rise in the biochemical parameters like those of hydroxyproline, hexosamine and protein content of the granuloma tissue was lot more in both topically and orally treated groups. Histopathological studies showed formation of scanty collagen tissue in the control group.

The test groups treated with poly-herbal topical formulations showed formation of dense collagen tissues with minimum presence of macrophages and formation of complete epidermis. The best groups treated with poly-herbal oral formulations also showed formation of dense collagen tissues, but slightly less than topically treated groups by poly-herbal formulations. The test group treated with topical formulation of *E. hirta* showed formation of collagen tissues with presence of macrophages, whereas those treated with oral *E. hirta* formulations showed collagen tissues less than in the topically treated groups of *E. hirta*. The polyherbal formulations containing equal proportions of the ethanolic extracts of the plants studied can be used both topically and orally for the healing of wounds. Single or multiple factors may play a role in any one or more individual phases, contributing to the overall outcome of the healing process (Guo and Di Pietro, 2010).

***Ficus hispida* Linn.**

Origins: *Ficus hispida* Linn. (Moraceae) is plants of *Ficus* species is a large genus of trees or shrubs, often climbers with milky juice, widely distributed throughout the tropical or hemispheres, but particularly abundant in south-East Asia and Polynesia (Singh et al., 2014).

Traditional uses: Traditionally, various parts of the plants of the *Ficus* spp. are used for medicinal purpose.

Phytochemical substances: Phytochemical screening of extract of leaves of *F. hispida* revealed glycosides, carbohydrates, sterols, saponins, tannins, flavonoids, and triterpenoids contents.

Mode of extraction: About 250 g of powdered material was soaked in 1000 ml methanol for 72 h in beaker and mixture was stirred every 18 h using a sterile glass rod.

Wound models: The rats were inflicted with excision wounds. A full thickness of the excision wound of circular area of 500 mm² and 2 mm depth was created along the markings using toothed forceps, scalpel and pointed scissors.

Mode of administration: The drugs were administered orally.

Wound healing: The healing proceeds with an increase in the number of collagen tissues. New tissues are formed in the wound area and the wound starts healing. The significant increase in wound healing by extract of *F. hispida* Linn. leaves at dose of 75 and 150 mg/kg body weight on days 0, 4, 8, 12, 16 and 20th successively is an indication of enhanced healing effect of the plant on wound.

***Milicia excelsa* (Welw.) C.C. Berg**

M. excelsa, family Moraceae popularly known as Iroko tree or African teak (Udegbunam et al., 2013) is a large deciduous tree 30 to 50 m high occurring naturally in humid forests of West Africa (Agyeman et al., 2009).

Traditional uses: Oral information obtained from traditional healers

in Uzo-Uwani in Nsukka area South East Nigeria showed that aqueous extract obtained from its leaves is used to treat fresh wounds.

Phytochemical substances: Phytochemical screening of the extract showed the presence of tannins, alkaloids, flavonoids and saponins.

Mode of extraction: The leaves were air dried, pulverized and macerated in 80% methanol for 48 h. After filtration, the filtrate was concentrated in a rotary evaporator at 40°C to obtain the methanol extract of *M. excelsa* (yield: 33.9% w/w). The methanol leaf extract of *M. excelsa* was used to prepare the ointment for topical application. *M. excelsa* ointment (50% w/w) was prepared using soft white paraffin. A neomycin-bacitracin (cicatrín®) ointment (50% w/w) was prepared using the same procedure stated above.

Wound models: Excision wounds were inflicted on rats. The areas of the dorsum to be excised (2 cm diameter) were traced with the aid of circular rubber seal and a blue marker. The traced dorsal areas were excised using scalpel blade and surgical scissors.

Mode of administration: The wounds of rats in groups 1 and 2 were treated topically with basic ointment (soft white paraffin) and cicatrín® ointment (50% w/w) while wounds of group 3 rats were treated with *M. excelsa* ointment (50% w/w).

Wound healing: The wounds of the *M. excelsa* ointment treated rats were dry and uninflamed from day 3 post wounding. The ability of the ointment to maintain a dry wound surface suggests that *M. excelsa* possessed both astringent and anti-inflammatory properties. These two effects may be attributed to the abundance of tannins, flavonoids and alkaloids in the leaves of *M. excelsa*.

It was noted at day 14, that the number of macrophages in the wounds treated with cicatrín® ointment or *M. excelsa* ointment was more compared to the number of neutrophils in wounds treated with basic ointment. This shows that wound healing was progressing faster in cicatrín® and *M. excelsa* ointment treated groups compared to the base group. The histologic study of the wound of the *M. excelsa* ointment treated rats showed more fibroblast proliferation and significant collagenization on day 14. These results obtained in the extract ointment treated group suggests that daily application of the ointment of *M. excelsa* promoted collagen expression (Bonte et al., 1993), angiogenesis (Shukla et al., 1999) and tensile strength of the wounds (Suguna et al., 1996).

***Plantago lanceolata* L**

Plantago lanceolata L. (PL; Plantaginaceae), is a perennial plant species with a worldwide distribution and large ecological amplitude (Farahpour et al., 2012).

Traditional uses: Therapeutic effects on gastrointestinal, blood and respiratory (asthma and dyspnea) disorders have been described for the *Plantago lanceolata* in Iranian ancient medical books (Zargari, 1990). PL is used internally to suppress coughs associated bronchitis and upper respiratory inflammation, to reduce skin inflammation, treatment of wounds and as a laxative (Baytop, 1999).

Phytochemical substances: Iridoid Glycosides (IGs) are a group of monoterpene derived compounds that have been recorded in over 50 plant families. The main IGs found in *P. lanceolata* are catalpol and its precursor aucubin (Jensen, 1991).

Mode of extraction: Dried PL flowers were collected and cold aqueous extract was prepared. Dried flowers were steeped for 6 h at 4°C in 300 ml distilled water, with constant stirring.

Wound models: Two circle-shapes, full thickness surgical wounds with 7 mm diameters in both side of the backbone, 1 cm away from backbone and 5 cm away from each other were made with biopsy punch.

Mode of administration: In group A, 0.75% PL extract was administered. Group B received 1.5% PL. Group D as placebo were administrated with Eucerin and vaseline and group C as control group did not received any administration. All extracts were applied in topical route.

Wound healing: Histological analysis of the treated healed wound group contained a large amount of fibroblasts proliferation, collagen synthesis, and neovascularization, which resulted in an increased wound tensile strength and accelerated healing of wound. Acetoeside, the main phenyl ethanoid from PL inhibits arachidonic acid induced mouse ear oedema (Murai et al., 1995). Therefore PL can inhibit oedema in wound site; as it did in present study. Some wound healing process could be accelerated under none oedema condition in wound site. More so, it is proved that PL has an anti-inflammatory activity (Wegener and Kraft, 1999; Herold et al., 2003) which might be a reason in accelerating wound healing.

Spirulina

The cyanobacteria *Spirulina* (Gur et al., 2013) which is consumed in daily diets of natives in Africa and America has been found to be a rich natural source of proteins, carotenoids and other micronutrients (Faroq et al., 2004).

Traditional uses: Interest in *Spirulina* increases due to the fact that it is a nutraceutical and that it is also a source of potential pharmaceuticals. As a result of this fact, it has many biological activities.

Phytochemical substances: The biological and pharmacological properties of *Spirulina* are attributed mainly to calcium-spirulan (Ca-SP) and C-phycoerythrin (C-PC) (Subhashini et al., 2004). Ca-SP, a sulfated polysaccharide consists of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate and calcium. C-PC is one of the major light harvesting biliproteins of *Spirulina* and is of great importance because of its various biological and pharmacological properties.

Mode of extraction: The crude extract of the cells (Crude *Spirulina* extract) (PSE) was prepared by freezing-thawing method. Filtered cells produced in the raceway ponds were frozen and thawed in order to disrupt their membrane, and afterwards it was extracted by hot water. Extraction and purification of C-phycoerythrin (C-PC) was achieved by using 10 g of lyophilized *S. platensis* powder which was suspended in 1000 mL of 1.5% CaCl₂·2H₂O (w/v) aqueous solution.

Wound models: Six circular full-thickness skin wounds (= 8 mm) were created using a sterile biopsy punch.

Mode of administration: Every day, six full-thickness skin defects were made on the back of the rats; 1: Placebo gel; 2: 0.5% PSE; 3: 2.5% PSE; 4: 1.25% PSE; 5: 2.5% C-PC; 6: 1.25% C-PC.

Wound healing: These last years, researchers speculate that the minerals, phycobiliproteins, vitamins, beta-carotene, fatty acids, polysaccharides, phenolic compounds and volatile compounds present in these microalgae may accelerate wound healing by acting as an antioxidant and scavenging destructive free radicals responsible for cell death (Qishen et al., 1988; Kim et al., 1998; Hirahashi et al., 2002; Reddy et al., 2003; Subhashini et al., 2004; Li et al., 2005; Patel et al., 2005; Singh et al., 2005; El-Baky et al., 2009; Plaza et al., 2009). These reports confirmed that some unidentified factors in the crude extract might have enhanced the healing process.

It is observed that 1.25% C-PC has a better effect on the 7th day compared to other preparations. On the 7th day, (treating group with 1.25% PSE), the crust was still persisting in three of the animals. The wound surface was covered by a thin epithelium; the underlying scar tissue was rich in blood vessels. There was a rather loose connective tissue below the scar. The muscle layer was still discontinuous.

However, in 1.25% C-PC treating group, the epithelium over the scar tissue which filled the wound region was recovered on the 7th day. The muscle layer was still missing but its place was filled with dense connective tissue. Based on the results of this study, it can be suggested that new proliferative molecules should be found by bioactivity guided isolation of PSE in the following studies. Slight differences were obtained with the PSE and C-PC. On the other hand, PSE contains a mixture of proteins and carotenoids which interact synergistically in mediating proliferation of skin cells and hence contribute significantly to wound healing and tissue regeneration death.

Strobilanthes crispus

Strobilanthes crispus (Al-Henhena, et al., 2011) is one of the herbs that have great potential in wound healing activity in animal. Acanthaceae is native plant to tropical countries and the leaves have been used in traditional medicine in Malaysia.

Traditional uses: Traditionally, *S. crispus* leaves were boiled with water and has been used as anti-diabetic, diuretic, antilytic, and laxative.

Phytochemical substances: These leaves contained catechins, alkaloids, caffeine and tannins.

Mode of extraction: Fresh leaves of *S. crispus* were tap washed followed by washing with distilled water and shade-dried for 7 to 10 days and was then finely powdered using electrical blender. 100 g of fine powder was soaked in 1000 ml of 95% ethanol in conical flask for 3 days in room temperature.

Wound models: An area of uniform wound 2 cm in diameter (circular area = 3.14 cm²), using circular stamp, was excised from the nape of the dorsal neck of all rats with the aid of round seal.

Mode of administration: Wounds of group 1 animals were topically treated with 0.2 ml of vehicle, gum acacia in normal saline (20 mg/ml) as placebo control group. Wounds of group 2 rats were topically treated with 0.2 ml of Intrasite gel as a reference standard control. Moreover, 0.2 ml of 100 mg/ml and 200 mg/ml of ethanol extract of *S. crispus* each were applied topically to the wound of group 3 and 4, respectively.

Wound healing: *S. crispus* treated groups showed complete

wound healing closure compared to placebo control group which showed incomplete wound healing closure. Granulation tissue of healed wound in *S. crispus*-treated group contained comparatively few inflammatory cells, and more collagen fiber, fibroblasts and proliferating blood capillaries (angiogenesis) compared with placebo-treated group which contain less collagen fibers, fibroblasts and blood capillaries, and more inflammatory cells. An ester glycoside compound of caffeic acid, a verbascoside, which was isolated from the leaves of *S. crispus* may be responsible for wound-healing activity and studies with plant extracts have shown that constituent like catechins, alkaloids, caffeine and tannins have analgesic effects. Mechanisms of wound healing may be contributed to stimulate the production of antioxidants in wound site and provides a favorable environment for tissue healing (Shukla et al., 1999). *S. crispus* has shown antioxidant activity (Norfarizan et al., 2009).

It has been reported that antioxidants may play a significant role in the wound healing process and may be an important contributory factor in the wound healing property. *S. crispus* leaf extract contain a wide array of free radical scavenging molecules and flavonoids were the major naturally occurring antioxidant components in this plant (Liza et al., 2010). The higher the flavonoid content, the stronger the antioxidant activity. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibers by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis (Getie et al., 2002).

***Ziziphus mauritiana* Lam.**

Z. mauritiana Lam. (Family: Rhamnaceae) is commonly known as jujube tree or Indian jujube, magarya in Hausa and whuya in Kilba (Nigeria) (Senthil et al., 2013).

Traditional uses: The leaves of the plant are used in the treatment of diarrhoea, wounds, abscesses, swelling, gonorrhoea, liver diseases, asthma and fever (Morton, 1987; Michel, 2002). The different parts of the plant are used as cuts and ulcers healer, pulmonary ailments, fevers, laxative, sedative, anti-nausea, anti-rheumatic areas, anti-diarrhoeal, wounds and abscesses healer, swelling, gonorrhoea curer (Michel, 2002), and also used as anthelmintic in ethno-veterinary medicinal system in Pakistan (Hussain et al., 2008). They are also used to treat pulmonary ailments, dysentery, fevers and skin diseases (Adzu, 2001).

Phytochemical substances: Phytochemical investigation of ethanolic and aqueous extracts of the test plant showed the presence of alkaloids, flavonoid, phenolic compounds and tannins in *Z. mauritiana* Lam.

Mode of extraction: Preparation of crude aqueous extract (CAE) of powdered *Z. mauritiana* was obtained by mixing 100 g of the powdered leaves with 500 mL of distilled water in a 1 L flask and boiled for 1.5 h. The yield was found to be aqueous extract 8.16% w/w.

Preparation of crude ethanolic extract: One kg (1 kg) of ground plant material was soaked in sufficient quantity of 70% aqueous ethanol by cold maceration at room temperature for three days. The yield was found to be ethanolic extract 8.4 % w/w.

Formulation of the herbal ointment: A simple ointment base as

per Indian pharmacopeia was prepared by fusion method. The weighed quantities of the extract was weighed, added to the molten ointment base and then homogenized in tile by trituration.

Wound models: A full thickness of the excision wound of uniform 2.5 cm diameter circular area was created along the markings using toothed forceps, scalpel and pointed scissors (excision wound model).

Mode of administration: Group I received no drug or extract (control). Group II received 5% ethanolic extract of the ointment base. Group III received 5% aqueous extract of the ointment base. Group IV received nitrofurazone ointment (0.2% w/w) (Standard).

Wound healing: It is observed that the wound contracting ability of the ethanolic extract in simple ointment (5% w/w) is significantly greater than that of the aqueous extract in simple ointment (5% w/w). On days 2, 4, 8, 12 and 16, animals treated with the ethanolic extract in simple ointment (5% w/w) showed results similar to animals treated with nitrofurazone ointment (0.2% w/w), (standard), with improving the wound healing process. It is observed that the wound contraction ability of the test formulation was significantly greater than that of the control and reference standard ($p < 0.05$). The enhanced capacity of wound healing with *Z. Mauritiana* Lam. could be explained on the basis of the anti-inflammatory effects of the plants that are well documented in the literature (Radhika et al., 2012). The antioxidant and anti-inflammatory activities of flavonoids in ethanolic extract and aqueous extract were believed to be one of the important mechanisms in wound healing (Marwah et al., 2007), and in the presence of tannin it improved the regeneration and organization of the new tissue and hastened the wound healing process (Leite et al., 2002).

DISCUSSION

The process of wound healing is promoted by several herbal extracts, which are composed of active agents like triterpenes, alkaloids, flavonoids, tannins, saponins, anthraquinones, and other biomolecules. Tannins act as free radical scavengers (Thakur et al., 2011) and 55% of overviewed plants in our study contain tannins. Triterpenoids and flavonoids promote wound healing due to their astringent and antimicrobial properties (Thakur et al., 2011) and we found that 45% of our plants overviewed contain terpenoids. Saponins, due to their antioxidant and antimicrobial activity (Thakur et al., 2011), appear to be responsible for wound contraction and elevated rate of epithelialization; they are present at a level of 36% in these overviewed plants. Finally, we know that flavonoids also possess potent antioxidant and free radical-scavenging effect (Thakur et al., 2011), enhancing the rate of antioxidant enzymes in granuloma tissue and 80% of plants studied here contain flavonoids.

The antioxidant and anti-inflammatory activities of flavonoids were believed to be one of the important mechanisms in wound healing and in the presence of tannin it improved the regeneration and organization of the new tissue and hastened the wound healing process (Senthil et al., 2013). The healing proceeds with an

increase in the number of collagen tissues. New tissues are formed in the wound area and the wound starts healing (Peraza-Sánchez et al., 2002). It was evident that wound tissue from animals treated had properly organized collagen fibers, less infiltration with macrophages and lymphocytes (Abajo et al., 2004). The significant increase in collagen deposition with fewer macrophages and more fibroblasts resulted to enhanced migration of fibroblasts and epithelial cells to the wound site (Chong et al., 2008). The histologic study of the wound treated rats showed more fibroblast proliferation and significant collagenization on day 14 (Udegbumam et al., 2013).

Well-formed collagen bundles in the treated wounds, support the efficacy of *E. guineensis* for example on fibroblast proliferation and synthesis of extracellular matrix during healing and we know that collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength (Chong et al., 2008). It was found that the tensile strength of new tissue was more in the treated groups with least significant signs of infection. In fact, formation of dense collagen tissues with minimum presence of macrophages and formation of complete epidermis was observed. Similarly, the rise in the biochemical parameters like those of hydroxyproline, hexosamine and protein content of the granuloma tissue was lot more in both topically and orally treated groups (Ganju and Pathak, 2013). The treated healed wound group contained a large amount of fibroblast proliferation, collagen synthesis, and neovascularization, which resulted in an increased wound tensile strength and accelerated healing of wound (Farahpour et al., 2012).

Sterols and polyphenols are also responsible for wound healing due to free radical-scavenging and antioxidant activity, which are known to reduce lipid peroxidation (Thakur et al., 2011), thereby reduce cell necrosis and improving vascularity. In fact, several types of injuries like burn, wounds, and skin ulcers usually generate superoxides and lipid peroxidation through the activation of neutrophils. Any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibers by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage (Geite et al., 2002).

Oil palm contains ascorbic acid, which acts as a cofactor for the synthesis of collagen (Chong et al., 2008). Granulation tissue of healed wounds contained comparatively few inflammatory cells, and more collagen fibers, fibroblasts and proliferating blood capillaries (angiogenesis) (Mahmood et al., 2010; Al-Henhena et al., 2011). The gamma-3 fatty acids exhibit anti-inflammatory properties by inhibiting the production of eicosanoids and other mediators, such as platelet-activating factor, interleukin-1 and tumor necrosis factor alpha (Chung et

al., 2002). It is possible that the presence of linoleic acid in some preparations contributed to a similar event as better presence of granulation tissue is seen in the study with linoleic acid (Chung et al., 2002).

Cactus extract up-regulates VEGF expression in wounded skin, stimulating in return angiogenesis for supplying nutrients and oxygen for skin regrowth (Chen et al., 2011).

The expression of MMPs revealed the enhanced healing pattern in the *E. guineensis* leaf extract treated rats group. MMP 8 and MMP 9 facilitate fibrin and eschar removal and the resultant peptides are known to possess chemotactic and angiogenic properties (Chong et al., 2008). Neutrophil-derived matrix MMP-8 is the predominant collagenase present in normal healing wounds. Expression of MMP 9 on early days suggests that it might have involved in keratinocytes migration and granulation tissue remodeling (Chong et al., 2008).

Treated sections with *Ageratum conyzoides* showed fewer inflammatory cells and more fibrosis than the controls. More so, serum glutamic pyruvic transaminase (SGPT) activity in the blood of some treated rats was reduced to 30.2% ($p < 0.05$) which confirmed the anti-inflammatory properties (Moura et al., 2005).

Conclusion

Biochemically wound healing process is promoted by active agents which compose aqueous and or alcohol herbal extracts and those components act synergistically since any of the plants overviewed reveals just one phytochemical constituent.

Conflict of interest

Authors have not declare any conflict of interest.

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