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Determination of the Anti-inflammatory Property of Tannins from the Rind of Calamansi (*Citrus microcarpa*, Rutaceae)

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Abstract:

Background: The research involved the screening of tannins and the determination of the anti-inflammatory activity of tannins from the rind of calamansi (*Citrus microcarpa*, Rutaceae). The problem dealt mainly with the determination of anti-inflammatory activity of tannins from calamansi rind.

Materials and Methods: The investigation started with the collection, drying and grinding of the rind. The rind was macerated in 95% ethyl alcohol. The filtrate was used in the screening and preliminary testing for tannins performed, and the remaining plant sample was macerated with distilled water and was used in the extraction and testing of tannins. The presence of tannins was determined by the following test: Ferric chloride test, gelatin test, bromine water test, lime water test, and lead acetate test. Physical, chemical, and biological test for the anti-inflammatory activity using rats as animal test was done. The biological test was performed through the plant extract by carrageenan-induced edema formation using albino rats. The samples that were administered to the rats were 250, 500 and 1000 mg/kg, respectively. The percent protection should not be <20% of anti-inflammatory activity compared to the positive control that was aspirin 300 mg.

Results: Gelatin test showed the formation of precipitate. Ferric chloride test resulted in the formation of brownish green color. The yellow color solution was obtained in lead acetate test. Bromine water test resulted in formation of a light brown precipitation with orange precipitation. Lime water test indicated slight brown precipitation. Organoleptic test for tannins showed a sticky, brownish, semi-solid with coffee – like odor substance. Tannin extracts were insoluble in acetone, chloroform, and ether. Nearly, 95% of alcohol has slight solubility and water was soluble of tannins. Biological testing results of anti-inflammatory activity with the samples that were administered to the rats in 250, 500, and 1000 mg/kg showed 51.93%, 52.72%, and 65.70% of anti-inflammatory protection, respectively. Aspirin tablets of 300 mg/kg showed 90.93% of anti-inflammatory protection.

Conclusion: We found that the rind of calamansi plant contains tannins, and these tannins can be extracted using maceration and with water as solvent. We also concluded that anti-inflammatory property of tannins from calamansi rind is effective in increasing doses of 250, 500 and 1000 mg/kg.

Key Words: Anti-inflammatory, aspirin, calamansi, rind, tannin

Introduction

Many people have been reviving their interest in finding ways on how to prolong lives with medicines using the natural ways. This is may be due to the depressing and unpredictable condition of the country wherein things like medicines are somehow being sold like gold. And so as to their interest and practically arise, they have been using and improving the old ways of treating or somehow preventing the spread of diseases through the use of what we call medicinal or herbal plants.

Plants are the most abundant resources found in different places. The plant produces a great number of compounds of various chemical structures. That is why it can be considered as the most pro-life laboratories in the world. Every part of it has been useful and has been discovered to contain constituents that can brief relief or cure to any kinds of diseases. From its roots to its stem lies a mysterious healing power.

With regards to its importance and popularity, their ability to possess synthetic compounds is indeed very important in the manufacture of medicines. Thousands and millions of them have been identified and classified. However, in spite their overwhelming popularity and long history, we know relatively little about the safety and effectiveness of these herbal remedies. A Scientific study should make these remedies far safer and more effective in the future. Global recognition of nature's green pharmacy should inspire individuals and nations to protect this extraordinary resource.

Citrus is categorized in the family of Rutaceae. It has Asian roots and are cultured all through the hotter parts of the world.[1] These plants are vast in number especially scattered all over the East Asia, especially Philippines and Malaysia. These plants have multiple uses that are sometimes unknown to man. These plants have been made popular for it can be a great prospect for evolution. Commonly these are fragrant due to the presence of glands producing essential oils in the leaves, flowers, and fruits. These oils are used to

flavor drinks, in perfumes or utilized for confectionaries. These oils are additionally used as a part of the creation of organic chemicals.[2] It also utilized for culinary, ceremonial, and medication purposes among East Asians. They said to have snake deterrent quality and have cleaning properties as shampoo among Malay and Malenasians. This paper is based on one of the 3 members of the *Citrus* genus: *Citrus hystrix* (DC), *Citrus microcarpa* (Bunge), and *Citrus aurantifolia* (Swingle) (also named *Citrus acida*, Roxb.).[1]

C. hystrix, locally known as "*limau purut*," is a small tree, with a pear-shaped fruit, the skin intensely green, or ultimately upon ripening yellowish and wrinkled. Before, the fruit is ripe the juice is gummy, but with ripeness becomes thin and watery, though never abundant.[1] Blossems are little, white, and have sweet lemony smell. The leaves look shiny and oily ranging from 7.5 to 10 cm long. A light yellowish green liquid represents the essential oil from the leaves.

Commonly *C. microcarpa* is used as a juice drink, or they are preserved with sugar or salt as dried natural fruits. This plant can be kept indoor or outdoor. They can reach up to 3-4 m. Their color turns yellow or orange when ripe. The size of this fruits are different compared to other *Citrus* and are very smaller. They are smooth, shiny, thin skin, sour with roundish appearance (about 2.5-3.7 cm across).[3] *C. microcarpa* has a peculiar musky fragrance which gives it its name.

C. aurantifolia is an organic product used as a fruit juice and utilized in foods and garnishing. Leaves are customarily used for poulticing, both against evil spirits and for skin complaints. In the early years, it is a thorny shrub. It can reach up to about 5 m tall.[4] The fruits are round, ranging from 2.5 to 5.0 cm in diameter, very juicy and sour. The thickness of its skin is about 0.3-1.2 mm, but shiny and smooth.[3] *C. aurantifolia* is truly common and numerous varieties are available and can be seen all through the region.

In the study, the crude extracts from the plants were obtained by first collecting the fresh plant samples and then followed by maceration and then extraction method of the crude extracts using 80% ethyl alcohol. After obtaining the necessary amount of the samples, it was tested for its physical, phytochemica, instrumental, pharmacologist, and biological properties (Figure 1).

Materials and Methods

The 100 g of finely powdered leaves from fresh, washed, air-dried, and ground calamansi were macerated in an Erlemeyer flask with sufficient amount of 80% ethyl alcohol for 24-48 h. They were then filtered using a muslin cloth to obtain its extracts. Two trials were made in the crude extracts. To screen tannins, an equivalent of 10 g of plant extract was evaporated to incipient dryness over a water-bath and was cooled. The residue was extracted using 20 ml of hot distilled water and

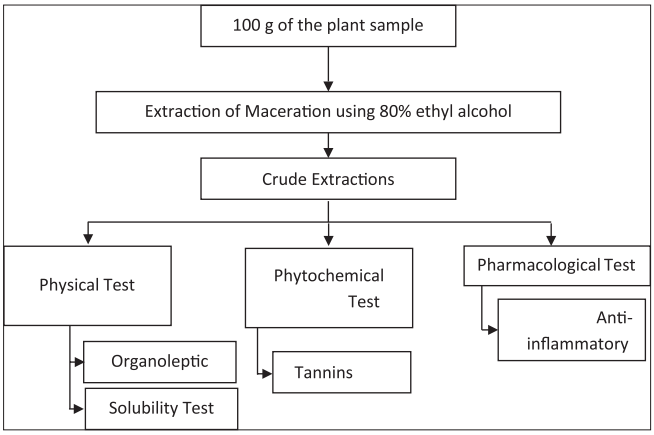

Figure 1: Paradigm of the study of calamansi leaves.

added with five drops of 10% sodium chloride solutions to precipitate the undesirable constituents. It was filtered and divided into three test tubes labeled as A, B, and C where test tube A served as a standard. For gelatin test, three drops of gelatin salt reagents were added to Tube B and then observed and compared with the standard. For ferric chloride test, three drops of ferric chloride solutions were added Tube C and was observed for any color changed and then was compared to the standard.

For pharmacological screening of the crude extracts of calamansi leaves, determination of the anti-inflammatory property was done. 10 male rats between 22 and 26 g which were kept in light or dark cycle with the temperature at 22°C, food and water *ad libitum* were used and made to fast for 18 h before drug administration. The rats were randomized, weighed, numbered, and grouped into positive control, negative control, and test groups (five groups and two mice per group):

- Negative control (normal saline solution [NSS])
- Positive control (aspirin 300 mg)
- Test Group I – 250 mg/kg (crude extract)
- Test Group II – 500 mg/kg (crude extract)
- Test Group III – 1000 mg/kg (crude extract)

After measuring the left hind paw of each rat using the plethysmometer, different volumes of different concentration of drug and volume of NSS to be administered to each rat was first computed using the following formulas:

Volume to be administered = Weight of the rat (kg) × 25°

The first group was negative control, which was given NSS. The second group of rats was used for positive control, which was given a dose of 300 mg of aspirin. The sample drug was administered to the corresponding rats with increasing doses of 250, 500 and 1000 mg/kg in test groups, respectively. After an hour, 0.05 ml of 1% carrageenan was injected into the plantar surface of its left hind paw. Exactly after 3 h, the left hind paw displacement of each rat was measured using the

plethysmometer. The percent inhibition was computed for each treated rats using the formula:

$$\% \text{ inhibition} = 1 - \frac{(\text{Test difference}) * 100}{\text{Negative control difference}}$$

OR

$$\% \text{ inhibition} = 1 \frac{\left(\text{Average difference}\right) * 100}{\text{Average of negative control}}$$

After through experimentations to prove the different pharmacological and microbiological properties of the plant sample, the following formulas were used:

$$\% = \frac{n}{N} \times 100$$

$$\%I = 1 - \frac{\text{Test difference}}{\text{Negative control difference}}$$

Where, *n* = Number of sample, N = Total number of sample used, I = Inhibition.

The percentage was used to determine the part of the sample on the basis of a whole divided into one hundred parts. It referred to the probability of the plant sample to be effective like that of the positive control.

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Where, t = t-test, X_1 = First mean, X_2 = Second mean, S_1 = First obtained standard deviation, S_2 = Second obtained standard deviation, N_1 = Number of samples in the first group, N_2 = Number of samples in the second group.

T-test was used to determine if the mean differences of the two samples were significant or not. The standard deviation was used to know and to determine the different variation of the samples. Degree of freedom was used to determine the tabular value for the test of the sample. The level of significance was used to denote that the researcher was giving 5% of error in their decision and further implied that they were 95% of confident of their decision to be right.

Results

Preliminary test for tannins

The presence of tannins was determined by the following test: Gelatin test and ferric chloride test. The expected result was blue or black that indicated the presence of hydrolyzed tannins, brownish color for the presence of condensed tannins. The actual results were presented in Table 1.

Interpretation

Formation of precipitate in the gelatin test indicated the presence of tannins. Moreover, formation of brownish green color indicated the presence of condensed tannins.

Extraction and partial purification of tannins

Extraction of tannin extract

About 50 g of the powdered dried sample were placed in a glass jar, it was added with sufficient amount of distilled water to cover the sample, and it was macerated for 3 days. It was then filtered and was transferred to an Erlenmeyer flask. The marc was discarded.

About 15 ml of ether was added to the filtrate. The solution was set aside until it separated into two layers. The upper layer contained the ethereal layer, which was the free acid while the aqueous layer at the bottom contained the tannin extract. A separatory funnel was used to separate the solution. The solution that contained the tannin was evaporated to dryness; the ethereal layer was discarded.

The tannin layer was placed in an evaporating dish, accurately weighed then covered with aluminum foil. The percentage yield of tannin control was computed as follows:

Percentage yield of tannin extract data:

Weight of evaporating dish = 39.58 g

Weight of evaporating dish + plant sample = 107.525 g

Weight of the residue = 67.945 g

Weight of plant sample = 1,033 g

Computation:

$$\text{Percentage yield} = \frac{\text{Weight of residue}}{\text{Weight of plant sample}} \times 100$$

$$\text{Percentage yield} = \frac{67.945 \text{ g}}{1{,}033 \text{ g}} \times 100$$

Percentage yield = 6.58%

Physical and chemical properties manifested by tannin extractives

Interpretation

The actual results of the test performed for the organoleptic test as shown in Table 2, indicated that it does not crystallize. Therefore, there was the presence of tannins because it was a non-crystalline substance.

Interpretation
The actual result of the test performed for solubility of tannins as shown in Table 3, indicated that the suitable or best solvent was water.

Results obtained from the chemical test of tannins
To confirm the presence of tannins in the plant sample, five tests were conducted: Ferric chloride test, gelatin test, bromine water test, lime water test, and lead acetate test.

Interpretation
The actual results of the test performed for the chemical test of tannins as shown in Table 4, indicated the presence of tannins. Therefore, tannins were present in the plant sample.

Determination of anti-inflammatory property of tannin form calamansi rind
Interpretation
The results of the test performed for the anti-inflammatory activity of the tannin extract shown in Table 5, indicates that tannin extracts are effective as an anti-inflammatory agent.

Discussion

Citrus mitis which is commonly known as *Kalamansi* is widely cultivated in the East Asia.[5] calamansi, also called Philippines lime procedures fruit all year around and breads true from seeds. This prolific species is so stable that even when propagated by seeds no other from may be reasonably isolated distinct from the common variety. The plants are also propagated by building to hasten the period of flowering and fruiting.[6] This plant is a smooth somewhat spiny tree growing from 3 to 5 m tall. The elliptical leaflets are 4-8 cm long. The skin of *Calamansi* is green to yellowish green or yellow. The peel adheres loosely to the fresh which has couple of seeds and is light orange in color. It is fairly sour and are utilized as a seasoning agent and for making "ade." *Marmalade* is made using the whole fruit. Concentrated juice is also popular. The fruit is crushed and used for cleansing the hairs.[5] Quisumbing[5] recorded the following constituents: Leaves:volatile oil; 0.90-1.06% rind: Aldehydes sesquiterpense β-pinene linalooi linely1 acetate tannin glycosides and cyanogenetic substances. He reported that the juice of the fruit is used in removing ink stains from garments and for washing the hair of ladies and can be utilized for blanching spots and as an accessible medication for itching.

He recorded the following medicinal uses of calamansi juice: As a febrifuge (has a rich source of vitamin C), as a medication for coughs and for itches, as a deodorant, and as an antiphlogistic. The oil from the calamansi leaves produces stronger carminative than the peppermint oil in small amounts even <1 cc. It is also used for a sore throat, refreshing drink for fever, nausea, and fainting.[5] Tannis are complex substances which occur in almost all plants. They are largely found in dried tissues or deposited as the end product of metabolism. It is also present in some stages of growth and finally destroyed. This is true in immature fruits and disappears upon ripening. They are often associated with simple sugar, and some of them may be glycosidic in character where when hydrolyzed decomposed to polyhydric phenol where their action is due.

Tannins are complex single chemicals possessing a phenolic structure capable of combining with proteins: Cinchotannic acid, gallotanniic acid rosattanic acid, and kramerotannic acid. They are an amorphous polyhtdroxy phenolic compound with molecular weight in the range of about 1000-5000 which possesses an astringent taste and ability to combine with hide to form leather. They are distributed in plants and occur in solution in cell sap often in distinct vacuoles. They are usually found in great quantities in dead or drying cells. Although some tannins may appear to be glycosidic in nature the majority probably is not. When hydrolyzed they yield relatively simple polyphenols: (a) Gallic acid, which is broken down to

Table 1: Results for preliminary test for tannins.

Test performed	Expected results	Actual results	Remarks
Gelatin test	Formation of precipitate	Formation of precipitate	Presence of tannins
Ferric chloride test	Formation of blue-black color or brownish green	Formation of brownish green color	Presence of condensed tannins

Table 2: Results for organoleptic test for tannins.

Criteria	Results
Color	Brown
Appearance	Sticky mass
Physical state	Semi solid
Odor	Coffee-like odor

Table 3: Results for solubility test of tannins.

Solubility	Expected results	Actual results
Water	Soluble	Soluble
95% alcohol	Slightly soluble	Slightly soluble
Ether	Insoluble	Insoluble
Chloroform	Insoluble	Insoluble
Acetone	Insoluble	Insoluble

Table 4: Result of chemical test of tannins.

Test	Expected results	Actual results	Remarks
Gelatin test	Formation of precipitate	Formation of precipitate	Presence of tannins
Ferric chloride	Blue-black or brownish precipitate	Brownish precipitate	Presence of condensed tannins
Lead acetate test	Yellow brown color	Yellow color solution	Presence of tannins
Bromine water test	Formation of brownish orange precipitate	Formation of a light brown precipitation with orange precipitation	Presence of tannins
Lime water test	Brown precipitation	Slight brown precipitation	Presence of tannins

Table 5: Biological testing results of anti-inflammatory activity.

Rat no.	Weight of rat (kg)	Drug	Dose (mg/kg)	Total dose (mg)	Volume NSS	Initial foot volume (ml)		Average of initial foot volume	Final foot volume (ml)		Average of final foot volume	Difference of foot volume	Average final and initial foot volume	% Protection	Remark
						T1	T2		T1	T2					
1	0.1923	NSS	-	-	4.8075	8.18	8.05	8.115	5.89	5.61	5.75	2.37	2.205	0	
2	0.1943	NSS	-	-	4.8575	6.73	6.44	6.585	4.40	4.68	4.54	2.045			
3	0.2198	Aspirin	300	0.2198	5.495	5.17	5.08	5.125	4.74	4.94	4.84	0.285	0.2	90.93	
4	0.2061	Aspirin	300	0.2061	5.15	6.03	7.23	6.63	6.89	6.14	6.515	0.115			
5	0.1952	Tannin extract	250	0.16	4.88	5.31	5.23	5.27	2.24	3.59	3.915	1.355	1.06	51.93	Positive
6	0.2139	Tannin extract	250	0.178	5.35	4.6	4.91	4.755	4.14	3.84	3.99	0.765			
7	0.1928	Tannin extract	500	0.32	4.82	5.31	5.16	5.23	4.89	3.99	4.44	0.795	0.755	65.70	Positive
8	0.1977	Tannin extract	500	0.33	4.94	4.90	5.12	5.01	4.67	3.92	4.295	0.715			
9	0.2427	Tannin extract	1000	0.809	6.07	5.8	4.38	5.09	3.86	3.60	3.73	1.36	1.0425	52.72	Positive
10	0.2052	Tannin extract	1000	0.684	5.13	4.7	5.36	5.13	3.74	4.87	4.305	0.725			

NSS: Normal saline solution

pyrogalol, (b) protocatechuic acid, which is broken down to catechol, and (c) ellagic acid and other phenols. The phenolic groups of tannins are responsible for their astringent and antiseptic as well as their coloration with iron salts.[7]

According to Mosby's pocket dictionary of medicine and allied health[8] inflammation the protective response of body tissues to irritation or injury. Inflammation may be acute or chronic; its cardinal signs are redness (rubor), heat (calor), swelling (tumor), and pain (dolor), often accompanied by loss of function. *Histamine, Kinnins,* and various other substances, mediate the inflammatory process.

The inflammatory response is a nonspecific response that is triggered whenever body tissues are injured. The inflammatory process begins with a chemical "alarm." When cells are injured they release inflammatory chemicals including *Histamine* and *Kinnins* and that (1) Causes blood vessels in the involved area to dilate and capillaries to become leakly, (2) activate pain receptors, and (3) attract phagocytes and white blood cells to the area (This phenomenon is called chemotaxis because the cells are following a chemical gradient). Dilation of the blood vessels increases the blood flow to the area accounting for the redness and heat observed. Increased permeability of the capillaries allows plasma to leak from the bloodstream into the issue spaces causing local edema (swelling) that also activates pain receptors in the area. If the swollen painful area is a joint its function (movement) may be impaired temporarily. This forces the injured part to rest which aids healing. Some authorities consider the limitation of joint movement to be an additional (fifth) cardinal signs of inflammation.

The inflammatory response: (1) Prevents the spread of damaging agents to nearby tissues, (2) disposes cell debris and pathogens and (3) sets of the stage for repair. Within an hour or so after the inflammatory process has begun neutrophils are squeezing through the capillary walls to enter the area and begin the clean-up detail by engulfing damaged or dead tissue cells and or pathogens. As the counter attack continues monocytes begin to leave the bloodstream and follow the neutrophils into the inflamed area. Monocytes are fairly poor phagocytes but within 8-12 h after entering the tissues, they become macrophages with insatiable appetites. The macrophages continue to wage the battle replacing the short-lived neutrophils on the battlefield. Macrophages are the central factors in the final disposal of cell debris as the inflammation subsides.

Besides phagocytosis, other protective events are also occurring at the inflamed site. Clotting proteins, leaked into area from the blood, are activated and begin to wall off the damaged area with fibrin to prevent the spread of pathogens or harmful agents to neighboring tissues. The fibrin mesh also forms scaffolding for permanent repair processes.

If the area contains pathogens that have previously invaded the body, the third line of defense also comes into play the immune response mediated by lymphocytes. Both protective antibodies and t-cells (lymphocytes) invade the area to act specifically and directly against the damaging agents.[9]

According to Fabros and Shukla the anti-inflammatory of tannin extractives from the Kamachile leaves have been proven effective through the causes of carrageenan-induced edema method because the minimum percent protection of anti-inflammatory drugs is (%20) and positive result wherein the 250, 500 and 1000 mg/kg dose, respectively. Thus, as percent increases the anti-inflammatory activity of the tannin extracts from the Kamachile leaves increases.[10]

They were also concerned with the preparation of the anti-inflammatory poultice from the alkaloid of comfrey leaves concluded that alkaloids was really present in the plants samples and can be prepared into an anti-inflammatory poultice. The presence of anti-inflammatory property in the leaves makes comfrey more than just an ornamental plants.

Poultice prepared from fresh leaves are used for sprains swelling bruises boils and abscesses. It is applied to the skin to reduce inflammation or in order cases to act as counter-irritant.[11]

The research of Aala confirmed presence of tannins, carbohydrates, and alcohol based from the result on the test of anti-inflammatory activity the sample administered orally to male Sprague-Dawley rats produce against edema at a dose of 1000 mg/kg.[12]

Adayo and Magnaye formulated a product on the basis of resin plaster replacing with aroma alkaloid. Finally, the purified extracts were made into an anti-inflammatory plaster was used for the treatment of inflammation was pursued because the aroma was mainly used for the treatment of anti-inflammatory.[13]

In the study of Agbayani *et al.*, the author stressed some points concerning the use of *Sweetenia mahogany* as an anti-inflammatory agent in comparison to indomethacin. And, I proved that the mahogany seed preparation was noted in higher close response relationship.[14]

The said study from the university of the Philippines, Manila, aimed to assess the anti-inflammatory activity of locally available mahogany seed comparing the effect of three dose level of mahogany seed preparation against a positive control of indomethacin and a negative control of pup in preventing or hastening the resolution of an edema induced by carrageenan injection and at the same time by nothing whether a dose relationship exist. *Coryline fruticosa* was also proven to be more effective as compound to aspirin in exerting an anti-inflammatory activity by Lio *et al.*[15]

Cascalang stressed the anti-inflammatory activity of the crude extract of Suha leaves had been proven effective through the use of carrageenan-induced edema method using the Sprague-Dawley rats as test animals.[16]

According to the researcher Asab of Centro Escolar University, the anti-inflammatory property of the alkaloidal present in kalokanting leaves (*Clifonia temalea*) give the positive test on the leaves of kalokanting leaves to find out what constituent were present. The result was positive for alkaloids Saponin carbohydrates and proteins. The amount of kalokanting leaves which is alkaloid to be incorporated should not be <0.25% and not more than 0.30% based on Belladona plaster. The alkaloidal extractives were used in formulating an anti-inflammatory was used in formulating an anti-inflammatory poultice.[17]

Citrus has been used for more medicinal purposes than most other fruit crops an impressive list of folk medicine treatments was compiled by Duke and Ducellier (1993) for several *Citrus* species. Remedies range from chew sticks per oral hygiene and toothache relief to contraceptives laxarives purgatives sedatives and treatment of a wide variety of common ailments such as diarrhea and vomiting. *Citrus pectin* is reported to reduce cholesterol 30% aortal plaque 85% and narrowing of coronary arteries by 88% in animals feeding studies.

In addition to various juice products from the pulp, *Citrus* peels are candied, fed to livestock and used to used perfumes and soap products. Limonene and petitgrain oils from peels or leaves are used as essential oils and have an insecticidal property recently discovered. Lemon oil is cold-pressed from lemon peels and used in everything from baked goods candy furniture polish and insecticides. Curacao and Cointreav liqueurs are made from sour orange fruit.

Calamansi is a small *Citrus* fruit that appears as a cross between a lemon and lime. It is widely cultivated throughout the Philippines and is abundant on Mt. Banahaw. It is native of the Philippines and does not occur naturally outside of the Philippines. The juice of calamansi is known as a refrigerant a remedy for coughs and itches a deodorant and an antiphlogistic. The calamondin is said to be native in China and then they were spread through Indonesia and in the Philippines many years ago. Calamansi is the most widely used *Citrus* juice source in the Philippines and is grown in India, Malaysia and all through southern Asia. The tree seems can be cultured in many types of soils. Mid-august through October is the peak season in the Philippines. Calamondin can be served with iced tea, meals, meats or to make avid juice, tart source, sweet pickles with sugars, *Marmalade* (Calamondin – papaya *Marmalade* In Hawaii), as flavor to some fruit that are stemmed or preserved, gelatin dessert or salads, custard pie, chiffon Preparation, hair shampoo, itching remedy for bites, bleaching agent for freckles, clearing up acne *Pruritis vulvea* and acne vulgaris, oral cough remedy, and antiphlogistic medicine and is a phlegm expeller in Malay. In the Philippines, bottles of calamansi juice with *Guna tragacanthare* are commercially available which must be kept in low temperature. Leaves contain 0.90-1.06% volatile oil.[19]

Tannins are amorphous substances occurring in plants having an astringents taste, and turning dark blue or green with iron salts. They occur in greatest quantity in the bark and the gall formations. They are soluble in water, alcohol, and a mixture of alcohol and ether. They are almost insoluble in absolute ether and chloroform. They give insoluble precipitates with organic bases such as alkaloids and with most of the salts and heavy metals.[20]

Tannins comprise a large group of complex substances that widely distributed in the plant kingdom, almost every plants contain tannins. When tannins occur in appreciable large quantities, they are usually localized in specific plants such as leaves, fruits, barks, or stems.[21]

All of the tannins are relatively resistant to digestion of fermentation, and either decrease the ability of animals to

easily consume the living plant, or as in deciduous trees, causes shed parts of the plants to decay as slowly that three is little like hood of infection to the living tree from the rotting dead material around the base.

In addition to their principal to their principal application in leather manufacturer and dyeing, tannins are red in the clarification of wine and beer, as a constituent to reduce viscosity of drilling muds for oil wells, and in boiled water to prevent scale formation. Because of its styptic and astringent properties, tannins have been administered internally to check diarrhea and intestinal bleeding and as antidote for metallic, alkaloidal, and glycosidic poisons, with which it dorms insoluble precipitates.[22]

Tannins are non crystallizable compounds, with which with water form colloidal solutions processing an acid reaction and a sharp puckering taste. They cause precipitation of solutions of gelatin and alkaloids, forming a dark blue greenish black soluble compound with ferric salts. They produced decreased color with potassium ferric cyanide and ammonia. They are precipitated by salts of copper, lead, and tin and also by strong aqueous potassium dichromate nature plant.[23]

Inflammatory is the non-specific immune response to any type of bodily injury. The inflammatory is nonspecific because it takes place in above and some way no matter what stimulus and occurs in the same manner even in second exposure to the same stimulus.[24]

Inflammation and repair can be divided into several phases. The characteristics of early inflammatory response differ from these of later response, and each phase involves differential biochemical, mediates, and cells that function together, (1) Destroy injuries agents and remove them from inflammatory rites; (2) Wales of and confire these agents so as to limit their effects on the host; (3) stimulates and enhance the immune response; (4) promote healing.[25]

Anti-inflammatory agent is an agent with alleviated swelling and local response to cellular injury characterized by capillary dilation, heat, and commonly pain and serving as a primary mechanism for control of noxious agents and elimination of damaged tissues.

The process of an inflammation occurs to the tissues of the body in response to an injury, such as sunburn, on insect bite, a wound of an infection.[23]

Many forms of arthritis and joint inflammatory matters can be managed through planed dietary and supplementation practices. Muscle therapy, joint mobilization and manipulation, acupuncture, and exercise are the additive requirements for prompt therapy.[26] Studies have shown that a lot of these natural by products provide similar effects as anti-inflammatory drugs in the market, and have lesser side effects. Intestinal tract ulcers in 10-30% of long term patients, and stomach lining erosions of intestinal tract in 30-50% of cases are some of the reported side effects of non-steroidal anti-inflammatory drugs (NSAID's).[27] As a result of these, NSAID use are associated with great number of deaths per year in the U.S.[28] New generation drugs like cyclooxygenase 2 inhibitor drugs can only be of help to lessen intestinal tract damage by 50%. Their toxicity to the kidneys and liver is still under investigation.[29] Anti-inflammatory medicines have been shown to hasten damage and erosion of joint cartilage, accelerating the osteoarthritic process. Conventional NSAIDs are also said to have liver and kidney damage in the case of long-term useage.[30]

Due to these facts being presented by researches they concluded that "The epidemiological data highlight the importance of using (acetylsalicyclic acid)/NSAID therapy only if truly necessary."[31] Similar to many synthetic anti-inflammatory medications, the active component of anti-inflammatory herbs can block the activity of the cyclooxygenase and lipoxygenase enzymes, thus preventing PG-2 and leukotriene B4 to synthesis the pro-inflammatory eicosanoid. They have been shown to alleviate pain and inflammation associated with different types of arthritis and traumatic joint injuries. Unlike synthetic drugs, intestinal tract erosion, acceleration of cartilage damage or toxicity of kidney and liver is not seen. For these reasons, the use of natural anti-inflammatory agents as potential takeover for synthetic medications or as a means to lessen their need for conventional anti-inflammatory pharmaceutical remedies is gaining popolarity.[32]

Conclusions

After conducting different tests and procedures from the rind of calamansi we concluded that the rind of calamansi plant contains tannins. This tannins can be extracted using maceration and with water as solvent. Anti-inflammatory property of tannins from calamansi rind was investigated using carrageenan-induced edema method and is effective in increasing doses of 250, 500, and 1000 mg/kg.

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