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Evaluation of combined effect of *Psidium guajava* and *Syzygium cumini* leaves on inflammation

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ABSTRACT

The present study was an attempt to investigate the anti inflammatory activity of combined extracts (1:1mixture) of *Syzygium cumini* and *Psidium guajava* as well as to compare the *invitro*anti-inflammatory activity of them by the method of protein denaturation assay and Proteinase inhibitory activity. Both the plants selected belong to the family *Myrtaceae*. Ethanolic extract of shade dried leaves were used for the study. Diclofenac sodium was used as the standard drug. The result showed that at a concentration of 500 µg/ ml, the percentage inhibition of protein denaturation, by the combinedethanolic extract (1:1 mixture) of *Syzygium cumini* and *Psidium guajava* was 78.91, while for Diclofenac, the corresponding value was 89.36. The percentage inhibition of Proteinase , by the combined ethanolic extract (1:1 mixture) of *Syzygium cumini* and *Psidium guajava* at a concentration of 500 µg/ ml, was 86.46 and for Diclofenac the percentage inhibition of Proteinase was 90 .66 at the same concentration. The combined extract of the plants selected was found to be more effective than individual plant extracts against inflammation. The protein denaturation inhibition and Proteinase inhibition of the combined extract was found to be closer to that of the standard drug Diclofenac. On comparison of two plants *Syzygium cumini* was found to be more active against inflammation than *Psidium guajava.* As the 1:1 mixture of the ethanolic extract is found to be more active, the combination of the two plants can be used to formulate drugs for various inflammatory disorders.

INTRODUCTION

Inflammation is the response of living tissue to injury or foreign bodies. It involves a well-organized cascade of fluid and cellular changes in the living tissues. It has both beneficial and detrimental effects locally and systemically. Causes of Inflammation are microbial infections, hypersensitivity reactions, physical agents, irritants, corrosive chemicals and tissue necrosis Non-steroidal anti-inflammatory drugs (NSAIDs) are the frequently used drugs to treat a wide range of inflammatory conditions. However, even at doses within prescribing recommendations, NSAIDs are associated with serious adverse effects in susceptible patients [1, 2]. The greatest disadvantage of potent synthetic drugs is their toxicity and reappearance of symptoms after discontinuation. Therefore, the screening and development of new anti-inflammatory drugs from indigenous medicinal plants is essential. Unlike synthetic drugs with a single active component that target on a specific mechanism of inflammation, herbal medicines contain a multitude of different molecules that act synergistically on targeted elements of the complex cellular pathway[3].

Medicinal plants possess a large variety of chemicals from which new anti-inflammatory agents can be isolated. Research on the biological activities of compounds, isolated from plants has led to the development of new drugs. *Syzgium cumini* (Synonym: *Eugenia jambolan* Linn.) family, *Myrtaceae* is a very large evergreen tropical tree. [Fig: 1]. *Syzygium cumini* is a medium-sized tree 10-30 m high, with a straight to crooked, short, stout trunk, 40-100 cm in diameter[4].All parts of the jambolan can be used medicinally and it has a long tradition in alternative medicine. From all over the world, the fruits have been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm. Ayurvedic medicine (Indian folk

Fig. 1 : *Syzygiumcumini*

Fig. 2 : *Psidiumguajava*

medicine) mentions its use for the treatment of diabetes mellitus. The leaves are used in dermopathies, constipation, leucorrhoea, and diabetes; fruits are used in the treatment of pharyngitis whereas barks are used as astringents, anthelmintic, and carminative. Seeds are used as astringents and diuretic[5].

Psidium guajava L. known as Guava is a medicinal plant belonging to the family Myrtaceae. [Fig: 2]. Guava is a small branched tree of about 10 m height with smooth mottled and peeling bark. Traditionally it is used for anorexia, cholera, diarrhoea, digestive problems, dysentery, gastric insufficiency, inflamed mucous membranes, laryngitis, skin problems, sore throat, ulcers, aches, bacterial infections, boils, bowel disorders, bronchitis, colds, colic, convulsions, coughs, dyspepsia, oedema, epilepsy, fever, gingivitis, haemorrhoids, itch, jaundice, menstrual problems, nausea, nephritis, respiratory problems, rheumatism, scabies, spasms, and sprains[6].

The aim of the present study was to compare the anti inflammatory activity of ethanolic extract of *Syzygium cumini* and

Psidium guajava leaves and to investigate the anti inflammatory activity of the combined extract of both the plants. The objective of the study was to perform the *invitro* anti-inflammatory assays by the method of protein denaturation assay and Proteinase inhibitory activity.

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of both plants were collected from the hilly regions of Idukki District, Kerala and were authenticated by Dr. Sr. Tessy Joseph, Professor, Department of Botany, Nirmala College, Muvattupuzha.

Extraction

The mature leaves of both *Psidium guajava* and *Syzygium cumini* are shade dried separately, powdered in grinder, to get coarse powder for extraction. 250 gm of the *Syzygium cumini* leaf powder was extracted in ethanol (2.5 L) in a Soxhlet apparatus and concentrated to yield the crude ethanol extract SC EL. Similarly, 250 gm of the crude powder of *Psidium guajava* leaf was extracted in ethanol (2.5 L) and concentrated to yield the crude ethanol extract, PG EL. The extracts were concentrated using vacuum evaporator.

INVITRO ANTIINFLAMMATORY ACTIVITY

1. PROTEINASE INHIBITORY ACTIVITY

Proteinase inhibitory activity was performed by a modified method. 0.06mg trypsin, 1ml 20Mm TrisHCl buffer(pH 7.4) and 1ml test sample of different concentration 62.5- 500µg/mL from a stock concentration of 10 mg/ml were the components of reaction mixture(2ml).The reaction mixture was incubated for five minutes ata temperature of 37ºC,followed by addition of 1ml of 0.8%(w/v) casein. The mixture was incubated for an additional 20 minutes, followed by addition of 2ml of 70% perchloric acid, which terminated the reaction. The cloudy suspension was centrifuged for 10minutes at 3000rpm.Finally measured the absorbance of the supernatant at 280 nm against buffer as blank. The experiment was performed in triplicates[7].The formula used for calculating the percentage of Proteinase inhibitory activity is

Percentage inhibition of Proteinase = (100-(((A of test- A of product control)/A of Control) ×100))

2. PROTEIN DENATURATION INHIBITION

Bovine Serum Protein Denaturation Method

0.45 ml of bovine serum albumin and 0.05 ml of test sample wasmixed to form the test solution. The test control was prepared by mixing 0.45ml bovine serum albumin and 0.05 ml of distilled water. Product control was a mixture of 0.45 ml of distilled water and 0.05 ml of test solution. Standard solution consists of 0.45 ml of Bovine serum albumin and 0.05 ml of Diclofenac sodium. The pH of all the above solutions was adjusted to 6.3 using 1N HCl. The samples were incubated the samples at 37^{0}C for 20 minutes and increased the temperature to 57^{0}Cfor 3 minutes. Cooled and added 2.5ml of phosphate buffer to the solutions [8]. The absorbance was measured at 416nm using UV-visible spectrophotometer at (SL119, Systronics).

Stock concentration: 10mg/ml

The percentage inhibition of protein denaturation was calculated by the formula

% inhibition= 100-[((optical density of test solution optical density of product control) ÷ optical density of test control)) ×100]

RESULTS

Proteinase inhibitory activity

The ethanolic extract of leaves of both *Psidium guajava* (PG EL) *and Syzygium cumini* (SC EL) as well as the 1:1 mixture of both the extracts exhibited a significant Proteinase inhibitory activity in a dose dependant manner. The percentage inhibition of Proteinase activity by ethanolic extract of the leaves of *Psidium guajava, Syzygium cumini,* 1:1 mixture of both and Diclofenac sodium are tabulated in Table1. Results are expressed as mean±SD (n=3).The comparison of Proteinase inhibitory effects of the extracts is depicted in Fig: 3

Protein denaturation inhibition

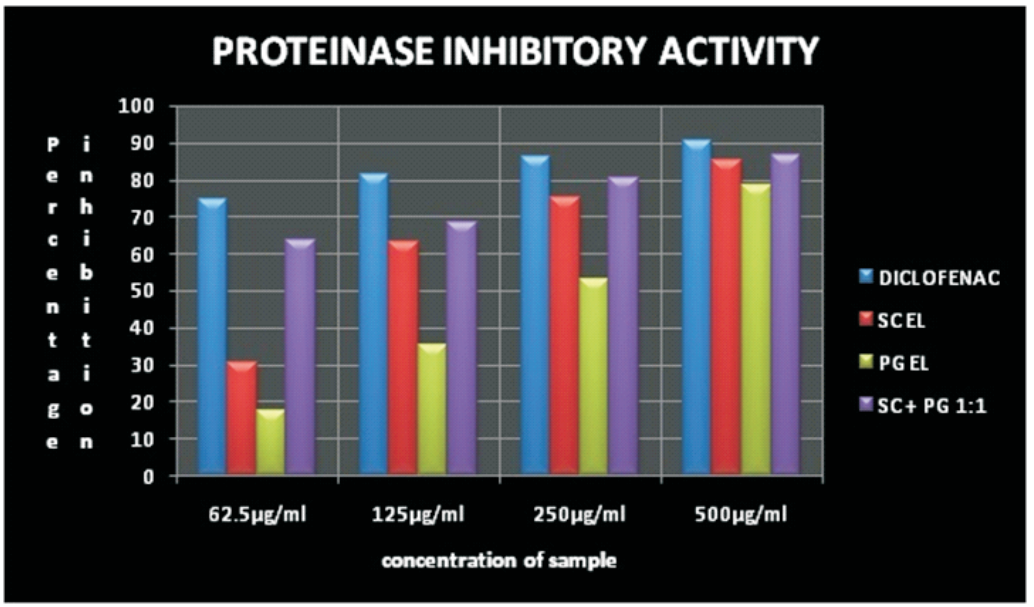

Fig. 3 : Proteinase inhibitory activity

Table 1 : PROTEINASE INHIBITORY ACTIVITY

Sl. No	Sample	Concentration (µg/ml)	Absorbance of test solution	Absorbance of product control	Percentage of inhibition
1	Diclofenac(standard)	62.5	1.1866±0.96	0.9558±0.87	74.67
		125	1.1427±0.99	0.972±0.86	81.26
		250	1.1431±1.13	1.0154±0.94	85.98
		500	1.9346±0.73	1.8495±0.89	90.66
2	Ethanolic extract of *Syzygiumcumini* (SC EL)	62.5	0.2992±0.78	0.0219 ±0.79	30.61
		125	0.3136±0.85	0.166±0.76	63.06
		250	0.4525±0.76	0.3521±0.89	74.87
		500	0.6436±0.96	0.5931±0.62	85.36
4	Ethanolic extract of *Psidiumguajava* (PG EL)	62.5	0.4878±0.79	0.1585±0.7 6	17.59
		125	0.4917±0.96	0.1646 ±0.68	35.2
		250	0.4857±0.65	0.2977±0.65	52.95
		500	0.9034±0.94	0.8167±0.87	78.30
5	1:1 mixture of SC EL & PG EL	62.5	0.3107±0.76	0.1646±0.97	63.44
		125	0.2937±1.07	0.1667±0.66	68.22
		250	0.3957±0.69	0.3178±0.99	80.51
		500	0.3199±0.86	0.2658±0.91	86.46

The 1:1 mixture of *Psidium guajava*and *Syzygium cumini* was found to possess good inhibitory effect against protein denaturation than either of the extracts alone. The Percentage inhibition of protein denaturation by ethanolic extract of the leaves of *Psidium guajava, Syzygium cumini*, 1:1 mixture of both and Diclofenac sodium are tabulated in table 2. The result showed the combined extract has good activity comparable with that of Diclofenac. Results are expressed as mean±SD (n=3). The comparison of protein denaturation inhibition of the extracts at different concentrations is shown in Fig: 4.

DISCUSSION

Inflammation is implicated in the pathogeneses of arthritis, cancer, stroke, neurodegenerative and cardiovascular disease. Long term use of NSAIDs can lead to various harmful effects. So

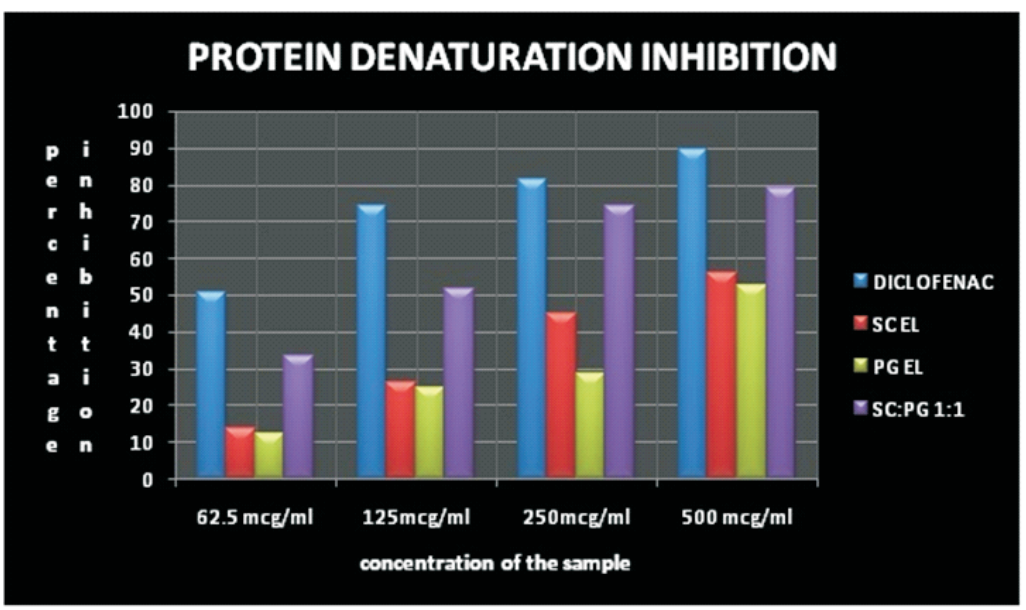

Fig. 4 : Protein denaturation inhibition

Table 2 : PROTEIN DENATURATION INHIBITION

Sl. No	Sample	Concentration (µg/ml)	Absorbance of test solution	Absorbance of product control	Percentage of inhibition
1	Diclofenac (standard)	62.5	0.227±0.62	0.1983±0.62	50.77
		125	0.0311±0.98	0.0161±0.62	74.27
		250	0.0553±0.82	0.0443±0.62	81.13
		500	0.0876±0.92	0.0814±0.62	89.36
2	Ethanolic extract of *Syzygiumcumini* (SC EL)	62.5	0.6681±0.67	0.2389±1.62	13.47
		125	0.6175±0.63	0.2499±1.12	25.89
		250	0.7911±1.02	0.2879±0.72	44.76
		500	0.8511±0.67	0.6322±0.66	55.87
3	Ethanolic extract of *Psidiumguajava* (PG EL)	62.5	0.7781±0.76	0.3419±0.84	12.06
		125	0.7611±0.69	0.3879±0.62	24.76
		250	0.8517±0.86	0.4967±0.99	28.43
		500	0.9692±0.92	0.747±0.98	52.03
4.	1:1 mixture of SC EL & PG EL	62.5	0.8711±0.82	0.5411±0.87	33.47
		125	0.8291±0.62	0.5899±0.74	51.77
		250	0.7665±0.88	0.6619±0.94	73.95
		500	0.8309±0.69	0.7017±0.83	78.91

it is necessary to explore plants to obtain traditional herbal medicines.

Proteinase has an important role in the development of tissue damage during inflammatory reactions. So Proteinase inhibitors can give protection against inflammation [8]. At the concentration of 500µg/ml, the standard anti inflammatory drug, Diclofenac, showed the Proteinase inhibition of 90.66%. The 1:1 mixture of ethanolic extract of *Psidium guajava* and *Syzygium cumini* exhibited Proteinase inhibition of 86.46% at a concentration of 500µg/ml. The Proteinase inhibition of ethanolic extract of *Psidium guajava* and *Syzygium cumini* at a concentration of 500µg/ml was 78.30% and 85.36% respectively, which is less than that exhibited by the combination of two plants, showing the enhanced activity of the mixture.

Proteins lose their tertiary structure and secondary structure when exposed to external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. This denaturation of biological proteins leads to the lossof their biological function. Inflammation may be caused due to denaturation of proteins.Ability of plant extract to inhibit protein denaturation can be used to investigate the anti-inflammatory activity [9]. At the concentration of 500µg/ml, Diclofenac, the standard anti inflammatory drug showed the inhibition of 89.36%. The 1:1 mixture of *Psidium guajava* and *Syzygium cumini* was found to possess good inhibitory effect of 78.91% against protein denaturationat a concentration of 500µg/ml.At the same concentration the ethanolic extract of *Psidium guajava* and *Syzygium cumini* inhibited protein denaturation to the extent of 55.87 % and 52.03 % respectively. The result shows that the mixture of the plant extracts are more effective in inhibiting protein denaturation.

In Ayurveda and traditional Chinese medicine combination of herbs are fundamental to their philosophy. The concept of polyherbal formulation differentiates herbal products from conventional medicines containing isolated constituents. When the combined action of constituents is greater than would be expected from individual contributions either synergistic or additive action may be expected. The idea of synergy within and between herbs is of importance today as herbs possess a variety of constituents responsible for their effects [10].

This work is an attempt to compare the anti-inflammatory activity of two plants from the family Myrtaceae as well as to investigate the anti-inflammatory activity of the combined extract of the plants.Even though the total ethanolic extract of *Psidium guajava* and *Syzygium cumini* exhibited good inhibition ofprotein denaturation and Proteinase activity, the activity was more for the combined extract of the two plants. On comparison of the individual plants *Syzygium cumini* was found to be more active against inflammation than *Psidium guajava.* There was a dose dependant increase in the anti-inflammatory activity, which was prominent for the 1:1 mixture of the ethanolic extract of *Psidium guajava* and *Syzygium cumini.*

This attempt to investigate the effect of combination of

Psidium guajava and *Syzygium cumini*, which is not reported till now, revealed the enhanced anti inflammatory activity of the 1:1 mixture of ethanolic extracts of *Psidium guajava* and *Syzygium cumini*, than the individual plant extracts. This enhanced activity of the mixture throws light on the concept of synergism, suggesting the importance of polyherbal formulation, which may lead to improved products with increased efficacy.

CONCLUSION

In the present study the ethanolic extract of leaves of *Psidium guajava, Syzygium cumini* and 1:1 mixture of ethanolic extract of both the *Psidium guajava* and *Syzygium cumini* leaves exhibited protein denaturation and Proteinase inhibition properties. This indicates the ability of the plants under study to act as anti-inflammatory agents. The anti inflammatory activity may be due to the presence of polyphenolic compounds. Among the three extracts studied, the combined extract of *Psidium guajava* and *Syzygium cumini* was found to be more active than either of the plants alone. Further studies are recommended to find the mechanism behind this synergistic or additive effect. This study also suggests that the active compounds isolated from these plants can be used as lead compounds for designing a potent anti-inflammatory drug that may be useful for treatment of various inflammatory disorders. Based on the future investigations the plants can be utilised as the components of a polyherbal formulation for treating inflammatory conditions.

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REFERNCES

1. Cameron C. Non-steroidal anti-inflammatory drugs (NSAIDs): Making safer treatment choices. *Best Practice Journal New Zealand*. 2013;(55): 8-18.
2. Teslim OA. Side Effects of Non-Steroidal Anti-Inflammatory Drugs: The Experience of Patients with Musculoskeletal Disorders. *American Journal of Health Research*. 2014;2(4):1 0-6.
3. Kumar S, Bajwa BS, Kuldeep S, Kalia A. Anti-Inflammatory Activity of Herbal Plants□: A Review. *International Journal of Advances in pharmacy, Biology and Chemistry* 2013;2(2):2 72-81.
4. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Syzygium cumini (L.) SkeelsMyrtaceae. *Agroforestry database: a tree species Reference and Selection Guide* version 40. 2009;0:1-5.
5. Pradhan M. Phytochemistry, Pharmacology and Novel Delivery Applications of Syzygiumcumini (L.). *International journal of Pharmacy and Pharmaceutical Research .2016;7(1):659-675*
6. Shirur Dakappa Shruthi, Adhikari Roshan, Sanjay Sharma. A review on the medicinal plant Psidium Guajava Linn. (Myrtaceae). *Journal of Drug Delivery and Therapeutics*. 2013;3(2):162-168
7. Oyedepo O.O and Femurewa A.J., "Anti-protease and membrane stabilizing activities of extracts of Fagrazanth oxiloides, Olaxsubscorpioides and Tetrapleuratetraptera", *International Journal of Pharmacongnosy*. 1995; 33: 65-69.
8. Rahman H, Chinna Eswaraiah M, Dutta A. *In-vitro* Anti-inflammatory and Anti-arthritic Activity of Oryzasativa Var. Joha Rice (An Aromatic Indigenous Rice of Assam). *American-Eurasian Journal of Agriculture and Environmental sciences*. 2015; 15(1):1 15-21
9. Leelaprakash G, Mohan Dass S. Invitro anti-inflammatory activity of methanol extract of enicostemma axillare. *International Journal of Drug Development & Research* 2011;3(3):1 89-96.
10. Evans W.C; Evans D; Trease G.E. *Trease and Evans Pharmacognosy*, Edinburgh: Saunders/ Elsevier, 2009,.15: 49-53