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# Extraction, Evaluation, and Formulation of *Hyptis capitata* Jacq. (Burunganon) Flower Crude Extract as Bactericidal Ointment

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Bacterial infection is not only a nationwide problem but a global health concern facing communities and populations across the world. The impact of bacterial infections extends beyond just the health sectors, affecting economies, healthcare systems, and overall societal well-being. This study developed and evaluate ointment from *Hyptis capitata* Jacq. (Burunganon) flower crude extract and

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its antibacterial potential. The phytochemical screening was to determine the presence of Alkaloids, phenolic, and cardiac glycosides. The physicochemical properties were analyzed to determine their appearance, homogeneity, odor, pH, spreadability, and water number, and the antibacterial properties were determined. Results showed that the Burunganon flower crude extract revealed the presence of alkaloids, flavonoids, phenolic and cardiac glycoside. Result in physicochemical screening of the formulated ointment showed that it had a yellowish color, good homogeneity, menthol like fragrance, slightly acidic, had a good spreadability, and the base had a good absorption of water. Meanwhile, the formulated ointment from burunganon flower crude extract showed inhibitory effect against *E. coli* and *P. aeruginosa*. Therefore, the Burunganon flower crude extract showed a presence of secondary metabolites that can be used in a wide range of pharmaceutical application, a promising candidate for development of bacterial agents, providing a natural and potentially effective alternative for combatting infections. Additionally, it shows that it has an good physicochemical properties that can be used in development of bactericidal ointment. More physicochemical test, different base used in formulation, further characterization, and incorporation of antibacterial were recommended.

*Keywords: Antibacterial; burunganon; crude extract; ointment; physicochemical.*

## 1. INTRODUCTION

Plants have various nutritional and medicinal values and they provide adequate supply of nutrients useful for the maintenance of health and prevention of diseases [1]. Plants are the renowned cradle of traditional medicine system that assuages human diseases and promotes health for thousands of years. Plants are a rich reservoir of a vast array of active constituents that have significant therapeutic applications like antiviral, anticancer, analgesic, antitubercular, Anti-bacterial, anti-inflammation and so on.

*Hyptis* genus is one of the largest plant genera. Plants of this genus are characterized by glandular trichrome that produce essential oils and have a Strong aroma [2-3]. There are about 290 species, which are adapted in tropical areas. The *Hyptis capitata* Jacq., knobweed or false iron wort belongs to the family Lamiaceae family and is among known traditional medicine [4-5].

Bacterial infection significantly impacts public health, affecting body sites and transmitted through various modes. Bacterial resistance to antibiotics is growing concern, impacting communities and populations globally. Today in the modern era, the pathogenic bacteria have developed resistance against existing antibiotics because of the extensive use of antimicrobial drugs against the infectious diseases [12-14].

Herbal plants are wonderful sources of traditional & modern medicine, useful for primary health care system. Instead of the alternative formulation like herbal medicine may also be prepared in the form of ointment [6]. Ointments are used topically for several purposes, example, as protectants, antiseptics, emollients, antipruritic, keratolytic and astringents. Ointment bases are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion [7].

Ointments have long been used to treat localized diseases but can be less effective for systemic therapy due to poor drug bioavailability [8]. Furthermore, dosing can be a problem due to variation in the formulation application area. The development of transdermal therapeutic systems using different patch technologies.

Worldwide, there is a long history of traditional usage of many Lamiaceae plants as medical plants. The compound of this plants has the ability to fight off bacterial infection, which is why they are valuable. The *Hyptis capitata* Jacq. is merely a pretty flowering plant that look people overlook if they are unaware of its potential to treat bacterial infections, illnesses, and other health related condition [9-11].

In this research study an antibacterial ointment was made from burunganon flower crude extract. With the help of its extensive repertory of bioactive chemicals, this plant has a constitutional that may able to provide a natural antibacterial ointment that is both effective and safe.

## 2. METHODOLOGY

The *Hyptis capitata* Jacq. (Burunganon) flower used in this study were collected at Brgy.

Poblacion Lope De Vega Northern Samar. Extraction of samples, phytochemical screening, formulation of ointment and the physicochemical test were conducted at the Technology Innovation Center while the antibacterial susceptibility test were done at the Integrated Research Laboratory both situated at the University of Eastern Philippines, University Town, Catarman, Northern Samar.

**Extraction of the crude extract:** The collected *Hyptis capitata* Jacq. (Burunganon) flower were washed, and air dry for one day. After air drying, the flower was place in an oven for 80 °C for 3 hours then its pulverized using electric blender. The powdered Burunganon flower are kept in dry bottle, macerated in a solvent (1:10 w/v ratio) for 3-5 days. The regulating suspension are filtered, and subjected to simple distillation within 78°C. After, simple distillation, the Burunganon flower crude extract is incubated using 45°C to 50°C temperature in order to remove the remaining alcohol presents in the sample. The flowers of Burunganon flower crude extract are collected and refrigerated until use.

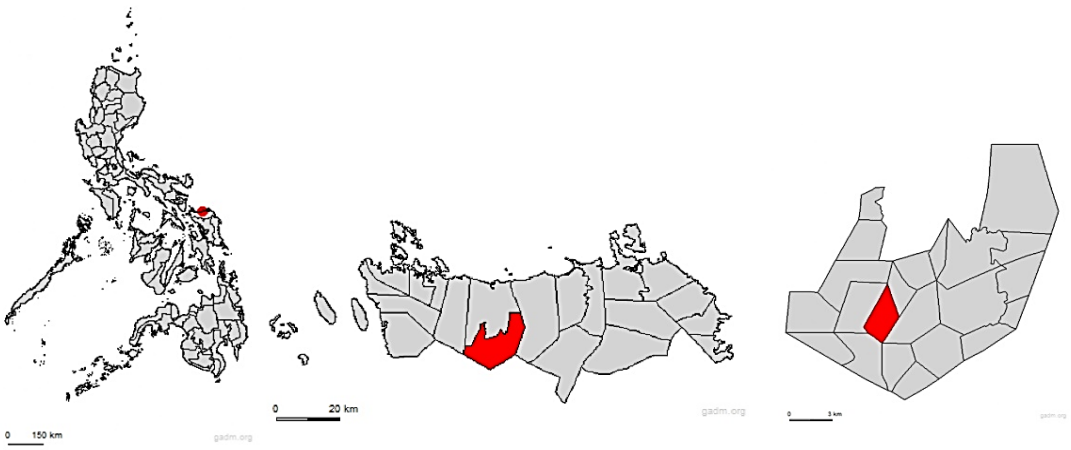


**Fig. 1. Map of Barangay Poblacion, Lope De Vega Northern Samar**

**Fig. 2. *Hyptis capitata* Jacq. leaves and its flower**

**Phytochemical screening of Burunganon flower crude extract for active secondary metabolites:** Standard Preliminary phytochemical qualitative analysis of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites in three trial such as; alkaloids, flavonoids, cardiac glycoside, phenolic. We used the procedures of Dianito et al. [12], Dagalea et al. [13], and Lim et al. [14] in this section – with minor modifications.

**Detection of alkaloids:** Picric test about a drop of picric solution was added to the Hyptis *capitata* Jacq. (Burunganon) flower crude extract. Formation of yellow colored or white precipitate indicates the presence of alkaloids.

**Detection of flavonoids:** Alkaline Reagent Test was used in determining the presence of flavonoids. Addition of *Hyp*tis *capitata* Jacq. flower crude extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which will become colorless on addition of dilute hydrochloric acid, indicates the presence of Flavonoids.

**Detection of cardiac glycosides:** In determining the presence of cardiac glycoside keller-kiliani test was used. About 4 mL of glacial acetic solution with few drops of 2.0% ferric chloride was mixed with the crude extract and addition of few drops of concentrated sulfuric acid. Formation of brown ring between the layers which showed the entity of cardiac steroidal glycosides.

**Detection of phenolic:** For the presence of phenolic Ferric Chloride Test was used. About 3 to 4 drops of ferric chloride solution were added to the *Hyptis capitata* Jacq. flower crude extract formation of bluish-black color indicates the presence of Phenols.

**Ointment formulation:** Initially ointment base was prepared by weighing accurately grated paraffin wax which will be placed in beaker. After melting of paraffin wax remaining ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment. The ointment was prepared by mixing accurately weighed Burunganon flower crude extract to the ointment base. Method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating baser until to form homogeneous ointment, finally transferred in a suitable container [15].

**Determination of the physicochemical properties of the formulated ointment:** The physicochemical analysis of the formulated ointment was determined through the method describe by Maru *et al.* [15].

**Appearance:** In this parameter the appearance of the ointment was evaluated through visual observation. This was done in three trials by five evaluators.

**Homogeneity:** It is done by applying an ointment to a piece of glass slide. Homogeneous ointment marked by absence blubs on the smearing, at structure and has uniform color of the dot initial smearing until the point end of basting.

**Odor:** To determine the odor of the formulated ointment it was place in glass slide. Then, five (5) evaluators was described the odor of the formulated ointment, this was done in three trials.

**pH:** The pH of the formulated ointment was determine using a pH meter. It was done in three trials.

**Spreadability:** The Spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as Spreadability. Lesser the time taken for separation of two slides results better Spreadability. Spreadability was Calculated by following formula:

$$S = M \times L/T$$

Where,

S = Spreadability
M=Weight in the pan (tied to the upper slide)
L= Length moved by the glass slide and
T=Time (in seconds) taken to separate the slide completely each other

**Water number:** Water number is the maximum amount of water that can be added to of base at a given temperature. It was determined by continuously stirring the base with the addition of distilled water. When no more water was absorbed into the base evidenced by droplets of water, remaining in the container were taken as end point.

**Determination of antibacterial property:** In determining the antibacterial of the formulated ointment, Dianito's [12] and Dagalea's [16] procedures were used. All equipment was sterilized using autoclave. First, all the

equipment's were sterilized using autoclave. Then to determine the bactericidal activity of the formulated ointment from Burunganon flower against *E. coli* and *P. aeruginosa*, agar disc-diffusion test was employed. The suspension of the tested bacteria was uniformly swabbed on agar Plates using sterile cotton swabs. Sterile blank discs will be Individually impregnated to the formulated ointment and was placed onto the in occulated agar plates. The plates were inverted and incubated at 37 ºC for 24 hours for bacteria. The antibacterial Activity was measured using Vernier caliper by measuring the diameter of zone of inhibition against the tested organisms.

## 3. RESULTS AND DISCUSSION

As shown in Table 1, the results showed the present of active secondary metabolites in the *Hyptis capitate* Jacq. (Burunganon) flower crude extract through phytochemical screening. Formation of yellow colored in addition of picric solution from indicates a positive result alkaloid. Alkaloids have a wide range of pharmacological activities including antimalaria, antibacterial, anti-inflammatory activities [13]. Antibacterial effects of alkaloids promote research and development of new antibacterial drugs with high efficiency [17].

Additionally, a positive result for flavonoids was revealed using the alkaline method. Presence of flavonoids indicates in the formation of intense yellow color in addition of sodium hydroxide solution become colorless in addition of dilute hydrochloric acid. Flavonoids contains antioxidant that acts a free radical scavenger this property can help reducing oxidative stress on the skin, preventing damage from environmental factors [18]. Ferric chloride test was used in determining the presence of phenolic in Burunganon flower crude extract. Bluish black color indicates the presence of phenolic in addition of ferric chloride solution. Phenolic compound is known to exhibit various biological activities such as antimicrobial, antioxidant, and anti-inflammatory properties that is significantly enhance its therapeutic benefits in formulation of an ointment [19].

Cardiac glycoside content in *Hyptis capitata* Jacq. (Burunganon) flower crude extract were determined using the killer-kiliani. Addition of glacial acetic acid with ferric chloride solution to the extract and followed by addition of sulfuric acid. Indication of positive results in cardiac glycoside is the formation of brown ring between the layers. Cardiac glycosides are unique group of secondary metabolites that they considered one of the most useful drugs in therapeutics. Presence of CG in formulation of ointment offer certain therapeutic benefits such anti-inflammatory, antimicrobial and wound healing [13, 20-22].

Based on the results showed in Table 2, the formulated ointment from Burunganon flower crude extract has a yellowish color as the result revealed of the five evaluators. Burunganon ointment exhibit good homogeneity by the absence of blubs in smearing in the glass slide. Topical products should be acidified and possess pH in the range of 4 to 6 to keep the moisture in and bacteria out and make the microbe harder to survive and grow [23].

Good spreadability indicates a good consistency [15] as what result showed in the formulated ointment. Based on the evaluation of the five evaluators it revealed that the formulated ointment from Burunganon flower crude extract has a menthol like fragrance. Menthol like odor acts as a counter-irritant by imparting a cooling effect and by initially stimulating nociceptors and then desensitizing them. Good capacity of the ointment base in absorption of water implies that it helps maintain the desired consistency and efficacy of the ointment [7].

**Table 1. Phytochemical screening results of *Hyptis capitata* Jacq. (Burunganon) flower crude extract**

| Secondary metabolites | Positive Indicator | Result | Interpretation |
|---|---|---|---|
| **Alkaloids** | Yellowish | Yellowish | Positive |
| **Flavonoids** | Intense yellow | Intense yellow | Positive |
| **Cardiac glycosides** | Brown ring | Brown ring | Positive |

**Table 2. Summary of the Physicochemical Properties of formulated ointment from *Hyptis capitata* Jacq. (Burunganon) flower crude extract**

| Physicochemical Properties | Observation | Interpretation |
|---|---|---|
| **Appearance** | **Yellowish** | **Yellowish** |
| **Homogeneity** | No bubbles | Good homogeneity |
| **pH** | 5.15 | Slightly acidic |
| **Spreadability** | 12.78 | Good spreadability |
| **Odor** | Menthol | Menthol |
| **Water number** | 46 | Good absorption |

**Table 3. Chart of the zone inhibition of formulated ointment from *Hyptis capitata* Jacq. (Burunganon) flower crude extract**

| **Zone of inhibition against *Escherichia coli* (mm)** | |
|---|---|
| Burunganon ointment | 20 mm |
| Positive control | 20 mm |
| Distilled water (- control) | 0 mm |
| **Zone of inhibition against *Pseudomonas aeruginosa* (mm)** | |
| Burunganon ointment | 16 mm |
| Positive control | 23 mm |
| Distilled water (- control) | 0 mm |

Table 3 shows that formulated ointment from *Hyptis capitata* Jacq. (Burunganon) flower crude extract showed an inhibitory effect against *Escherichia coli and Pseudomonas aeruginosa*. Formulated ointment exhibits an average zone inhibition of 20 mm against *Escherichia coli* and an average inhibition of 16 mm against *Pseudomonas aeruginosa*. Meanwhile, the positive control have an average zone inhibition of 20 mm in three replicates against *Escherichia c*oli. Moreover, the positive control has an average zone inhibition of 23 mm, against *Pseudomonas aeruginosa.* Results of the susceptibility test revealed that the formulated ointment from burunganon crude extract show an antibacterial property.

## 4. CONCLUSION

The findings of this study revealed that the crude extract of *Hyptis capitata* Jacq. (Burunganon) flower can be used to formulate an antibacterial agent. Furthermore, the formulated showed presence of secondary metabolites indicates that Burunganon flower crude extract can be used in a wide range of pharmaceutical application, a promising candidate for development of bacterial agents, providing a natural and potentially effective alternative for combatting infections. Additionally, it revealed that the formulated ointment show a good physicochemical property and it can be used in development of bactericidal ointment.

These findings were call to more physicochemical test such as stability, drug content, non-irritancy, viscosity, and consistency as well as further characterization of the extract using UV-vis and FTIR. Formulating ointment with different amount of extract, using another type of base and further conduct antibacterial analysis against other bacteria to further determine the antibacterial capacity of the ointment from Burunganon flower.

The development of this new natural ointment is landmark achievement with wide reaching implications. This work serves as an gate opener for the business, agricultural, and medical sectors, to develop the industry. Each sector plays a vital role in leveraging this innovation to enhance patient care, support economic growth, and promote sustainable practices. By capitalizing this opportunity, Northern Samar can improve the quality of life for its resident and establish itself as a leader in pharmaceutical innovation and promote conservation and preservations of these natural resource.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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