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Erythema Inhibiting Potential of Banana (*Musa paradisciaca Linn.),* Guava (*Psidium guajava Linn.)* and Lima Bean (*Phaseolus lunatus)* Leaf Extracts on Acute Models of Inflammation

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The study was conducted to determine the erythema inhibiting potential of banana, guava and lima bean leaf extract on acute models of inflammation in male and female guinea pigs.A total of sixty three (63) 2-3 month old apparently healthy male and female guinea pigs, weighing 180-200g served as experimental animals of the study. Test animals were exposed to four treatment materials consisting of Treatment 1 (Saline), Treatment 2 (Banana), Treatment 3 (Guava) and Treatment 4 (Lima Bean) leaf extracts. Patch and scratch methods were used in evaluating the effects of the test materials on acute inflammatory conditions 24 hours and 72 hours post-treatment. All data gathered were statistically analyzed using ANOVA, LSD and Frequency distribution. Results revealed a varying erythema and primary irritation index formation using the Patch and Scratch Test Method 24 to 72 hours post-treatment. Lima bean provided the most recorded anti-erythema (8/9) agent indicated by a score of 0 (No erythema) using the Patch Test 72 hours post-treatment. Lima bean provided support to three erythema scores of slight to moderate to severe types and one edema score classified as moderate to severe. Banana and guava were also observed to have anti-erythema effect in 6/9 and 4/9 treated animals. Statistical analysis of the patch test however provided no significant differences between plant treatments as anti-erythema agent. Banana (8/9) 72 hours post-treatment provided the most effective anti-erythema using the Scratch Test Method. Banana, however, has limited efficiency on slight to moderate types of erythema. Anti-erythema effects of banana however were found to be comparably the same with the effects of guava while lima bean was comparably the same with the effect of saline. The computed primary irritation index that possibly accounted for the erythema formation were mostly recorded in saline (1.5) followed by lima bean (1.0), guava (0.67) and banana (0.61), all of which fall under the slightly irritating agent. Conclusively, the observed results indicate that most of the plant materials were effective for acute inflammatory conditions.

Keywords: Erythema, patch, scratch, acute inflammation

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Introduction

Erythema is a part of the visual changes indicative of injury and disease in the event of inflammation. Thus, the sensation of heat is caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities, also resulting on the increased redness (due to the additional number of erythrocytes passing through the area). (Punchard *et al.*, 2004; Cheville 1999; Guyton and Hall, 2006 and Tizzard 2004).

With the availability of synthetically prepared anti-inflammatory drugs most of these first few visual tissue changes were properly alleviated. The conventional drug available in the market however, produces various side-effects hence, newer drugs with less or no side-effects are continuously being searched. Developments of potent anti-inflammatory drugs from the natural products are now under consideration. Natural products are rich source for discovery of new drugs because of their chemical diversity. A natural product from plants plays a major role to cure many of these diseases associated with inflammation. Plants are good source of a wide variety of compounds, such as phenolic compounds, terpenoids, and nitrogen containing compounds, vitamins, and secondary metabolites which have antioxidant, antimicrobial, anti-inflammatory, antitumor, antimutagenic, anti carcinogenic and diuretic activities. The use of these plants had gained popularity internationally, nationally and locally. According to WHO, 70% to 80% of the population in many developed countries have used some form of alternative or complementary medicine which are traditionally plant products. There are hundreds of phytoconstituents reported to have many pharmacological activities although most of these reports are of academic interest and very few find entry in clinical trials (Kumar *et al.*, 2013).

Recently, three top plant materials specific for skin disease were identified in the municipality of Echague, Isabela. These include banana, guava and lima bean all of which were proven safe by the people in alleviating skin conditions, yet their extent on the use for acute inflammation was not given emphasis (Guzman, 2015).

It was therefore the purpose of this study to investigate the erythema inhibiting and protective effect of banana, guava and lima bean leaf extract on induced acute inflammatory models.

Methodology

Authentication, Collection and Preparation of Plant Materials

All plants that were used in the study were taxonomically identified and authenticated at the Botany Division Office, Philippine National

Museum, Padre Burgos Drive, Manila City, 1000, Metro Manila. Fresh mature leaves of guava, banana and lima bean were collected at the Municipality of Echague, Isabela. Two hundred grams (200g) of the fresh plant material were finely cut and were submerged in 300 ml of 95% alcohol, stoppered for 24 hours and filtered and the collected filtrates were distilled through distillation process, the collected distillate were the final products used for the analysis.

Management of the Test Animals

A total of sixty three (63) 2-3 month old healthy male and female guinea pigs, weighing 180-200g served as the experimental animals of the study.

Experimental animals were acclimatized for 8 days before the start of the study and maintained in laboratory environment, fed with standard crow pellet diet with water given free access to animal.

At the start of the experiment, all experimental animals were weighed and kept in an individual observation cages. All animals were fasted from food and water 10 hours before each test.

Experiment Proper

Patch Test. The skin lateral to the spinal groove were shaved and cleaned. The left sides of the groove were utilized as the negative control site and the right side as the test drug site. The sites were cleaned with 70% alcohol to make a patch incision (approximately 1 cm in diameter) using scalpel blade. The test drug and negative control on the inoculation sites were given drops appropriately. Sites were covered with sterilized gauze (1x1 cm in size) kept in place by the use of surgical tape. The test animal was left for 24-72 hours during the time from which they rendered immobile.

Patches were removed after 24 hours exposure and reactions were evaluated according to the scores in Table 1. Another reading or scoring was done after 72 hours. Average scores of the 24 and 72 readings were computed.

Scratch Test. The procedure and scoring method was the same as in patch test but with a light modification.

The skin of the test animal were abraded by lightly scratching the skin five to seven times with a gauge of hypodermic needle, followed by application of the test drug and the saline solution (negative control).

Test Scoring

The scoring was done through the following parameters:

Table 1. Evaluation of Skin Reaction through Erythema and Eschar Formation.

Erythema formation	**Score**
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (best redness) to slight eschar formation (infinity in depth)	4
Total Erythema Score	4

The averages of the scores for patch and scratch test was combined. This combined average is referred to as primary irritation index.

Table 2. Primary Irritation Index (Classification System).

PII	Classification
<0.5	Non-irritating
0.5 – 2.0	Slight irritating
2.1 – 5.0	Moderate irritating
> 5.0	Severe irritating

Analysis of Data

All data collected were subjected to ANOVA and subsequently to LSD (Least Significant Differences) for the determination of the differences between means. Frequency distribution of each scores was also determine for each test.

Experimental Design and Experimental Layout

The study utilized a Complete Randomized Design (CRD) for layout and distribution of the experimental animals. Each treatment consisted of 9 animals. Each of treatment animals were distributed in four treatments consists of 3 replicates, with 3 animals each replicate. All test animals were distributed randomly in the following treatment:

Patch and Scratch test
Treatment 1 – Saline (Control)
Treatment 2 – Banana
Treatment 3 – Guava
Treatment 4 – Lima Bean

Results and Discussion

The anti-inflammatory effect of banana, guava and lima bean were evaluated using the Patch, Scratch Test. Patch and Scratch results were based on observed erythema and primary irritation index 24 to 72 hours post-treatment.

Erythema Formation Using the Patch Method

Table 3 below provides the evaluated skin reactions after 24 and 72 hours following application of the three plant extracts. A score of 1 (very slight erythema/ barely perceptible) is the most reading obtained in banana (4/9) and guava (5/9) and the control saline (5/9) after 24 hours. Erythematous condition subsided to score of 0 (no erythema) after 72 hours in banana (6/9) while only five of the slight erythema recorded for guava (5/9) subsided to score of 0 (no erythema). Majority of the lima bean (5/9) treated animals however, obtained a score of 2 (well-defined erythema) 24 hours post-treatment. Yet, erythematous condition was completely eliminated as indicated by score of 0 (no erythema) in 8 out of the 9 treated animals after 72 hours. The control saline provided the same scores of 1 and 2 erythematous conditions after 72 hours.

Although results obtained a varying erythema formation 24 hours post exposure to the treatment materials, results revealed an effective result for lima bean due to its ability to cause (8/9) reduction in erythema condition of the test animals 72 hours after. That even with the recorded degree of erythema formation scores of 2 (well-defined erythema), 1 (very slight erythema) and 3 (moderate to severe erythema), still the majority of the treated animals recovered after 72 hours. Banana (6/9) and guava (4/9) also provided a 0 score (no erythema) but at lesser number of recorded animals after 72 hours.

Table 3. Formation of Erythematous Lesions 24 and 72 Hours Post-treatment of Saline, Banana, Guava and Lima Bean Leaf Extracts Using Patch Test Method

Treatment	Hours of observation	No. of experimental animals	Scores of erythema formation(freq.)					Total erythema Score Types	Ave. scores after 24 & 72 hours	Erythema score Indication
			0 (%)	1 (%)	2 (%)	3 (%)	4 (%)			
Treatment 1	24	9	0(0)	5(55)	1(11)	2(22)	1(11)	4(44)		
Saline	72	9	0(0)	5(55)	4(44)	0(0)	0(0)	2(22)	1.67a	Well- defined
Treatment 2	24	9	2(22)	4(44)	2(22)	1(11)	0(0)	3(33)		
Banana	72	9	6(66)	3(33)	0(0)	0(0)	0(0)	1(11)	0.78b	Barely perceptible
Treatment 3	24	9	2(22)	5(55)	2(22)	0(0)	0(0)	2(22)		
Guava	72	9	4(44)	4(44)	1(11)	0(0)	0(0)	2(22)	0.83b	Barely perceptible
Treatment 4	24	9	0(0)	3(33)	5(55)	1(11)	0(0)	3(33)		
Lima bean	72	9	8(88)	1(11)	0(0)	0(0)	0(0)	1(11)	0.95b	Barely perceptible

Note; Score 0=No erythema; 1=Very slight erythema; 2=Well defined erythema; 3=Moderate to severe erythema; 4=Severe erythema

Means with the same letter are not significantly different;

**significant at 1% level

Regardless of the detailed scores obtained, total erythema provided three types of erythema score of 1 (very slight erythema), 2 (well defined erythema) and 3 (moderate to severe erythema) for banana and lima bean and a total of two erythematous condition of score of 1 (very slight erythema) and 2 (well defined erythema) after 24 hours while control group provided all types of erythema with scores of 1 to 4 after 24 hours. A total of erythema score type reduction was identified for banana and lima bean having a previous score of 3 and 4 both turned to a score type of slight erythema while guava treated animals obtained the same number of erythema types falling under scores of 1 and 2 after 72 hours. Such result may possibly indicate that lima bean and banana have the same effect in reducing the three score types of erythema.

Results of the average scores after 24 and 72 hours revealed a lowest erythema score in banana (0.78) followed by guava (0.83), lima bean (0.95) and saline (1.67). All score results however fall under the slightly irritating agent. This may be due to the astringent and anti-microbial property of the three plants (Agarwal *et al.,* 2013; Sanda *et al.,* 2011; Seidu *et al.,* 2014).

Statistical analysis revealed that the three plant materials used are not significantly different with each other. Meaning, the three plant materials have similar erythema effect. The three plant materials however are significantly different with the control saline an indication that the three plant materials respond differently with the effect of saline.

Erythema Formation Using the Scratch Method

Table 4 provides the evaluated skin reactions using the scratch method after 24 and 72 hours. A score of 1 (very slight erythema) was obtained for guava (6/9), banana (5/9) and lima bean (5/9) while a score of 2 (well-defined erythema) was obtained from saline after 24 hours. Eight out of 9 (8/9) and 6/9 of the banana treated animals were completely eliminated of the erythematous condition indicated by a score of 0 (no erythema) after 72 hours. Majority (5/9) of the lima bean treated animals obtained a score of 1 (very slight erythema) while few (4/9) of the remaining animals obtained a score of 0 (no erythema).The score of 0 (no erythema) for banana and guava clearly indicates that the two test materials are effective in eliminating erythematous conditions.

Table 4. Formation of Erythematous Lesions 24 and 72 Hours Post-treatment of saline, Banana, Guava and Lima Bean Leaf Extracts Using Scratch Test Method

Treatment	Hours of observation	No. of experimental animals	Scores of erythema formation(frequency)					Total erythema Score Types	Ave. scores after 24 & 72 hours	Erythema score Indication
			0 (%)	1 (%)	2 (%)	3 (%)	4 (%)			
Treatment 1 Saline	24	9	1 (11)	1 (11)	6 (66)	1 (11)	0 (0)	3		
	72	9	3 (33)	4 (44)	2 (22)	0 (0)	0 (0)	2	1.33a	Well-defined
Treatment 2 Banana	24	9	3 (33)	5 (55)	1 (11)	0 (0)	0 (0)	2		
	72	9	8 (88)	1 (11)	0 (0)	0 (0)	0 (0)	1	0.44b	No erythema
Treatment 3 Guava	24	9	3 (33)	6 (66)	0 (0)	0 (0)	0 (0)	1		
	72	9	6 (66)	3 (33)	0 (0)	0 (0)	0 (0)	1	0.50b	No erythema
Treatment 4 Lima bean	24	9	4 (44)	5 (55)	5 (55)	0 (0)	0 (0)	2		
	72	9	4 (44)	5 (55)	0 (0)	0 (0)	0 (0)	1	1.06a	Barely perceptible

Note; Score 0=No erythema; 1=Very slight erythema; 2=Well defined erythema; 3=Moderate to severe erythema; 4=Severe erythema
Means with the same letter are not significantly different; **significant at 1% level

Regardless of the number of animals treated, total erythema score revealed that guava and lima bean produced the same number of erythema type with a score of 1 (very slight erythema). Banana provided 2 types of erythematous condition after 24 hours but reduced to a score of 1 (very slight erythema) after 72 hours. The control saline provided 3 types of erythema after 24 hours and reduced to 2 types of erythema after 72 hours. Results indicate that guava and lima bean provides the same effect in reducing erythema condition after 24 to 72 hours.

The average erythema score after 24 to 72 hours provided a lowest score of 0.40 for banana, followed by guava (0.50), lima bean (1.06) and saline (1.33). Values of lima bean and saline were barely perceptible while banana and guava have no erythema formation.

Statistical analysis however, provided no significant differences in banana and guava, an indication that the two materials have the same performance as anti-erythema agent while lima bean has the same effect with the control saline. Banana and guava are significantly lower than lima bean and saline, an indication that the two groups of materials vary in their effect.

Table 5. Calculated Erythema Primary Irritation of Index in Saline, Banana, Guava and Lima Bean Treated Animals 24 and 72 Hours Post-treatment.

Treatment	Method	Hours		PII	Indication
		24	**72**		
Treatment 1 Saline	Patch Test	17	13		
	Scratch Test	16	8		
				1.50a	Slightly irritating
Treatment 2 Banana	Patch Test	11	3		
	Scratch Test	7	1		
				0.61c	Slightly irritating
Treatment 3 Guava	Patch Test	8	6		
	Scratch Test	6	3		
				0.63bc	Slightly irritating
Treatment 4 Lima Bean	Patch Test	16	1		
	Scratch Test	14	5		
				1.00b	Slightly irritating

Note; PII indication <0.5=Non irritating; 0.5-2.0=Slight irritating; 2.1-5.0=Moderate irritating; >5.0=Severe irritating
Means with the same letter are not significantly different.
**significant at 1% level

Primary irritation index for erythema formation provides the irritant effects of the test materials. The scores and reading evaluated for the two tests for each plant were combined. The following averages were computed at decreasing order: 1.5 saline, 1.0 lima bean, 0.63 guava and 0.61 banana. All of the said results however fall under the slightly irritating agent. Statistical analysis revealed that the effect of banana is not significantly different with the effect of guava. Such result is an indication that the mode of action of banana as an irritant is comparable with the effect of guava while guava and lima bean are not significantly different also indicating no differences in the effect of the two treatments.

In general, majority of the different test materials were found effective only to erythema scores of 1 to 3 indicated by slight erythema to moderate to severe erythema 72 hours post-treatment. Such indicates that

the activity of the three plant materials was found to be limited on the moderate to severe cases of erythema.

Conclusion

Conclusively, lima bean is the most effective anti-erythema agent in hygienically protected wound types of slight to moderate to severe as indicated in the Patch Test while most effective anti-erythema for the scratch test method was identified in banana and guava respectively. Observed results indicate that most of the plant materials are effective for acute inflammatory conditions.

As per findings of the study and from the conclusions made, the following are recommended:

1. The plant materials were found to have specific beneficial effects on acute inflammatory conditions, hence, could be adapted or used in the absence of a standard veterinary drug.
2. Scoring made in the study is subjective that a more accurate and measurable procedure evaluating erythema formation is recommended.
3. To strengthen conclusive results of this study, it is suggested that further studies be conducted with the inclusion of a positive control for comparison.

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