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Safety and Efficacy of Herbal Ointment formulated with Methanolic Extract of *Mikania cordata* as Treatment for Acute Superficial Injury.

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ABSTRACT

The main objective of this study is 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 and gave a satisfactory result. The prepared ointment was also stable at 4°C, 25°C and 37°C. Further, the formulation was evaluated for its antibacterial 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 reveals that the herbal ointment of *Mikania cordata* is safe and as pharmacologically competent as Mupirocin and can be used as treatment for superficial injuries.

INTRODUCTION

Injuries are the leading cause of mortality and morbidity in the world. An estimated 5.8 million people died from injuries in the world in 1998 [1]. 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, injuries judged to have been purposely inflicted, either by another or oneself such as assaults, intentional

shootings and stabbings, and suicides ^[2]. Healing is a survival mechanism and represents an attempt to maintain a normal anatomical structure and junction ^[3]. Many immunosuppressant, cytotoxins and non-steroidal anti-inflammatory drugs suppress wound healing. Management of wound healing is a complicated and expensive step ^[4]. 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 ^[5].

Natural products play an important role in drug development programs in the pharmaceutical industry ^[6]. 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^[6].

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 ^[7]. 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 ^[8]. Ointment bases are mainly anhydrous and generally contain one or more medicaments in suspension or solution or dispersion. Ointment bases may be hydrocarbon (oleaginous), absorption bases, water removable and water soluble type ^[9].

M. Cordata locally known as Bikas or heart heartleaf hempvine belongs to the family *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 northan 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 would be reduced by specific enhancement of drug-detoxifying enzymes in the liver of rats treated with the plant extract ^[10]. 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%) ^[11]. 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 ^[12]. Further, study of alkaloidal fraction from *M. cordata* on diclofenac-induced gastric ulcer showed 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 ^[13]. Study on stress-induced alterations in central neurotransmitters showed 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 ^[14]. A different study on its effects on the central nervous system also showed the root extract induces profound behavioral changes, especially disappearance of aggressive behavior. It also showed strong narcotic and analgesic effects ^[15].

In the continuation of strategies to discover a new drug, as well as a follow up to a previous study ^[16], we conducted a pilot study (Phase I and II clinical trial) on the safety and efficacy of herbal ointment of the methanolic leave extract of *Mikania cordata* as treatment for acute superficial wounds among construction workers in Davao City, Philippines.

MATERIALS AND METHODS

Preparation of plant materials

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°C.

Phytochemical screening

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 [17].

Evaluation of antibacterial activity of Extract

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°C 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 10⁸ 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 [18].

Evaluation of the antioxidant activity of the extract

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°C 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 with a preparation of ascorbic acid solution (50mg of ascorbic acid dissolved in 50ml of methanol).

Preparation of ointment

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°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C 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 [19]. The prepared herbal ointments were put in ointment jars, labeled and were stored at room temperature.

Physical evaluation of formulated ointment

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 measured⁷. The ointment was tested for homogeneity by visual inspection: it was tested for its appearance with no lumps [20]. Loss on drying was determined by placing ointment in petridish on water bath and dried for 105°C.

Spreadability

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.

Extrudability

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.

Diffusion study

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

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

Study site

The study was conducted in a construction site at Davao City. Davao City is a city in Mindanao, Philippines which is the center of Metro Davao, the second most populous and the third most significant metropolitan area in the country with an area of 944 sq miles (2,444 km²), elevation 73' (22 m, weather 30°C, Wind E at 11 km/h, 74% Humidity. The monthly temperature is highly variable, varying between 15.6°C and 32.1°C in the different seasons.

Study population

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. Written informed consent was obtained from each volunteer before the commencement of study and approval from the Ethical Committee on Human Research of Davao Medical School Foundation Hospital, Davao City was obtained.

Experimental Procedure

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 Tool²¹ 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 *Mikania cordata* 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

Where,

Bm = Baseline measurement

Fm = Final measurement

Statistical Analysis

The collected data was reviewed, coded and analyzed using the Statistical Package for Social Sciences (SPSS) software version 17.0. The means and standard deviations were calculated and comparisons between treatments were done using Pearson's correlation and student's t-test. The results were expressed where appropriate as Mean \pm 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

Phytochemical screening

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 [22-23]. 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 [24]. Triterpenoids, tannins and flavonoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation [25-28].

Evaluation of antibacterial activity of Extract

In the preliminary antimicrobial sensitivity screening, the ethanolic extract of *Mikania cordata* compared favourably with Mupirocin ointment for its antibacterial activity and 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 ± 0.82 mm zone of inhibition compared to 21.00 ± 0.00 mm 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 ± 0.96 mm zone of inhibition compared to 20.00 ± 0.50 mm 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 aureus* 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 [28]. It has been implicated as the commonest etiologic agent of boils, carbuncles, breast abscess and infantile-impetigo [30]. 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 ± 0.00 mm and 30.00 ± 1.00 mm for *E. coli* and *P. aeruginosa* respectively, compared to the extract ointment which showed a mean zone of inhibition index of 9.00 ± 1.50 mm and 0.00 mm for *E. coli* and *P. aeruginosa* respectively.

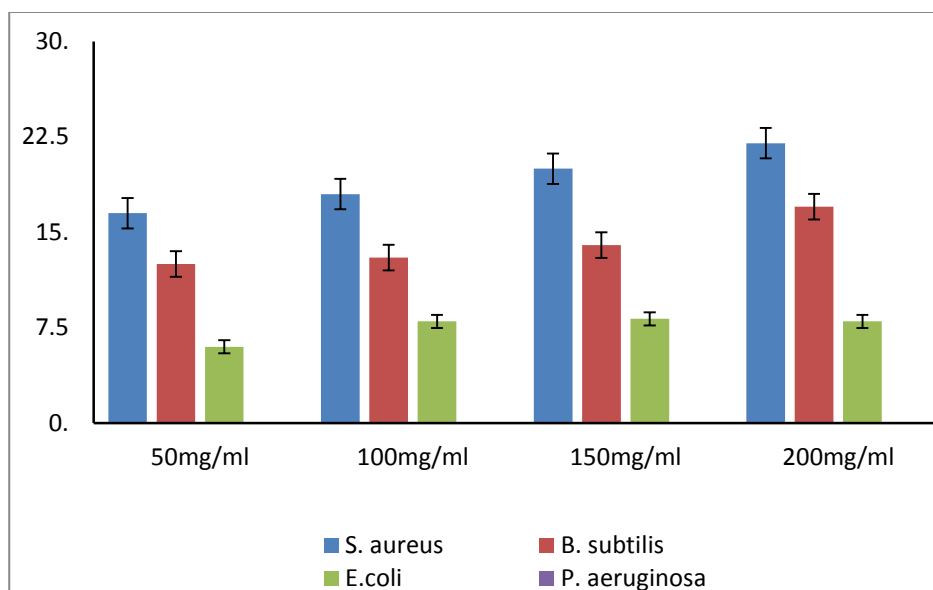


Figure 1: Preliminary *in vitro* antibacterial activity of methanolic extract of *M. cordata* (Zone of inhibition in mm)

Evaluation of antioxidant activity of the extract

The antioxidant activity inferred that the herbal ointment showed similar activity as that of standard ascorbic acid. This antioxidant activity is believed to be due to the presence of flavonoids and tannins [12].

Physical evaluation of formulated ointment

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 \pm 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 \pm 0.21 (day 1), 6.3 \pm 0.09 (day 15) and 6.1 \pm 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.

Dermatological effects on healthy individuals

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 dermatological effect in Phase I trial, Phase II trial was initiated.

Wound healing effect

Wound healing is a fundamental response to tissue injury that results 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 \pm 0.37 and a percentage reduction index of 28.26 \pm 4.14 when compared with the pretreatment index of 3.99 \pm 0.39. The standard treatment drug mupirocin also showed a significant difference ($p < 0.01$) with a treatment index of 4.47 \pm 0.26cm (posttreatment) compared to 3.56 \pm 0.32cm (pretreatment) and a wound percentage reduction index of 21.68 \pm 3.76.

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 may be either due to their individual or additive effect that fastens the process of wound healing.

However, further studies are in progress to determine the direct mechanism of action where the ethanol extract will be 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 see if the result of this study can be applied in the treatment/dressing of deep wounds. The present findings provide scientific evidence to some of the ethnomedicinal properties of this plant.

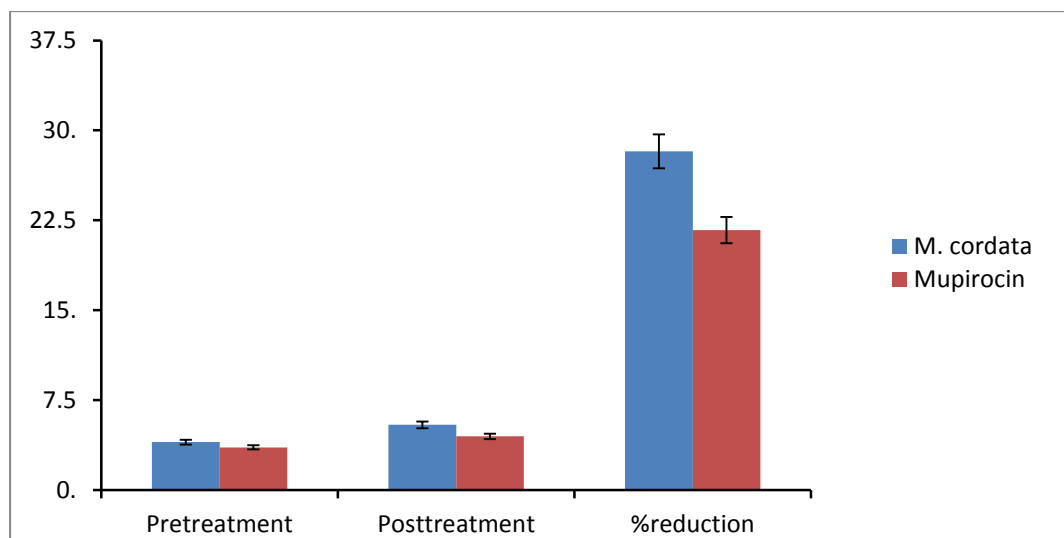


Figure 2: Comparison of the Healing effects of *M. cordata* and Mupirocin in the treatment of superficial injuries after seven days

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