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RESEARCH ARTICLE

# Formulation and evaluation of ointments containing methanolic leaf extract of *Momordica balsamina* Linn (Cucurbitaceae) for wound healing

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## ABSTRACT

Drugs obtained from natural or synthetic sources are not administered to patients as such. They are usually presented in a form which is stable, effective, efficacious and acceptable to the consumers. The aim of this study was to formulate the methanolic leaf extract of *Momordica balsamina* as an ointment for wound healing purposes, and evaluate the properties of the products. A 0.1 or 0.2% w/w *Momordica balsamina* extract was incorporated into Emulsifying Ointment BP, Cetrimide Emulsifying Ointment BP and Cetomacrogol Emulsifying Ointment BP. These are examples of anionic, cationic and non-ionic ointment bases respectively. The consistency, pH, spreadability, and extrudability of the herbal ointments were evaluated by suitable methods and techniques. The antibacterial property was assessed by the agar well diffusion method using *Staph aureus*, and by the ability of the ointments to heal open wounds inflicted on male and female albino rats. Results show that the ointment formulated with the anionic base exhibited the fastest rate of wound healing compared to others. The inhibition zone diameter of this ointment against *Staph aureus* was 14±2.31 mm. The ointment equally possessed spreadability and extrudability profiles comparable to those of cationic and nonionic bases or penicillin skin ointment used for comparison. It is also non-irritant to the skin of the experimental animals. On the basis of these, Emulsifying Ointment BP could be employed as a suitable base for formulating ointment containing the extract.

**Keywords:** Herbal medicines; *Momordica balsamina*; Herbal ointments; Evaluation

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## 1. Introduction

Herbal medicine refers to the use of leaves, flowers, berries, seeds, barks and roots of plants for medicinal purposes. Plant parts, among other uses, serve as good antibiotics and chemotherapeutic agents. They are used in formulated or unformulated forms (Sofowora, 2012).Various parts of the plants are used for treating diseases. A lot of people patronize herbal medicines because they are cheap and readily available compared to the orthodox medicines. A combination of herbs is often used to enhance effectiveness and produce additive or synergistic effects with little or no side effects (Chhetri *et al*, 2010). Herbal remedies are useful tools for discovering new chemotherapeutic agents for the treatment of many disease conditions (Sasidharan *et al*, 2011).

Herbal drugs have been presented in the form of powders for infusion or decoction, syrups, ointments, creams, capsules, and tablets (Carter, 2000; Sofowora, 2012). Ointments are viscous semisolid preparations applied topically for treating various conditions of the skin and other surfaces. They come in medicated or unmedicated forms (Shelke and Mahajan, 2015; Carter, 2005). Ointments formulated as antiseptics inhibit the growth of bacteria. Although *Momordica balsamina* plant possesses antibacterial properties, it has not been presented in a conventional dosage form. The natives used it as fresh infusion or decoction for wound healing and other medicinal purposes. Such products are often unstable and unstandardized. The aim of this study was to formulate a conventional, stable and standardized ointment containing the methanolic leaf extract of this plant, and evaluate the physical properties and antibacterial activity of the product.

## 2. Materials and Methods

**Materials:**

*Momordica balsamina* leaves were collected from Jos North area of Plateau State, Nigeria. The animals used were purchased from the Animal House of the University of Jos. Other materials were used as purchased from accredited marketers by the Institution.

**Preparation of ointments containing *Momordica balsamina* leaf extract:**

The formula for preparing batches of ointments containing *Momordica balsamina* leaf extract is shown in Table 1. A 20 g quantity of each official ointment was prepared according to the method in the BP (1993). Each ointment base contained in a crucible was melted on a water bath at 70 $^{o}$C. The molten base was allowed to cool with constant stirring. When the base was about to set, 2.5 or 5.0 g of *Momordica balsamina* leaf extract was incorporated and stirring continued until the product congealed. The herbal ointments were stored in wide mouthed ointment jars until required for evaluation.

**Microbiological evaluation of ointment using *Staphylococcus aureus:*** The antibacterial activity of the formulated ointments was evaluated using agar well diffusion method. A 14 g quantity of nutrient agar (NA) was mixed with 250 ml of distilled water contained in a 500 ml conical flask. The flask and its contents were preheated on a hot plate at 100$^{o}$C until the contents were properly dispersed. Then 20 ml was distributed into universal bottles and autoclaved at 121$^{o}$C for 1 h. Then pure isolate of *Staphylococcus aureus* was inoculated into a nutrient broth and incubated at 37 °C for 24 h. With the aid of a micropipette, a 200 µl quantity of the broth containing *Staphylococcus aureus* was transferred into the universal bottles, mixed and poured into labelled sterile Petridishes and allowed to set. Wells of 5 mm diameter were made in the dishes with a flame-sterilised cork borer. Then 100 µl of a mixture consisting of 1 g each of the formulated herbal ointment in 5 ml of liquid paraffin was introduced into each well. Penicillin skin ointment was used as positive control, while the ointment base without the herbal extract was mixed with liquid paraffin and used as negative control. All the tests were replicated to minimize experimental errors. The cultured plates were allowed to stand for 30 min and subsequently incubated at 37°C for 24 h. The inhibition zone diameter (mm) of each Petri dish was measured with the aid of a plastic ruler.

**Effect of temperature and ageing on physical appearance of ointments:** A 1g quantity of each herbal ointment was weighed (Mettler P165, Switzerland) and placed in two sets of Petri dishes. One set was refrigerated at 4$^{o}$C, while the second set was kept on a water bath at 25$^{o}$C. After 1 h, the two sets were examined for changes in colour and consistency. The tests were repeated after 24 h. The experiments were carried out in duplicate. The procedure was also carried out at temperatures of 30, 35, 40 and 45 $^{o}$C and the observations recorded. The experiment was repeated on a five-hour period instead of a one-hour period and the observations recorded.

**Apparent viscosity of the herbal ointments:**

The apparent viscosity of the herbal ointments was determined using a digital rotational viscometer (Model BDV 1 S, China)). The reading was taken at 27 ±2 $^{o}$C using spindle number four (4) which was rotated at 30 revolutions per minute (rpm). The apparent viscosity (mPa.s) was read off and recorded. The apparent viscosity was also determined by dispersing 1 g each of the ointments in 10 ml of liquid paraffin. The viscosity of the plain liquid paraffin was determined with the digital viscometer at 28$^{o}$C using spindle number one (1) at 60 rpm. Equally, the viscosity of the herbal ointment containing liquid paraffin was also determined and values read and recorded as above.

**Determination of pH of the ointments:**

The pH of the ointments was determined by dispersing 1 g of each ointment in 10 ml of liquid paraffin. The pH of the plain liquid paraffin was determined using the digital pH meter (Model PHS-25, China) at 28$^{o}$C and that of the herbal ointment containing liquid paraffin was also determined and the values recorded.

**Spreadability test:**

A 1g quantity of the ointment contained on a glass slide was weighed. The glass slide was turned against a second that was bigger than the glass slide. A third glass of the same dimension as the bigger glass was pre-weighed and placed on top of the smaller glass slide and a weight of 1.3 kg was placed on them for 1min. The weight of the pre-

weighed glass was added to the 1.3 kg to make up the final weight that caused the spread. The area of the spread was calculated using the following formula:

$A = \frac{\pi}{4} x D^2$ ..... 1

A = area of spread

D = diameter of spread

**Extrudability test:**

A small quantity of each ointment was loaded into a 5 ml syringe with the aid of a spatula after removing the plunger. The plunger was replaced and a small quantity was extruded through the orifice to ensure proper packing and smooth extrusion. The loaded syringe was clamped on a retort stand using a cotton wool as a cushion. A load of 1.3 kg was placed on the plunger of the syringe and a stop watch was used to record the time required for the contents of the syringe to be completely extruded. The results are the average of replicate experiments.

**Content homogeneity test:**

A 1 g quantity of each ointment was taken at three different points from the container. The sample was dispersed in 5 ml of methanol contained in a 50 ml measure and left overnight for proper dispersion. Methanol was added to make up the volume to 20 ml. The dispersion was filtered through Whatman No 4 filter paper and the filtrate made up to 40 ml with methanol. Then 0.2 ml of this solution was made up to 10 ml with methanol to yield a 1:2000 dilution. The absorbance of the dilute solution was read in a UV/VIS-spectrophotometer (Metertech, Model SP- 8001, Taiwan) at 220 nm and recorded.

**Wound healing test:**

Approval for the use of experimental animals was obtained from the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Jos. Fifteen albino rats were separated into five groups of three animals according to their body weight range and sex. They were kept in cages and fed with standard feed and water *ad libitum* for a week. They were anaesthetized by injecting them intraperitonially with ketamine (100 mg/kg). The animals were subsequently inflicted with wound by incision of the skin using new blade and forceps. Both the loaded and unloaded ointments, as well as those used as controls were applied on the open wounds on the skin of the animals as soon as the wounds were inflicted. Evaluation of the wound healing was initiated 24 h after the wounds were inflicted. The wound areas and changes in body weight of the animals were monitored daily for twelve days.

## 3. Results and Discussion

Table 2 shows the results of the qualitative antimicrobial assay of the formulated ointments. The inhibition zone diameter in the plate containing the anionic ointment formulated with 0.2%w/w extract was 14± 2.31 mm. The corresponding value for the plate containing penicillin ointment used as standard was 18.5 ± 0.71 mm. No inhibition zone diameter was obtained in the plates containing the other ointments. The cationic and nonionic ointments containing the extract did not exhibit any inhibition zone diameter.

It is possible that the constituents of the extract responsible for antibacterial activity were not released in the cationic and nonionic bases, whereas the anionic base enhanced their release. The methanolic extract may be less firmly bound to the anionic base than the other bases. This, coupled with the presence of sodium lauryl sulphate as a surfactant, may have promoted the activity of this extract against the test organism (Carter, 2005; Hassan et al., 2015).Cetrimide Emulsifying Ointment BP possesses some inherent antibacterial activity. This inherent antibacterial activity may not have effect on insensitive bacteria. When the ointment base was mixed with the extract there was no observed activity against the test organism as indicated by the absence of inhibition zone diameter. This may be due to lack of extract release from the ointment bases or incompatibilities among the constituents (Carter, 2000). This result emphasizes the importance of preformulation studies in the design and development of dosage forms (Sahitya et al, 2012). The methanolic extract of *Momordica balsamina* leaf, which demonstrated antibacterial activity in our preevious report (Kadiri et al, 2020) is unable to replicate the same activity in some ointment bases.

The effect of temperature on the stability of the formulated ointments is shown in Table 3. The herbal ointments are stable to heat at temperatures up to 37 $^{o}$C, but become hard, sticky and melt at 4, 40 and 45 $^{o}$C respectively. There was no difference in the physical behaviour of the herbal ointment when exposed to the same temperature conditions for 1 or 5 h. In a temperate region, the ointment may be sufficiently hard for application, whereas in regions with extreme environmental weather conditions, such as in the Northeastern Nigeria, the ointments may melt, spill or undergo phase separation.

Table 4 shows the physical properties of the formulated ointments. The viscosity of the ointments ranged from 18851 to 18856 mPas. There is slight increase in viscosity as the extract was incorporated. The pH of the ointments is mild for the skin, except for Cetrimide Emulsifying Ointment which is irritant to the skin. Results indicate that the cationic herbal ointment is the most spreadable of all the ointments, with a spreadability of 4.28 ± 0.17cm$^2$, compared to the closest value of 3.96±0.05 cm$^2$ obtained for penicillin ointment used as positive standard. The penicillin ointment possessed a high extrudability value of 360.15 ± 126 mg/sec while the cationic ointment possessed a low value of 53.3±7.2 mg/sec. The values obtained for the other ointments were smaller than these. Viscosity, spreadability and extrudability values of semisolids are parameters used to assess the flow or application characteristics of semisolids (Singh et al, 2013). The higher the value of these in an ointment, the better is the application characteristics. The cationic herbal ointment exhibited high spreadability and extrudability compared to the other herbal ointments. However, this ointment did not possess antibacterial or wound healing property. The homogeneity of the extract in all the ointment batches was fairly uniform, with values of 119.5±4.5, 115.9±6.5and 110.1±4.5 mg/g for the anionic, cationic and non-ionic herbal ointments respectively. The

effect of *Momordica balsamina* extract on the healing of wounds inflicted on the experimental animals is shown in Tables 5 and 6. Changes in the weight of the animals during the wound healing process are shown in Table 7. The anionic herbal ointment exhibited a fast onset of wound contraction compared to the rest of the ointments. Towards the end of the healing process however, the penicillin ointment used as positive control possessed the best healing profile. This ointment possessed the smallest residual wound area of 1.5±0.4 $mm^2$ and the highest percentage wound contraction of 98.3 %. This was evident by the increased physical activities of the rats. Although the animals treated with plain anionic ointment base used as negative control possessed a high value of percentage wound contraction than some of the herbal ointments, they were sickly and less active than the animals in the rest of the groups, indicating that their wounds may have been infected by microorganisms.

The World Health Organization (2006) defined health as the state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity. Even though the animals in the negative control group seemed to get along with the other groups, there was visual evidence of disease or infirmity since they looked sickly and are inactive. All the animals treated with the herbal ointments could not regain their weight fully within the period of the experiment, except those in the positive control group which gained more weight. The slight gain in weight observed in the negative control group may be due to inactivity of the animals and inflammation of the wound area.

**Table 1:** Formula for Preparing Various Batches of Herbal Ointments

| Ingredient | Batches | | | | | |
|---|---|---|---|---|---|---|
| | Ia | IIa | IIIa | Ib | IIb | IIIb |
| *M. balsamina* methanolic extract (g) | 2.5 | 2.5 | 2.5 | 5 | 5 | 5 |
| Emulsifying Ointment BP (g) | 20 | - | - | 20 | - | - |
| Cetrimide Emulsifying Ointment BP (g) | - | 20 | - | - | 20 | - |
| Cetomacrogol Emulsifying Ointment BP (g) | - | - | 20 | - | - | 20 |
| Total (g) | 22.5 | 22.5 | 22.5 | 25 | 25 | 25 |

**Table 2:** Qualitative Test Result of the Formulated Ointment Against *S. aureus*

| **n = 2** | **Inhibition Zone Diameter (mm)** | | | |
|---|---|---|---|---|
| **Test organism** | **Anionic** | **Non-ionic** | **Cationic** | **Penicillin oint** |
| *S. aureus* | 14 ± 2.31 | 0 | 0 | 18.5 ± 0.71 |

**Table 3:**Effect of Temperature and Time on the Stability of the Formulated Ointments

| Temperature ($^oC$) | Physical behaviour | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | | | 5 h | | |
| | Anionic | Cationic | Non-ionic | Anionic | Cationic | Nonionic |
| 4 | Hard | Hard | Hard | Hard | Hard | Hard |
| 25 | Normal | Normal | Normal | Normal | Normal | Normal |
| 30 | Normal | Normal | Normal | Normal | Normal | Normal |
| 35 | Normal | Normal | Normal | Normal | Normal | Normal |
| 37 | Stains | Stains | Stains | Stains | Stains | Stains |
| 40 | Sticky | Sticky | Sticky | Sticky | Sticky | Sticky |
| 45 | Melts | Melts | Melts | Melts | Melts | Melts |

**Table 4:** Physical Properties of *Momordica balsamina* Extract Ointments

| S/No | | | Ointment batches | | |
|---|---|---|---|---|---|
| | | | Anionic | Cationic | Non-ionic |
| 1. | Viscosity (mPa.s) | Herbal oint | 18856±7.15 | 18854±6.11 | 18851.83±2.56 |
| | | Plain | 18855.83±4.22 | 18850±2.37 | 18850.17±2.79 |
| 2. | pH | Plain +Lp | 6.88 | 6.22 | 4.42 |
| | | Herbal oint + Lp | 7.11 | 6.71 | 6.92 |
| 3. | Spreadability ($cm^2$) | | 3.05±0.2 | 3.57±0.25 | 3.01±0.3 |
| 4. | Extrudability (mg/sec) | | 25.7±1.36 | 53.3±7.2 | 30.4±0.72 |
| 5. | Homogeneity (mg/g, 111mg/g ) | | 119.5±4.5 | 115.9±6.5 | 110.1±4.5 |

**Table 5:** The Effect *of Momordica balsamina* Extract Ointment on Rats Wound

| S/No | Group | Wound area in $mm^2$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2nd day | 4th day | 6th day | 8th day | 10th day | 12th day |
| 1. | I | 94.6±20 | 73.5±8.7 | 54.7±19 | 21.6±2.8 | 8.4±3.7 | 4.7±3.6 |
| 2. | II | 93.6±22 | 86.8±27.6 | 62.4±21 | 27.2±9.9 | 12.2±2.5 | 4.5±2.6 |
| 3. | III | 84.1±12.3 | 81.2±4.6 | 63.6±11 | 17.2±4 | 6.5±3.2 | 2.2±0.7 |
| 4. | IV | 89.3±24.9 | 79.4±20 | 48.3±7.2 | 16.5±6.4 | 5±1.9 | 1.5±0.4 |
| 5. | V | 71.4±6 | 64.9±2.6 | 58.9±8.8 | 16.2±5.5 | 5.2±3 | 2.5±2.1 |

**Table 6:** Percentage Wound Contraction and Physical Observation of Treated Rats

| S/No | Group | Percent (%) Wound Contraction | | | | | Physical observation |
|---|---|---|---|---|---|---|---|
| | | 4th day | 6th day | 8th day | 10th day | 12th day | |
| 1 | I | 22.3% | 42.3% | 77% | 91% | 95% | active and healthy |
| 2 | II | 7.2% | 33.3% | 71% | 87% | 95% | active and healthy |
| 3 | III | 3.4% | 24.3% | 79.6% | 92.2% | 97.5% | active and healthy |
| 4 | IV | 11% | 46% | 81.5% | 94.4% | 98.3% | very active and healthy |
| 5 | V | 9.1% | 17.5% | 77.3% | 92.7% | 96.4% | less active and sickly |

I= Anionic; II = Cationic; III = Non-ionic; IV = Penicillin skin ointment; V = Plane ointment base

**Table 7:** The Effect of *Momordica balsamina* on Weight Changes in Wounded Rats

| S/No | Group | Rats Group Average Weight (g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1st day | 2nd day | 3rd day | 4th day | 6th day | 8th day | 10th day | 12th day |
| 1 | I | 382.7±32 | 364.6±33 | 343.5±31 | 353.8±29.6 | 358.2±29.7 | 359.2±29.7 | 363.2±32.5 | 375.1±29.7 |
| 2 | II | 330.8±12 | 305.8±12 | 308±9.3 | 295±11 | 274±11.7 | 261.7±13.8 | 271.4±20 | 306.3±15 |
| 3 | III | 194±6 | 178.6±9.3 | 168.9±8.9 | 177.7±8 | 182.8±6 | 173.4±5.4 | 182.7±4.8 | 185.7±3 |
| 4 | IV | 245±32 | 236±36.7 | 235.5±48 | 227.4±46 | 214.2±43 | 226±60 | 241.6±56 | 248.8±56.5 |
| 5 | V | 171.6±15 | 161±14.8 | 163±26.6 | 161±31 | 150±31 | 165.6±32 | 168.5±34 | 176±31.8 |

I= Anionic; II = Cationic; III = Non-ionic; IV = Penicillin skin ointment; V = Plane ointment base

## 4. Conclusion

The methanolic leaf extract of*Momordica balsamina*was formulated into wound healing ointments. The extract formulated with Anionic Emulsifying Ointment BP possessed excellent physical and antibacterial properties. The inhibition zone diameter exhibited by this ointment against *Staphylococcus aureus* was comparable to the value obtained for penicillin skin ointment. The application characteristics of this herbal ointment were adequate for wound healing. The ointment was able to heal wounds inflicted on experimental rats, although the healing efficiency of penicillin ointment was superior to that of the herbal ointment. The extract,when incorporated into Cetrimide Emulsifying Ointment BP or Cetomacrogol Emulsifying Ointment BP, did not exhibit any appreciable antibacterial activity either *in-vitro* or in experimental animals. Further work would include the use of hydrocarbon base, water-soluble and water-miscible bases with the extract.

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