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RESEARCH PAPER

Formulation of Hand Sanitizer Gel using the Semi-Purified Flavonoids from the outer coverings of the Red Creole variety of *Allium cepa* Linn.: A preliminary investigation

John Paul Tolentino Toting*, Jan Karlo Tiongson Ecalne, Jemimaiah Recinto Arceo, Romalyn Joson, Yasmine Tobias, Cecilia Diaz-Santiago, Regina Alberto-Jazul

Department of Pharmacy, Centro Escolar University – Malolos
Km. 44 Mc Arthur Highway, City of Malolos, Bulacan

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Correspondence
John Paul Tolentino Toting
Department of Pharmacy, Centro Escolar University – Malolos
Km. 44 Mc Arthur Highway, City of Malolos, Bulacan

E-mail
jptoting@aol.com

Abstract

This research focuses on the formulation of hand sanitizer gel using the semi-purified flavonoids from the outer coverings of the Red creole variety of *Allium cepa* L. (Family: Alliaceae). The agar cup diffusion method was used in determining the antibacterial activity of formulation with 40% semi-purified extract as compared to the two (2) locally available leading hand sanitizer brands. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Enterobacter aerogenes, Proteus vulgaris, Salmonella typimurium, Klebsiella pneumoniae, Bacillus subtilis,* and *Bacillus cereus* were utilized as test organisms. The formulation exhibited antibacterial activity against 8 of 10 bacteria used in the experiment, while Brand A exhibited antibacterial activity against 1 of 10 bacteria and Brand B manifested an antibacterial activity against 4 out 10 of bacteria utilized in the microbial assay. Moreover, based on the result of the primary skin irritation test, the formulation was perceptibly not capable of causing irritation to the skin when applied topically. The researchers recommends that thorough investigation of the semi – purified flavonoid extract using instrumental method of analysis and isolation of the pure flavonoid should be conducted in order to determine the specific flavonoid that exhibits the antibacterial activity.

Introduction

Hand washing is an essential method in reducing the number of various microorganisms from the hands. During the 19th century, Dr. Ignatz Semmelweiss became disturbed by the inflated mortality rates in the hospital. He theorized that the decaying matter on the physicians' hands, who recently conducted autopsies, was brought into contact with the genitals of the women giving birth. With these, he proposed a hand washing technique using chlorinated lime thereby reducing mortality rates to 3% (Mathur, 2011).

The use of antibacterial soaps became universal in industrialized nations. But despite of its popular use, one of the disadvantages of using soap is that it requires water to wash if off especially in areas where water for washing is not available. This led to the development of Hand Sanitizers (BHC, 1999). Hand Sanitizers grew in popularity for the past 10 years. However, it was been reported that hand sanitizers, especially those containing triclosan, causes toxicity in the endocrine system (USFDA, 2009).

Onion, scientifically known as *Allium cepa* from the family Alliaceae has been extensively studied for its medicinal use but writings and studies regarding its cosmetic application is limited.

This study aims to develop a hand sanitizer gel with a potent antibacterial activity consisting of natural, less toxic, and non-irritating substances. This study utilized the agar cup diffusion method in determining the antibacterial activity of the formulated gel and t-Test method was used to statistically interpret the results.

Material and Methods

***Allium cepa* semi-purified flavonoid extract**

About 100g of the dried outer coverings of the Red Creole variety of *Allium cepa* were osterized and then macerated using 80% Ethanol for 48 h. The extract is evaporated to incipient dryness. The sample is dissolved using 2M HCl and ethyl acetate is added until the extract is colorless. Separation of the acetate layer from the aqueous layer is done with the use of separatory funnel. The ethyl acetate layer obtained is evaporated to incipient dryness. The obtained residue is the semi-purified flavonoids.

Computation of the Percentage Yield

The collected semi-purified flavonoids were weighed and the percentage yield was computed based on the formula:

$$\% \text{ Yield} = \frac{\text{Weight of Semi} - \text{purified Flavonoids}}{\text{Weight of dried sample}} \text{ x } 100$$

Physical Evaluation

The semi-purified flavonoid extract was subjected to organoleptic and solubility tests. The odor, color, and appearance of the extract were noted for the organoleptic test. The solubility of the semi-purified flavonoids was determined by adding 0.1g of the extract to each test tube containing water, 80% ethanol, chloroform, and acetone respectively.

Confirmatory Tests for the presence of Flavonoids

a. *Bate – Smith and Metcalf Test*
   0.5mL of concentrated HCl was added to the sample and warmed on a water bath for 15 minutes and was observed for an hour. The appearance of strong red or violet color indicates a positive result.

b. *Shinoda's Test*
   Three pieces of magnesium turnings were added to the sample followed by few drops of concentrated HCl. A pink, orange, or red to purple coloration indicates the presence of flavonoids.

c. *Sodium Hydroxide Test*
   2 ml of the 10% aqueous sodium hydroxide was added to produce a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid is an indication for the presence of flavonoids.

d. *Ethyl Acetate Test*
   10mL ethyl acetate was added and subjected over steam bath for 3 minutes. Using 4mL of the filtrate, 1mL diluted ammonia solution was added and shaken. Yellow coloration of the solution will indicate the presence of flavonoids.

e. *Ferric Chloride Test*
   Few drops of 10% ferric chloride solution were added. A green-blue or violet coloration will indicate the presence of a phenolic hydroxyl group.

f. *Lead Acetate Test*
   1.2 mL of lead acetate was added and shaken. The formation of flesh-brown colored precipitate indicates the presence of flavonoids.

Determination of the Antibacterial activity of the semi-purified flavonoids

The Disc diffusion method using Mueller-Hinton Agar was utilized in this test. Being the most common hand-transmitted bacteria, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Enterobacter aerogenes, Proteus vulgaris, Salmonella typimurium, Klebsiella pneumoniae, Bacillus subtilis,* and *Bacillus cereus* were utilized. The zones of inhibition were measured and the following numerical scale was used to interpret the result:

$\geq$20 mm - Susceptible,
<20 mm - Resistant.

In order to identify the significant concentration of the semi-purified flavonoid, the t-test analysis was utilized.

Formulation of the Hand Sanitizer Gel

The hand sanitizer gel was formulated as follows:

Semi-Purified Flavonoids 40.00%

Methylcellulose	1.00%
Carbomer 934	0.35%
1N KOH	q.s. pH 7
Propylene Glycol	16.70%
Methylparaben	0.015%
Distilled water	q.s.100.00%
Rose Oil	5 drops

Comparison of the antibacterial activity of the formulated Hand Sanitizer Gel against the locally available hand sanitizer brands

The locally available hand sanitizer gels were labelled as Brand A and Brand B. The disc diffusion method was utilized in determining the zones of inhibition using the same sets of bacteria. The zones of inhibition were measured and the following numerical scale was used to interpret the result:

≥20 mm	Susceptible
<20 mm	Resistant

The t-test was also utilized in comparing the antibacterial activity of the formulated hand sanitizer gel against Brand A and Brand B.

Primary Skin Irritation Test

The mouse ear irritation test was used utilizing 5 female BALB/c mice that were acclimatized for 7 days. Each mouse was lightly anaesthetized with ether and 10µL of a liquid test sample was applied to the dorsal aspect of one ear; the sample was gently spread over the skin. The other ear served as an untreated control. Daily applications were made on four successive days; on the fifth day of the experiment, no application was made.

Results and Discussion

Percentage Yield

The collected residue weighed 1.48 grams out of 100 grams (1.48%) dried outer coverings of *Allium cepa* Linn. bulb.

Physical Evaluation Results

The semi-purified flavonoid extract was deep red in color, has sweet tamarind-like odor, and is gummy in appearance. The solubility of the extract is shown on Table 1.

Table 1: Solubility test results of the semi-purified flavonoids

Solvent	Result
Acetone	Very soluble
80% Ethanol	Freely soluble
Water	Sparingly soluble
Petroleum Ether	Insoluble
Chloroform	Insoluble

Confirmatory Tests Results

The extract showed positive result in all of the confirmatory tests done indicating the presence of flavonoids in the sample. Table 2 shows the summary of the rest results.

Table 2: Confirmatory test results for the presence of Flavonoids

Test	Theoretical Result	Actual Result	Interpretation
Bate – Smith and Metcalf Test for Leucoanthocyanins	Strong red or Violet	Strong Red	+ for Leucoanthocyanidins
Shinoda's Test	pink, orange, or red to purple coloration	Crimson / Red	+ for Flavonoids
Sodium Hydroxide Test	Color change form Yellow to Colorless	Colorless Solution	+ for flavonoids
Ethyl Acetate Test	Yellow coloration	Yellow coloration	+ for Flavonoids
Ferric Chloride Test	Green-blue or Violet coloration	Violet coloration	+ for Phenols
Lead Acetate Test	Yellow or Flesh brown precipitate	Flesh precipitate	+ for Flavonoids

Antibacterial Activity of the Semi-Purified Flavonoids

30 mg, 40 mg, and 50 mg semi-purified flavonoid extract was dissolved in 1 mL distilled water which is equivalent to a concentration of 30%, 40%, and 50% respectively. Table 3 shows the result of the microbial assay by measuring the zones of inhibition.

Table 3: Antimicrobial activity of the 30%, 40%, and 50% Semi – Purified Flavonoid Extract

Bacteria	Zones of Inhibition					
	30%	Interpretation	40%	Interpretation	50%	Interpretation
Staphylococcus aureus	0	Resistant	28.0	Susceptible	26.0	Susceptible
Escherichia coli	10.1	Resistant	25.5	Susceptible	30.5	Susceptible
Pseudomonas aeruginosa	0	Resistant	21.0	Susceptible	21.0	Susceptible
Micrococcus luteus	0	Resistant	55	Susceptible	55	Susceptible
Enterobacter aerogenes	20	Susceptible	55	Susceptible	58	Susceptible
Proteus vulgaris	13	Resistant	48	Susceptible	49	Susceptible
Salmonella typhi	17	Resistant	47	Susceptible	47	Susceptible
Klebsiella pneumoniae	20	Susceptible	48	Susceptible	50	Susceptible
Bacillus cereus	13	Resistant	41	Susceptible	42	Susceptible
Bacillus subtilis	0	Resistant	20	Susceptible	21	Susceptible

Table 4: T – Test Table for the Comparison of 30% & 40% and 40% & 50% Semi-purified Flavonoid Extract

	Sample Mean 1	Sample Mean 2	SD1	SD 2	No. of observations	Computed t-value	Significance
30% and 40%	9.31	37.95	8.58	15.36	10	5.15	Significant Difference
40% and 50%	39.95	37.95	15.36	14.11	10	0.30	No significant difference

Table 5: Microbial Tests Result of the Semi-purified Flavonoids Extracted from the Outer Coverings of the Red Creole variety of Allium cepa Bulb

Bacteria	Formulation		Brand A		Brand B	
	ZoI (mm)	Interpretation	ZoI (mm)	Interpretation	ZoI (mm)	Interpretation
Staphylococcus aureus	27	Susceptible	20.5	Susceptible	29	Susceptible
Escherichia coli	20	Susceptible	0	Resistant	0	Resistant
Pseudomonas aeruginosa	14	Resistant	0	Resistant	20	Susceptible
Micrococcus luteus	50	Susceptible	0	Resistant	26	Susceptible
Enterobacter aerogenes	48.5	Susceptible	0	Resistant	0	Resistant
Proteus vulgaris	42. 5	Susceptible	0	Resistant	13	Resistant
Salmonella typhi	42	Susceptible	0	Resistant	0	Resistant
Klebsiella pneumoniae	49 .5	Susceptible	0	Resistant	0	Resistant
Bacillus cereus	37.5	Susceptible	0	Resistant	0	Resistant
Bacillus subtilis	18.5	Resistant	0	Resistant	21	Susceptible

Table 6: T – Test Table for the Comparison of Brand A, Brand B, and the Formulation

	Sample Mean 1	Sample Mean 2	SD1	SD 2	No. of observations	Computed t-value	Significance
Brand A and Formulation	2.05	34.95	6.48	13.95	10	5.15	Significant Difference
Brand B and Formulation	10.85	34.95	13.87	12.16	10	4.13	Significant Difference

The result of the t-test in Table 4 shows that the antibacterial activity of 30% and 40% (5.15) concentration has significant difference while the 40% and 50% (0.30) has significant difference.

Result of the comparison of the Antibacterial activity of the Formulated Hand Sanitizer gel and the locally available hand sanitizers

The Formulation exhibits antibacterial activity (Table 5) against 8 of 10 bacteria used in the experiment, Brand A exhibits antibacterial activity against 1 of 10 bacteria and Brand B manifest an antibacterial activity against 4 out of bacteria utilized in the microbial assay.

The t-test result in Table 6 shows that the formulated hand sanitizer gel and brand A (5.15), and the formulated hand sanitizer gel and brand B (4.13) has significant difference.

Primary Skin Irritation Test Result

On the control ear, no sign of irritation or change in the appearance of the ear was observed. On the treated ear, few blood vessels were noted beginning on day 2. These results indicate that the formulated hand sanitizer gel is probably not perceptibly irritant to human skin, as observed in the treated mouse ear.

Conclusion

The formulated hand sanitizer gel with 40% concentration of semi-purified flavonoids from *Allium cepa* L. family Alliaceae has an antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Micrococcus luteus, Salmonella typhimurium, Enterobacter aerogenes, Klebsiella pneumoniae,* and *Proteus vulgaris*. The formulated hand sanitizer gel has stronger antibacterial activity against the two locally marketed hand sanitizer brands in the Philippines and the formulation is perceptibly not capable of causing irritation to the skin when applied topically.

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