Antimicrobial and wound healing potential of Marham-e-Aatshak (A Herb-o-Mineral formulation)

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Abstract: *Marham-e-Aatshak* (MA) is a Unani ointment, with wide use for treating chronic and infectious wounds since long time. This study was designed to screen the antimicrobial and wound healing potential of MA to validate the ethno-therapeutic claims. The agar diffusion method was used to study the antimicrobial action of MA as well as for all of its ingredients. Inhibition zone diameters were measured and MIC values were calculated. Wound healing activity was studied in models of both, excision and incision wounds. Wound contractibility was measured at different intervals in excision wound model; similarly tensile strength was measured in incision wound model. MA and its ingredients showed remarkable inhibitory activity against most of the organisms. In excision wound, a significantly enhanced wound contraction and significantly reduced epithelialization period was observed. In incision wound, significant increase in the mean breaking strength in the test group was observed. The results indicate that MA is capable of fighting against wound infections and able to potentiate the natural healing process.

Keywords: Marham-e-Aatshak, wound healing, Unani medicine, Herb-o-mineral.

INTRODUCTION

Wound is defined as a breach in the integrity of epithelium. However, the disruption could be deeper, extending to the dermis, subcutaneous fats, fascia, muscle or even the bone (Majoosi, 1889; Ibn-e-Sina, 1992a; Enoch and Leaper, 2005). Tissue repair is a complex and multi-step process involving dynamic biochemical processes involved in tissue restoration (Shai and Maibach, 2005; Jorge *et al*., 2008).

Wound infection is the most important factor, acting locally or systemically, which delays the healing process. *Staphylococci, Streptococci* and *Candida* species are commonly present as normal flora of the skin and unless the skin is disinfected, they may gain entrance to the body via an incision (Pelczar *et al*., 1993; Mohan, 2000). Antibiotics currently in use for the treatment of wound infection are associated with a number of adverse effects and drug resistance. Many efforts have been made by the researchers to discover new antimicrobial compounds and new wound healing drugs. Scientists are trying to develop newer drugs from natural sources for which they are looking towards indigenous systems of medicine (Essawi and Srour, 2000).

Unani medicine is a well known traditional system of medicine, recognized by WHO, which owes its origin to Greece and its theoretical frame work based on the teaching of Greece physician, Hippocrates. It developed into an elaborated medical system by Arabs and Iranian physicians. Traditional systems of medicine of many countries in middle and far East enriched the Unani system. In Unani system of medicine, plants, animals, metals and minerals, in single or compound forms, are being used as drugs to cure various ailments of mankind since ancient time.

Marham-e-Aatshak (MA) is an important compound Unani formulation, which has been used topically for syphilitic ulcers, chronic and infected wounds and other types of wounds and boils (Anonymous, 1986; Said, 1997). It was claimed by Unani physicians that it can cure and heal up the syphilitic ulcers and boils very quickly (Arzani, YNM Khan, 1996). The ingredients of MA (Root of *Smilax china*, copper sulfate, lead oxide, castor oil and beeswax) have been reported for their various pharmacological activities, i.e. *S. china* for antimicrobial (Mateen *et al*., 2010) and antioxidant activities (Lee *et al*., 2001), copper sulfate for its antimicrobial (Ibrahim *et al*., 2008) and wound healing properties (Sen *et al*., 2002), castor oil for its antioxidant (Ilavarasana *et al*., 2006) and wound healing activities (Carson *et al*., 2003), where as beeswax for antioxidant activities (Molina *et al*., 2001). Lead compounds have been advised topically for the treatment of malignant tumors in the past (Drill, 1965). In Unani system of medicine, it is used as a key ingredient,

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mainly in ointments and other locally applied formulations for chronic and malignant wounds and ulcers to resist or reduce the corrosive action of ingredients (Najm-ul-Ghani, YNM; Razi, 1991; Ibn-e-Sina, 1992b; Khan, 1996; Baghdadi, 2005).

A large number of plants and their bioactive compounds have been investigated for their antimicrobial and wound healing activities and found to be highly effective in this context. However, a large proportion of plants and other Unani drugs still remain unexplored. Moreover, there is a curious lacuna in the field of compound Unani formulations; a very little attention has been paid to evaluate their pharmacological activities. In view of scientific validation of Unani formulations as claimed about MA, an attempt has therefore been made to validate the efficacy and claims of Unani physicians about antimicrobial and wound healing property of MA.

MATERIALS AND METHODS

Collection of materials

The dried rhizomes of *S. china* (SC) and beeswax (BW), 500gm each were procured from Khari Bawli (Delhi, India), and identified on the basis of classical organoleptic and morphological descriptions, mentioned in Unani literatures at the Department of Ilmul Advia (Pharmacology), Faculty of Medicine (Unani), Jamia Hamdard. The taxonomic and microscopic authentication of *S. china* was done in the Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India. Copper sulfate (CS) was of analytical grade (500gm) and obtained from SD Fine-Chem Ltd, Mumbai, India, while lead oxide (LO), 500gm and castor oil (CO) 2.0L were of laboratory grade and obtained from Thomas Baker Chemicals Pvt. Ltd, Mumbai, India. Gentamicin and Tetracycline discs were purchased from Axiom Laboratories, New Delhi, India, while Ciprofloxacin from High Media Laboratories, Pvt. Ltd. Mumbai, India. Framycetin sulphate cream (Soframycin®, B.No.E0512, Aventis Pharma Ltd, Ponda, Goa, India), used as reference standard for wound healing activity, was procured from a local chemist shop in New Delhi. All the chemicals and reagents were procured and study was performed in the academic year 2010-11.

Preparation of ointment

Marham-e-Aatshak (MA) was prepared by adopting the classical method as described in Unani literatures (Anonymous, 1986; Said, 1997). In first instance, the castor oil (260mL) and fine powder of lead oxide (25g) were placed together in a pan and heated; when the oil blackened, wax (25g) was added and discontinued heating after melting the wax and proper mixing with oil. The finely powdered copper sulfate (12g) was added with stirring to the mixture. Lastly, powdered dried rhizomes of *S. china* (25g), after sieving through 100-mesh, was added to the semi-hot mixture and preserved in a glass jar after vigorous trituration.

Characterization of MA (Developed ointment formulation)

Viscosity of MA

The test sample was taken in a 250mL beaker and the viscosity was determined using CAP- 2000 Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, Massachusetts, USA) using spindle number 1 to 4 at different speeds from 0.3 to 60 rpm, respectively (Damodharan *et al*., 2010).

Centrifugation

Physical stability of MA (ointment) was determined by centrifugation in 10-mLfalcon tube at 10000 rpm for 10 minutes, as described by Baie and Sheikh (2000), using a Sigma centrifuge (Sigma scientific instruments PVT. LTD. Chennai, India). The MA formulation was observed visually for any breakage, cracking or any deterioration.

pH of MA

The pH of 10% ointment solution was measured with digital pH meter(Decibel instruments, Chandigarh, India).

Spreadability of MA

Spreadability of the formulation was determined using a special apparatus consisting of wood block having fixed glass slide with pulley at one end. 3.0g formulation was placed between the ground plate and another glass plate of same dimension with a hook. Further formulation between the plates was pressed for five minutes with One Kg weight for obtaining a uniform film. Excess ointment was removed and the upper hooked surface of glass plate was pulled with 240g weight. Total time taken by the top plate, to reach a distance of 10 cm. was recorded (Damodharan *et al*., 2010). Spreadability was measured as:

$S = w \times l / t.$

Where Srepresents spreadability, w is weight in grams, tied on upper plate, l denotes length of glass plates in cm and t represents time required to slide the entire length of plates.

Antimicrobial assay

Microorganisms

Four bacterial strains *Staphylococcus aureus* ATCC (25923), *Streptococcus pyogenes* MTCC (1925), *Pseudomonas aeruginosa* ATCC (27853), *Enterococcus faecalis* ATCC (29212) and one fungal strain-*Candida albicans* ATCC (5314) were used for the antimicrobial activity. These organisms were procured from the Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India.

Antimicrobial activity of MA

Agar diffusion method (Murray *et al*., 1995) was used to evaluate the antimicrobial activity. Bacterial cultures were grown over night at 37ºC in Muller Hinton Broth (Himedia, Mumbai), whereas fungal at 28ºC for 72h in

Potato Dextrose Broth (Himedia, Mumbai) and used as inoculum. A final inoculum (100µL) of suspension containing 108CFU/mL of bacteria and 104 spores/mL of fungus were spread on Muller Hinton Agar plate (MHA) and Potato Dextrose Agar (PDA) medium, respectively.

Marham-e-Aatshak (MA) and its all five ingredients (in different concentrations) were screened for antimicrobial activity. Stock solutions (512mg/mL, each) of MA in chloroform ($CHCl_3$), CS in distilled water and LO in acetic acid were prepared, respectively. Similarly, stock solutions (200mg/mL each) of BW in petroleum ether and methanolic extract of SC in methanol were prepared, respectively, whereas stock solution of CO was prepared by mixing equal volume of CO and chloroform. Five serial dilutions, ranging from 16-256mg/mL for MA, CS and LO, 6.25-100mg/mLfor BW and SC, and5-40 for CO were prepared respectively, in their respective solvents.

Sterile discs (6.0mm in diameter) made of Whatman paper no. 1, were impregnated with 10µL of all six concentrations of each drug individually, and placed on seeded agar after complete drying. Discs dipped into pure solvent without drug (Petroleum ether, chloroform, methanol and acetic acid) were used as negative control. Gentamicin (10µg/disc), tetracycline (30µg/disc) and ciprofloxacin (5µg/disc) were used as positive control for bacteria, whereas fluconazole (10µg/disc) and ketoconazole (10µg/disc) for fungus. The test plates were incubated at 37ºC and 24 h for bacteria, whereas 28ºC and 72h for fungus. After incubation, plates were observed for zone of inhibition, and their diameters were measured through millimeter scale. Minimum inhibitory concentration (MIC) values of all drugs were also determined for microorganisms by the same disc diffusion assay. MIC is defined as the lowest concentration of drugs solution that inhibits growth of microorganism on agar plates.

Wound healing activity

Animals

Thirty-six (18 for incision wound model and 18 for excision wound model) female inbred albino rats (Wistar strain) of weight ranging 150-200g were used in this experiment. They were obtained from Central Animal House Facility, Jamia Hamdard and were individually housed and maintained at standard environmental condition. Animals were fed with rodent diet and water ad libitum during the course of entire experiment. The study was conducted in accordance and under strict supervision of Institutional Animal Ethical Committee (IAEC), Jamia Hamdard, Hamdard Nagar, New Delhi, India under Reg.No.173/ CPCSEA.

Incision wound model

The animals were anaesthetized with intra-peritoneal injection of phenobarbitone (50mg/Kg body weight), and their back were shaved with depilatory cream and cleaned with dry gauze piece. Six cm long para vertebral incision was made on either side of the vertebra in full thickness of the skin of each animal (Ehrlich and Hunt, 1968). After haemostasis, wounds were cleaned with sterile wet cotton and then closed using a surgical thread (No. 000) and a curved needle (No. 11) with interrupted sutures one cm apart (Lodhi *et al*., 2006; Singh *et al*., 2006; Swamy *et al*., 2007). Immediately after operation, the rats were placed in the collars to prevent damage to the wounds. No Aseptic measures or antimicrobials were employed in the experiment. The animals were divided in to three groups (n=6).

Group I served as the control and left untreated, while Group II and III served as standard and test, which were treated with 1% w/w framycetin sulphate cream (Manjunatha *et al*., 2005) and MA, respectively. The test drug and standard drug were applied topically once daily for eight days. The sutures were removed on 9th day and the rats were sacrificed on day 10th. The skin was removed with one cm on each side of the wound. Tensile strength was recorded with a TA XT2 texture analyzer. Increase in the tensile strength was served as a measure of wound healing potential. Tensile strength was calculated using the following equation (Baie and Sheikh, 2000):

Tensile strength = Breaking load (force)/Area of the skin
Where, Area = Thickness × Width of the skin

Excision wound model

Rest eighteen animals were divided into three groups (n=6) as grouped for incision model. The rats were anaesthetized under light ether anaesthesia (Morton and Malone, 1972) and their back were shaved with depilatory cream and cleaned with dry gauze pieces. A circular piece of skin with full thickness (approximately 500 mm2) was excised from the pre-determined area in the dorsal inter-scapular region of rats (Mukherjee *et al*., 2000). No aseptic measures or antimicrobials were employed in the experiment.

Table 1: pH and spreadability of MA at different temperature

Temperature (ºC)	pH	Spreadability (cm g/s)
25	6.3	5.9
30	6.2	6.3
40	6.1	7.2

The test and standard drugs were applied topically once daily, from the day of operation, till complete epithelialization. The wound contraction and epithelialization period were observed.

Measurement of contraction and epithelialization of wound

The contraction as a measure of wound closure,was estimated by tracing the wound area with transparent paper on 3rd, 6th, 9th, 12th, 15th and 18th and thereafter every alternate day till wounds were completely covered with epithelium (Manjunatha *et al*., 2005). These wound tracings were retraced on a millimeter scale graph paper to determine the wound area. On each measurement day, the wounds of the animals were photographically documented. Wound contraction (WC) was calculated as a percentage change in the initial wound size i.e.

$$WC\ (\%) = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

Epithelialization period was calculated as the number of days required for eschar to fall away so than no wound area is left behind (Manjunatha *et al*., 2005; Kamath *et al*., 2006).

STATISTICAL ANALYSIS

All the values are expressed as mean ± SEM. Data was statistically analyzed using one-way ANOVA with post-test followed by Dunnett's t-test. P values less than 0.05 and 0.01 were considered significant and $p<0.001$ were considered highly significant.

RESULTS

Preparation of MA

The MA was developed successfully by adopting classical method and found to be dark brown in colour with optimum consistency.

Characterization of MA

Viscosity of MA

Since, it relates to the spreadability of the formulation and contact time on the skin surface (Punit *et al*., 2012), the viscosity of the MA was determined by Brookfield viscometer and found to be 196 cps.

Centrifugation

Centrifugation is an excellent tool for the evaluation of accelerated deterioration of cream and ointments. The formulated ointment was centrifuged at 10000 rpm for 10 min to analyze the accelerated deterioration. At same speed and time, the formulation was found to be stable on centrifugation.

pH of MA

The pH of 10% w/v solution of MA at 25ºC, 30ºC and 40ºC was found to be 6.3, 6.2 and 6.1, respectively (table 1), which lies in the normal range of the pH (5.5-6.5) of the skin therefore making the formulation less sensitive and ensure better acceptability to the skin (Naira and Karvekar, 2011). A gradual decrease in the pH of MA was observed with increase in temperature.

Spreadability of MA

The spread ability result of MA is shown in table 1. The MA has good spread ability character (5.9 cm g/sec at 25ºC) and it was increased with temperature.

Antimicrobial activity of MA

The disc diffusion method was used to determine the zones of inhibition of MA and its all ingredients, which showed significant antimicrobial activity against almost all organisms (table 2). The activity shown by MA against *S. pyogenes* and *S. aureus* was found comparable to standard antibiotic (Ciprofloxacin 5µg/disc and gentamicin 10µg/disc). In addition, it exhibited modest inhibition against *E. faecalis* and *C. albicans,* however; no activity was shown by MA against *P. aeruginosa*. Methanolic extract of root of SC showed highest activity against *S. pyogenes,* which was similar to that of ciproflaxacin. It also exhibited remarked activity against *S. aureus* and *P. aeruginosa.* The CS was found to be as effective as ciproflaxacin and showed highest activity against all organisms tested in this study. The most pronounced activity of CS was against *C. albicans* and was found more active than the standard antifungal drugs used in this study (Fluconazole and ketoconazole). The LO showed different activity towards test organisms. It showed remarkable activity against *S. pyogenes, E. faecalis* and *P. aeruginosa* and modest activity against *S. aureus*. It also exhibited remarkable activity against *C. albicans*. The CO showed lowest activity only against *S. aureus* at higher concentration, while it was inactive against rest of the tested organisms. The BW showed no inhibition zone against any of the organisms tested in the study. Completely dried discs treated with chloroform, methanol, petroleum ether, acetic acid and distilled water, which were served as negative control, showed no inhibition zone against any of the test organisms.

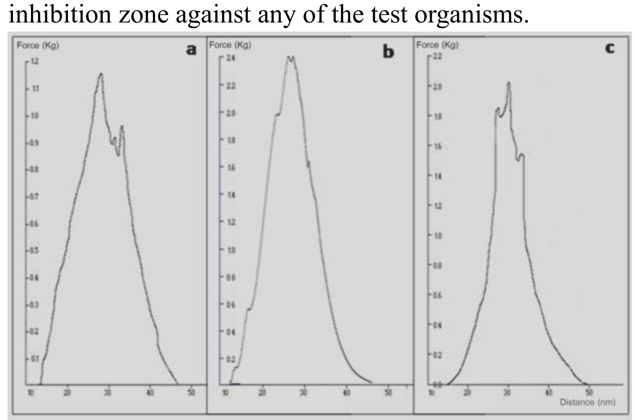

Fig. 1: Effect of MA on tensile breaking strength, breaking forces applied to the healed wounds using texture analyzer (a) Control; (b) Standard and (c) Marham-e-Aatshak. *X*-axis represents distance in millimeter (mm) and *Y*-axis represents force in Kilogram (Kg).

S. pyogenes was found to be most sensitive to MA and SC, while *S. aureus, P. aeruginosa, E. faecalis* and *C. albicans* were found to be most sensitive to CS. An increase in inhibition was found at higher concentration, and when the concentrations were decreased from 512-16 mg/mL, slight decrease was observed in inhibition zones. MICs of these test drugs are summarized in table 3. MA and CS produced low MIC values and growth inhibition of *S. aureus* and *S. pyogenes* with the concentration of 4.0mg/mL.

Table 2: Antimicrobial activity of MA and its ingredients from the disc diffusion method

Drugs tested	Concentration (mg/mL)	Inhibition zone diameters (mm)				
		Bacterial stains				Fungal strain
		S. a.[a]	*S. p.*[b]	*E. f.*[c]	*P. a.*[d]	*C. a.*[e]
Marham-e-Aatshak	512	18	25	13	-	14
	256	16	22	12	-	10
	128	15	16	11	-	7
	64	14	13	10	-	-
	32	12	12	9	-	-
	16	10	10	8	-	-
Smilax china						
	200	19	23	12	17	15
	100	16	20	10	14	11
	50	12	17	7	11	7
	25	10	13	-	9	-
	12.5	7	10	-	7	-
	6.25	-	6	-	-	-
Copper sulfate						
	512	22	22	18	24	25
	256	19	20	16	20	15
	128	16	15	14	15	10
	64	14	13	13	13	-
	32	12	11	11	10	-
	16	10	9	8	9	-
Lead oxide						
	512	13	16	18	17	19
	256	9	11	12	12	13
	128	7	9	10	8	7
	64	-	6	7	6	-
	32	-	-	-	-	-
	16	-	-	-	-	-
Castor oil						
	50%	10	-	-	-	-
	40%	9	-	-	-	-
	30%	8	-	-	-	-
	20%	7	-	-	-	-
	10%	6	-	-	-	-
	5%	5	-	-	-	-
Beeswax						
	200	-	-	-	-	-
	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
Gentamicin	10µg/disc	19	10	14	29	
Tetracyclin	30µg/disc	21	15	-	12	
Ciprofloxacin	5µg/disc	26	26	18	40	
Fluconazole	10µg/disc					23
Ketoconazole	10µg/disc					24
Negative control	Chloroform	-	-	-	-	-
	Methanol	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
	Acetic acid	-	-	-	-	-

Values are mean ± SEM (n=3). '–': No inhibition; [a]*Staphylococcus aureus;* [b]*Streptococcuspyogenes;* [c]*Enterococcus faecalis;* [d]*Pseudomonas aeruginosa*; [e] *Candidaalbicans*

Wound healing activity

Incision wound model

The tensile strengths of wounds were determined in all the three groups on day 10thas given in table 4. The tensile strengths of groups II and III were compared with group I (control). The mean breaking strengths were significantly ($p<0.01$) increased in the animals treated with standard (Group II) and test drug (Group III) as compared to the control group (Group I).

Table 3: The MIC values (mg/mL) of MA and its ingredients against test organisms

Test organisms	MIC values (mg/mL)				
	MA	SC	CS	LO	CO
S. aureus	4	12.5	4	128	5%
S. pyogenes	4	6.25	4	64	-
E. faecalis	16	50	8	64	-
P. aeruginosa	-	12.5	4	64	-
C. albicans	128	50	64	128	-

'−': No inhibition; MA: Marham-e-Aatshak; SC: *Smilax china*; CS: Copper sulfate; LO: Lead oxide; CO: Castor oil.

Excision wound model

The ointments were topically applied once daily, for 21 days, starting from the day of surgery, till the complete epithelialization. The reduction in wound size was observed and the mean percentage of wound contraction was calculated on 3rd, 6th, 9th, 12th, 15th and 18th days post wounding. The results of wound healing study showed that upon application of the MA, there were significant reduction in wound area as compared to the control ($P<0.01$), which was comparable to the standard drug framycetin (table 5). A significant increase ($P<0.01$) in the percentage of wound contraction was recorded in Group II & III when compared to the control group. In control group wound contracted to the extent of 82.61% by day 15th, on the other hand MA showed wound contraction of 98.09% which was significant ($P<0.01$) when compared to the control group (table 6). A significant decrease ($P<0.01$) was found in the epithelialization period of wounds in MA treated group when compared to the control group, which was comparable to the standard drug (table 6).

DISCUSSION

The results of the present study have revealed that the MA possesses remarkable antimicrobial activities against all tested microorganisms except *P. aeruginosa*, where it showed no inhibition effect. Present study also confirmed the inhibitory activity of *S. china* and copper sulfate, as all ingredients of the MA were screened individually for their antimicrobial activity. *S. china* showed highest activity against *S. pyogenes* comparable to that of antibiotic (Ciprofloxacin) and modest to good activity against rest of the organisms. Presence of many biologically active compounds i.e. flavonoids, tannins, terpenoids and alkaloids have been reported in *S. china* (Venkidesh *et al*., 2010), and these compounds have been reported to possess strong antimicrobial activity (Sher, 2009). Copper sulfate itself has a great antimicrobial potential, and more extensively used for disinfection (Aarestrup and Hasman, 2004). In the present study, copper sulfate was found to be almost as effective as standard antibiotic (Ciprofloxacin), moreover it showed stronger activity than that of standard antifungal (Fluconazole and ketoconazole) against *C. albicans*. Castor oil and beeswax were used as bases in Marham-e-Aatshak and also undertaken to screen their antimicrobial activity. Castor oil showed low activity only against *S. aureus*, while bees wax was found to be totally inactive against microorganisms tested. It is likely that the presence of stearic, oleic and linoleic acids in castor oil may have contributed to its activity. However, previous studies revealed that the three (stearic, oleic and linoleic acids) lacked antibacterial and antifungal power (Novak *et al*., 1961), and the same was observed in the present study. A previous study revealed that the beeswax with a mixture of honey and olive oil showed clear zone of inhibition (Al-Waili, 2005), however, no study has been reported on antimicrobial activity of beeswax alone.

The results of the wound healing activity showed that application of MA lead to a significant increase in the tensile strength in incision wound model when compared to the control group. The effect was comparable to the standard drug framycetin. In excision wound model, a marked reduction in wound area on day 3rd, 6th, 9th, 12thand 15th was observed. Also an increased percentage of wound contraction along with the decreased period of epithelialization was observed. The study findings revealed that the healing started steadily in the group treated with the test drug, the healing on 3rdday was found to be 17% comparable to standard drug, while it was only 10% in control group. It would be appreciable that throughout the study, MA did not cause any pain or irritation to the animals as they did not showed any scratching or biting of wound site when the ointment was applied and no sign of restlessness was observed in the animals treated with MA. Even several reports on toxic properties of lead oxide, it has been effectively used since long externally in Aurvedic, Unani and Chinese system of medicine for its anti-microbial properties usually after detoxification processes as per their traditional literatures (Bose *et al*., 1983; Ernst, 2002; Cooper *et al*., 2007)

It is worth mentioning here that angiogenesis is a key factor for wound healing and the copper is an important facilitator of angiogenesis as well as extra cellular matrix remodeling via Copper-sensitive pathways (Sen *et al*., 2002). Also, the castor oil contains ricinoleic acid that has been reported to stimulate the synthesis of prostaglandin E2 in the tissues *in vitro* (Gao *et al*., 1999), hence it can regulate wound healing (Baie and Sheikh, 2000).

Table 4: Effect of the MA on tensile breaking strengths

Formulations	Mean area of skin (cm^2)	Mean force applied (Kg)	Tensile strength (Kg/cm^2)
Control	0.67±0.012	1.213±0.075	1.810±0.117
Standard	0.67±0.014	2.186±0.020	3.262±0.133**
Marham-e-Aatshak	0.67±0.019	1.945±0.027	2.902±0.012**

Values are expressed as mean ± SEM (n=6); *significant, **highly significant, when compared with control

Table 5: The effect of the MA on post wounding reduction in wound area in excision wound model.

Post wounding days	Post wounding reduction in wound area (cm^2)		
	Control	Standard	Marham-e-Aatshak
0	4.98±0.007	4.981±0.006	4.99±0.005
3rd	4.451±0.009	3.961**±0.038	4.11**±0.044
6th	3.586±0.019	2.956**±0.008	3.068**±0.037
9th	2.24±0.005	1.87**±0.005	2.0915**±0.014
12th	1.451±0.007	0.67**±0.003	1.141**±0.007
15th	0.866±0.006	0.049**±0.01	0.095**±0.002
18th	0.126±0.003	0.001**±0.000	0.002**±0.002
21st	0.034±0.002	0.000**	0.000**

Values are expressed as mean ± SEM (n=6); *significant ($p<0.05$ and $p<0.01$), **highly significant ($p<0.001$), when compared with control.

Table 6: Effect of MA on wound contraction and period of epithelialization in excision wound model.

Groups	Percentage (%) wound contraction (Days)							Period of epithelialization (Days)
	0	3rd	6th	9th	12th	15th	18th	
Control	0.00	10.62 ±0.06	27.99 ±0.012	55.02 ±0.08	70.86 ±0.16	82.61 ±0.2	97.46± 0.14	21.33±0.21
Standard	0.00	20.46± 0.01**	40.65± 0.2**	62.45 ±0.1**	86.54± 0.19**	99.01± 0.15**	99.97± 0.11**	15.57±0.21**
Marham-e-Aatshak	0.00	17.63± 0.07**	38.51± 0.09**	58.08± 0.04**	77.13± 0.10**	98.09± 0.11**	99.95± 0.19**	16.67±0.36**

Values are expressed as mean ± SEM (n = 6); *significant, **highly significant, when compared to control.

Moreover, there is a definite role of free radicals in the pathogenesis of wound (Singh *et al*., 2006) and *S. china,* castor oil and beeswax (as discussed earlier) have been reported to have free radical scavenging activity.

CONCLUSION

It is evident from present investigation that the test formulation showed remarkable antimicrobial activity and possessed significant wound healing potential in wounds, which can be safely used in different types of injuries. However, a further study is required on toxicity and to ascertain the exact mechanism of action of the developed ointment (MA).

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