Safety and Efficacy of Herbal Ointment formulated with Methanolic extract of *Mikaniacordata* as Treatment for Acute Superficial Injury

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Abstract

The main objective of this study was to investigate the safety and efficacy of herbal ointment formulated with methanolic extract of Mikania cordata as Treatment for acute superficial injury. Ointment was prepared by fusion method using emulsifying ointment as base. The formulation was then tested for its physicochemical properties like loss of drying, pH, spreadability, extrudability, together with diffusion study, which gave a satisfactory result. The prepared ointment was also stable at 4^0C, 25^0C and 37^0C. Further, the formulation was evaluated for its anti-bacterial activity against Staphylococcus aureus, Pseudomonas sp., Bacillus sp., by agar diffusion method by using Mupirocin (6%w/w) as a standard. The formulation showed significant ($p<0.01$) activity against Staphylococcus aureus and Bacillus sp. compared to Mupirocin ointment which showed a significant ($p<0.01$) activity against Pseudomonas sp. and E. coli. Antioxidant activity of the extract through reducing power assay showed that the scavenging activity of the formulation increased with increase in concentration due to the presence of flavonoids and tannins. The dermatological effect of the herbal ointment was carried out by applying a considerable amount of the ointment on the skin surface area of ten healthy individuals in phase I clinical trial and showed a satisfactory result with no allergic or irritation reaction. Evaluation of the healing potency of the extract was carried out on twenty individuals with acute superficial injuries in phase II clinical trial and showed a significant ($p<0.01$) wound treatment index of 5.43 ± 0.37 and a percentage reduction index of 28.26 ± 4.14. The standard treatment drug mupirocin also showed a significant difference ($p<0.01$) with a treatment index of 4.47 ± 0.26 cm and a wound

percentage reduction index of 21.68±3.76. Paired sample correlations showed no significant difference (p>0.01) between those treated with M. cordata and those treated with mupirocin. Overall result of this study revealed that the herbal ointment of Mikania cordata is safe and as pharmacologically competent as Mupirocin and can be used as treatment for superficial injuries.

Keywords: *Mikania cordata, wound healing, superficial injury, herbal ointment, antimicrobial activity, zone of inhibition*

Introduction

Injuries are the leading cause of mortality and morbidity in the world. In 1998, 5.8 million people, as estimated, died from injuries in the world. It is a major public health problem and costly to society in terms of both human suffering and economic loss. Injuries can be divided into unintentional and intentional injuries. Unintentional injuries are injuries that occur without anyone intending that the harm be done such as those that result from car crashes, falls, drowning, and fires; whereas intentional injuries are injuries judged to have been purposely inflicted, either by another or oneself, such as assaults, intentional shootings and stabbings, and suicides (AnupamBishayee & Chatterjee, 1994). Healing is a survival mechanism and it represents an attempt to maintain a normal anatomical structure and junction (AshikMosadik, Faisal, Alama, 2000). Many immunosuppressant, cytotoxins and non-steroidal anti-inflammatory drugs suppress wound healing. Management of wound healing is a complicated and expensive step (Bates-Jensen & McNees, 1995). Restoration of damaged tissue (wound) is an important process which plays vital in survival of life; it is imminent for the basis of all surgical manipulations (Bedi, Tonzibo, & N'Guessan, 2003).

Natural products play an important role in drug development programs in the pharmaceutical industry (Carter, 1987). In Asian countries, especially for the rural population, herbal drugs come into the first choice for treatment. Nowadays, developed countries have a tendency to turn into traditional medicines, especially herbal drugs that showed a highly significant improvement. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness (Carter, 1987).

The delivery of drugs through the skin has long been a promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and non-invasive nature of the treatment (Alalor, Igwilo, and Azubuike, 2012). Along with other dosage forms, herbal drugs are also formulated in the form of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. These include the skin and the mucus membranes of the eye, vagina, anus, and nose. An ointment may or may not be medicated. Medicated ointments contain a medicament dissolved, suspended, or emulsified in the base. Ointments are used topically for several purposes, e.g. as protectants, antiseptics, emollients, antipruritic, keratolytics, and astringents (Daniels & Knie, 2007). Ointment bases are mainly anhydrous and generally contain one or more medicaments in suspension or solution or dispersion. Ointment bases may be hydrocarbon (oligeanous), absorption bases, water removable, and water soluble type (Deters, Dauer, Schnetz, Fartasch, and Hensel, 2001).

M. Cordata locally known as Bikas or heart heartleaf hempvine belongs to the family of *Asteraceae* that grows its vines at the soil surface or tree. *M. cordata* is initially a cover crop, but as time goes by it becomes a weed of rubber plantation. It is a smooth vine; its leaves are long-petioled, deltoid-ovoid or ovate heart-shaped, 4 to 10 centimeters long, with pointed tip, rounded, heart-shaped, or truncate base, and toothed margins. Heads are 4-flowered, cylindric, 6 to 9 millimeters long, borne in compound inflorescences. Achenes are smooth, glandular, linear-oblong, and 2.5 to 3 millimeters long. Pappus is composed of one series. It is distributed in thickets at low and medium altitudes, ascending to 1,600 meters in most islands and provinces, from northern Luzon to Palawan and Mindanao. It is probably a native of tropical America, but now, pantropic in distribution.

Study showed the intracellular contents of active intermediates of various xenobiotics including chemical carcinogens to be reduced by specific enhancement of drug-detoxifying enzymes in the liver of rats treated with the plant extract (Fernandez, Capdevila, Dalla, and Melchor, 2002). Essential oil of the leaves of *M. cordata* yielded four major constituents: a-pinene (20%), germacrene D (19.8%), beta-pinene (8.7%) and alpha-thujene (7.1%) (Getie, Gebre Mariam, Reitz and Neubert, 2002). Study of the methanolic fraction of

M. cordata root extract also showed significant anti-inflammatory effects in exudative, proliferative, and chronic phases of inflammation and also showed an antipyretic activity (Goren, Woerdenbag, and Bozok-Johansson, 1996). Further, study of alkaloidal fraction from *M. cordata* on diclofenac-induced gastric ulcer showed that the bioactive principles of *M. cordata* have anti-ulcerogenic effects. The results validate the use of the plant in Bangladesh for treatment of gastric ulcer (Gupta and Gupta, 2004). Study on stress-induced alterations in central neurotransmitters showed that pretreatment with *M. cordata* root extract prevented decreases in adrenaline and increases in 5-HT, while dopamine was further increased. Dose-dependent biochemical responses may be the possible mechanism of the anti-stress activity of this plant (Harshmohan, 2005). A different study on its effects on the central nervous system also showed that the root extract induces profound behavioral changes, especially disappearance of aggressive behavior. It also showed strong narcotic and analgesic effects (Hugo & Russel, 1983).

In the continuation of strategies to discover a new drug, as well as a follow up to a previous study (Johnson & Case, 1985), we conducted a pilot study (Phase I and II clinical trials) on the safety and efficacy of herbal ointment of the methanolic leave extract of *Mikaniacordatab* as treatment for acute superficial wounds among construction workers in Davao City, Philippines.

Objectives

To determine the safety and efficacy of herbal ointment methanolic leave extract of *Mikaniacordata* as treatment for acute superficial wound among construction workers in Davao City, Philippines.

Methodology

The leaf and root parts of *Mikania cordata* were collected from a local farm in Davao city and identified at the Department of Pharmacognosy, University of Immaculate Conception, Davao City. Dried and powdered plant materials (100g) were extracted with 80% methanol (500ml) in soxhlet apparatus for 24 h. The solvent was removed *in vacuo*using a rotation evaporator at low temperatures to give concentrated

extract. The extract came as semi-solid greenish paste. The residue was stored at 4^0C.

The extract was evaluated for the presence of flavonoids, tannins, alkaloid, saponins, gum, and sterols/triterpenes as per the standard procedure to reveal the presence of various active phytoconstituents. (Dewi, 2011)

The antibacterial activity of *Mikania cordata* at concentrations of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45^0C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*B.subtilis, E. coli, P. aeruginosa and S. aureus*) containing approximately 10^8 cfu/ml in a sterile petri dish and allowed to set. Wells of 6mm diameter were created with a sterile cork borer and filled to about three- quarters full with solutions of the methanolic extract of the leaves of *M. Cordata.* The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, n=2). Mupirocin and propylene glycol were used as positive and negative controls respectively.

The *in vitro* bacterial response to the extract was evaluated using the diameter of the zones of inhibition as follows: resistant: 10mm and below, intermediate: 11-15mm, and susceptible: 16mm and above[118].

The antioxidant activity of Mikania cordata was determined by reducing power assay. 50mg of the extract (2%, 4%, 6%w/w) was dissolved in 50ml of methanol. Different concentrations (0.5, 1, 2, 3, 4, 5ml) were pipetted into test tubes and made up to 10ml with methanol. 2.5ml of phosphate buffer and 2.5ml of potassium ferricyanide was added to each test tube and incubated at 40^0C for 20min. After incubation, 2.5ml of Trichloro acetic acid was added to the mixture and centrifuged for 5min. Finally, 0.5ml ferric chloride and 2.5ml of water was added to the mixture. The absorbance was measured using Double beam spectrometer at 700nm. The result was compared to a preparation of ascorbic acid solution (50mg of ascorbic acid dissolved in 50ml of methanol).

Working formula (emulsifying ointment base) used include extract (10%w/w), Liquid paraffin (20%w/w), emulsifying wax (30%w/w), and white soft paraffin (50%w/w). The constituents of the base were placed together in a melting pan and allowed to melt together at 70^0C. After melting, the ingredients were stirred gently maintaining temperature of 70^0C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment was done by incorporating 10g of the semisolid ethanolic extract of *M. cordata* into the base by triturating in a ceramic mortar with a pestle to obtain 100g of herbal ointments containing 10% w/w of *M. cordata*extract (Majumdar and Kamath, 2005). The prepared herbal ointments were put in the labeled ointment jars, and were stored at room temperature.

Physical assessments were carried out on the ointments and cream over a period of 30 days using the following parameters: Appearance, Odour, Texture and Color. The pH of the formulation was determined by using Digital pH meter. 0.5g of the weighed formulation was dispersed in 50 ml of distilled water and pH was measured (Chris, Igwilo, & Azubuike, 2012). The ointment was tested for homogeneity by visual inspection; it was tested for its appearance with no lumps (McKenzie, Pinger, & Kotecki, 2002). Loss on drying was determined by placing ointment in petridish on water bath and dried for 105^0C.

This is a term expressed to denote the extent of area to which the ointments readily spreads on application to skin or affected part. The spreadability is expressed as the time in seconds taken by two slides to slip off from the ointment and placed in between the slides under the direction of certain load. The lesser the time taken for separation of the two slides, the better the spreadability. Spreadability was calculated using the formula:

$$S = (M.L/T)$$

Where: S = Spreadability
M = Mass/weight tied to upper slide
L = Length of glass slides
T = Time taken to separate the slides.

A simple method was adopted for this study. The formulations were filled in the collapsible tubes after the

ointments were set in the container. The extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of ointment in 10 seconds.

The diffusion study was carried out by preparing agar nutrient medium of any concentration; it was poured into a petridish. A hole was bored at the centre and the ointment was placed in it. The time taken for the ointment to diffuse was observed and noted.

Stability study was carried out for the prepared formulation at different temperature conditions (4^0C, 25^0C and 37^0C) for 3 months.

The study was conducted in a construction site in Davao City, Mindanao, Philippines The second most populous and the third most significant metropolitan area in the country with an area of 944 sq miles (2,444 km^2), elevation 73′ (22 m, weather 30^0C, Wind E at 11 km/h, 74% Humidity. The monthly temperature is highly variable, varying between 15.6^0C and 32.1^0C in different seasons.

Thirty healthy adult male volunteers between the ages of 20 to 35 years (mean age 24.8± 0.6yrs) were recruited into the study. Exclusion criteria included smoking, alcohol consumption, hypertension, diabetes mellitus, obesity, musculo-skeletal disorders, sickle cell disease and goiter. The written consent was obtained from the volunteers before the conduct of study and the approval of Ethical Committee on Human Research of Davao Medical School Foundation Hospital, Davao City was obtained.

The study involved two phases of clinical trials – Phase I and Phase II as follows:

Phase I: n=10, included healthy individuals not diagnosed with acute superficial wounds. Physical examination of the area to be tested was done before and after the treatment period. Signs of dermatological effects were noted as change in skin color, localized rash, irritation, swelling, or hives.

Phase II: Included individuals diagnosed with acute superficial wound by a registered physician. The wound area was examined and measured using the Bates-Jensen Wound

Assessment Tool[21] prior to treatment and was recorded as pre-test data. Selected individuals were further divided into two groups, A and B, as follows:

Group A: n=10, those treated with 10mg (6%w/w) of Mupirocin twice daily for seven days. The wound area was then recorded taken into consideration factors such as depth, edges, undermining necrotic tissue type, necrotic tissue amount, exudates type, exudate amount, color of skin surrounding the wound, peripheral tissue edema and induration, granulation tissue and epithelialization using Bates-Jensen Wound Assessment Tool.

Group B: n=10, those treated with 10mg (6%w/w) of *Mikaniacordata* formulated ointment twice daily for seven days. The wound area was evaluated using same parameters as was described for those treated with Mupirocin.

Wound area percentage reduction was calculated using

$$Bm - Fm\ X\ 100/Bm$$

Where,

Bm = Baseline measurement

Fm = Final measurement

The collected data were reviewed, coded and analyzed using the Statistical Package for Social Sciences (SPSS) software version 17.0. The means and standard deviation were calculated and compared between treatments were done using the Pearson correlation and student t-test. The results were expressed appropriately in Mean ± Standard error of mean. The mean values (for pre and post data) of the test group was compared with those of the positive control group with the level of statistical significance taken at the level of $p<0.01$.

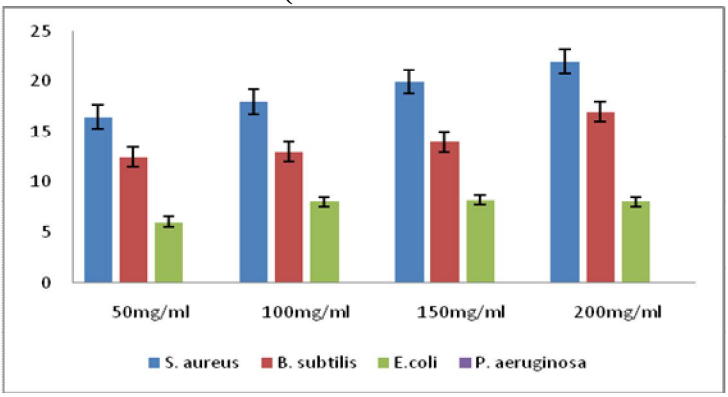
Results and Discussion

Preliminary phytochemical test revealed the presence of flavonoids, glycosides, saponins, reducing sugars, carbohydrates, tannins, sterol/triterpens, 2,3-pentanedione, glycerin, acetic acid, 2,3-butanediol, caryophyllene oxide, and a small amount of sequelene: compounds that are considered pharmacologically relevant. Tannins promote wound healing through several cellular mechanisms; chelating of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts (Panigrahi, Jhon, Shariff, & Shobanirani)

(Prabhuduta Panda, 2010). 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 fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting DNA synthesis (Bhattachara & Pal, 1992). Triterpenoids, tannins and flavonoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial propert, which seems to be responsible for wound contraction and increased rate of epithelialisation (Bhattacharya & Pal, 1992, 1998; Scoritchini M. & Pia Rossi M., 1991; Trease G & Evans SM, 2002)

In the preliminary antimicrobial sensitivity screening, the ethanolic extract of *Mikania cordata when* compared favorably with Mupirocin ointment for its antibacterial activity, showed excellent dose-dependent activity against *Staphylococcus aureus* and *Bacillus subtilis*($p<0.001$) with a mean antimicrobial index of 16.50±0.58 at 50mg/ml, 18.00±0.00 at 100mg/ml, 20.00±1.41 at 150mg/ml and 22.00±0.00 at 200mg/ml against *S. aureus* with an index value of 24.00±0.82mm zone of inhibition compared to 21.00±0.00mm zone of inhibition observed in the Mupirocin treatment group (1%w/v). The extract also showed a mean antimicrobial index of 12.50±0.50 at 50mg/ml, 13.00±1.29 at 100mg/ml, 14.00±0.50 at 150mg/ml and 17.00±0.50 at 200mg/ml against *B. subtilis* with an index value of 19.50±0.96mm zone of inhibition compared to 20.00±0.50mm zone of inhibition observed in the mupirocin treatment group (1%w/v). *Staphylococcus aureus* and *Bacillus subtilis* are known etiologic agents of several skin and mucous membrane infections in man. The activity against *Staphylococcus areus* is of significant interest since *S. aureus* is not easily eliminated, especially, in the deeper skin layers, sweat gland, sebaceous gland, and the hair-follicles by routine washing and scrubbing even with some antiseptic soap (Trease G & Evans SM., 2002). It has been implicated as the commonest etiologic agent of boils, carbuncles, breast abscess, and infantile-impetigo (Ya C, Gaffney SH, Lilley TH and Haslam E, 1988).

Figure 1:
Preliminary *in vitro* antibacterial activity of methanolic extract of *M. cordata* (Zone of inhibition in mm)



The extract did not show any activity against *Pseudomonas aeruginosa*, but showed a non significant ($p<0.01$) activity against *E. coli*. The standard drug Mupirocin ointment showed a relatively high activity against *E. coli and P. aeruginosa* with a mean zone of inhibition index of 27.00±0.00mm and 30.00±1.00mm for *E. coli* and *P. aeruginosa* respectively, compared to the extract ointment which showed a mean zone of inhibition index of 9.00±1.50mm and 0.00mm for *E. coli* and *P. aeruginosa* respectively.

The antioxidant activity inferred that the herbal ointment showed a similar activity as that of standard ascorbic acid. This antioxidant activity is believed to be due to the presence of flavonoids and tannins (Goren N, Woerdenbag H and Bozok-Johansson C, 1996).

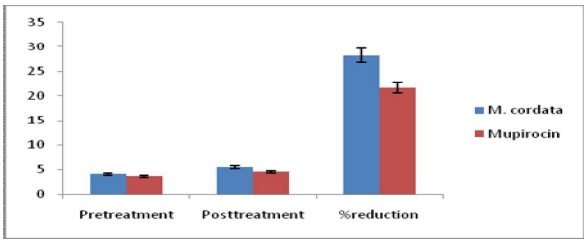
The prepared formulation showed a smooth and homogenous appearance with a pH value of 6.9. The pH value is within the normal range of the human skin (6.8 – 7.3 ±1.0) and is acceptable in the avoidance of the risk to irritation upon application to the skin. The ointment showed no observable changes ($p>0.01$) in pH consistency and phase separation after storing it for a period of thirty days with a mean pH index of 6.4±0.21 (day 1), 6.3±0.09 (day 15) and 6.1±0.38 (day 30). The color was consistently dark green, odor was characteristic, loss of drying was 40%w/w, spreadability was 12 seconds,

extrudability was 180g, and diffusion study after 60 min was 0.8cm. These physical parameters were within acceptable ranges.

Skin assessment of *M. cordata* showed a satisfactory result with no allergic or irritation reaction such as itching, dryness, burning, redness, stinging, pain or swelling, following a twice daily application of 10mg (6%w/w) of the extract ointment on the same skin surface area for a period of seven days. With no observable dermatologic effect in Phase I trial, Phase II trial was initiated.

Wound healing is a fundamental response to tissue injury resulting in restoration of tissue integrity. This is mainly achieved by the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the major component that ultimately contributes to wound strength. Comparisons between the pretreatment and post treatment group for *Mikania cordata* in phase II clinical trial showed a significant ($p<0.01$) post treatment index of 5.43±0.37 and a percentage reduction index of 28.26±4.14 when compared with the pretreatment index of 3.99±0.39. The standard treatment drug mupirocin also showed a significant difference ($p<0.01$) with a treatment index of 4.47±0.26cm (posttreatment) compared to 3.56±0.32cm (pretreatment) and a wound percentage reduction index of 21.68±3.76.

Figure 2:
Comparison of the Healing effects of *M. cordata* and Mupirocin in the treatment of superficial injuries after seven day



Paired sample correlations showed a slight increase in the healing activity of *M. cordata* compared to Mupirocin, but this increase was not statistically significant ($p>0.01$). Hence, based on the result of this research, it is safe to assume that the

herbal ointment of *Mikania cordata* is safe and as pharmacologically competent as Mupirocin and can be used as treatment for superficial injuries. The wound healing potency of *M. cordata* may be attributed to the phytoconstituents present in it, which can be either due to their individual or additive effect that hastens the process of wound healing.

However, further studies are in progress to determine the direct mechanism of action, where the ethanol extract is subjected to further fractionation and purification, to identify and isolate the active compound (s) responsible for these pharmacological activities. Further studies are also in progress to determine the safety and efficacy of the herbal ointment in the treatment of burns, carbuncles and furuncles, as well as to see if the result of this study can be applied in the treatment/dressing of deep wounds. The present findings provide scientific evidences of ethnomedicinal properties of this plant.

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