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EFFECT OF COMBINATION OF TWO PLANTS ON ANTI-OXIDANT ACTIVITY

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ABSTRACT:
Anti-oxidants are essential for maintaining optimum health and well being by acting against damage caused by free radicals. Regular consumption of vegetables and fruits with anti-oxidant activity can reduce the risk of many chronic diseases.
Objective: The main objective of the study was to evaluate the antioxidant activity of Psidium *guajava* and *Syzygium cumini* leaves individually and as a combination of ethanolic leaf extracts of both in 1:1 ratio.
Methods: The antioxidant activity of ethanolic leaf extracts of *Syzygium cumini* and *Psidium guajava* and its 1:1 combination were determined by different methods such as Superoxide free radical scavenging assay, Hydrogen peroxide scavenging assay and Hydroxyl radical scavenging assay.
Result: The 1:1 combination of ethanolic leaf extract of *Syzygium cumini* and *Psidium guajava* showed higher antioxidant activity than the individual extracts. Out of the two individual extracts, *Syzyium cumini* showed more activity than the *Psidium guajava* extract. A dose dependent increase in activity was found in all methods. The percentage inhibition of combined extract by hydroxyl radical scavenging assay and hydrogen peroxide scavenging assay was 72.70 and 76.15 respectively. Superoxide free radical scavenging assay, showed inhibition of 63.5percentage.
Conclusion: From the result it is concluded that leaves of both the plants possess anti-oxidant activity and *Syzygium cumini exhibited more activity than Psidium guajava.* But the combination of both is superior to individual extracts in exhibiting anti-oxidant activity. The results showed the possibility of synergistic or additive effect of combination of ethanolic extracts of *Syzygium cumini* and *Psidium guajava* leaves in the ratio 1:1.
Key words: *Syzygium cumini, Psidium guajava,* Superoxide free radical scavenging assay, Hydrogen peroxide scavenging assay, Hydroxyl radical scavenging assay

INTRODUCTION:

As mentioned in Ashtanga Hridaya all the plants in this earth are considered as medicinal in Indian tradition. Medicinal plants provide medicine to maintain health, prevent disease and cure ailments. Throughout the world medicinal plants are used in traditional systems of medicines. Herbs are used as medicines from time immemorial. The phytochemical ingredients present in plants make them useful for medicinal purpose [1]

Anti-oxidants at low concentration compared with that of oxidisable substance, significantly delays or stop the oxidation of that substrate by scavenging the free radicals. Natural anti- oxidants occur in all parts of plants. These compounds include phenolic acids, flavonoids, carotenoids, tocopherols etc.

Psidium guajava L. known as Guava is a medicinal plant belonging to the family *Myrtaceae. Psidium guajava* is a well known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. *Psidium guajava* is also known as the 'poor man's apple' of the tropics [2] .The leaves and bark of *Psidium guajava* tree have a long history of medicinal uses and is used in this era also. *Syzgium cumini* Linn

(synonym: *Eugenia jambolan* Linn.) is a very large evergreen tropical tree belonging to the family *Myrtaceae.* The synonym of the plant is black plum or jambolan which is also named as jamun. Jamun is a native of India and East Indies. All parts of the jambolan can be used medicinally and it has a long tradition in alternative medicine [3].

OBJECTIVES: The main objective of the study was to evaluate the antioxidant activity of Psidium *guajava* and *Syzygium cumini* leaves individually and as a combination of ethanolic leaf extracts of both in 1:1 ratio

METHODS:

1. Collection and identification of the plants *Psidium guajava* and *Syzygium cumini:*

The plants *Psidium guajava* and *Syzygium cumini* were authenticated by Dr.Sr.Tessy Joseph, HOD, Department of Botany, Nirmala College, Muvattupuzha.Voucher specimens are kept in the herbarium of Nirmala College, Muvattupuzha. The voucher specimen numbers are NCH/2014/NCP/3812 for *Psidium guajava* and NCH/2014/NCP/3811for *Syzygium cumini.*

The leaves of both the plants were collected in the month of April from hilly areas of Idukki district. The leaves were shade dried and powdered to get coarse powder for extraction. It was stored in polythene bags at room temperature.

2. Extraction:

Coarsely powdered *Syzygium cumini* and *Psidium guajava* leaves were subjected to Soxhlet extraction with 95% ethanol. The ethanol extracts were concentrated and evaporated to dryness. The percentage yield of the extracts was calculated. The dried extracts were kept in the refrigerator. The extracts were denoted as SCEL for *Syzygium cumini* and PGEL for *Psidium guajava.*

3. *In vitro* antioxidant studies:

a) Superoxide free radical scavenging assay:

Different concentrations of samples 125-2000 µg/mL were prepared from a stock solution of concentration 10 mg/mL. The standard used was L-ascorbic acid. Mixed 0.1 ml of extract, 0.05ml of riboflavin solution (0.12mM), 0.2 ml of EDTA solution (0.1M) and 0.1 ml NBT (Nitro-blue tetrazolium) solution (1.5mM) in a test tube and the reaction mixture was diluted up to 3ml with phosphate buffer (0.067M). A control without the test compound but with an equivalent amount of distilled water was taken. The absorbance of solution was measured on UV visible spectrophotometer at 560 nm after illumination for 5 minutes and 30 minutes in fluorescent light. ΔOD was calculated and the percentage inhibition was determined [4,5]

$$\% \text{ inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X100$$

b) Hydroxyl radical scavenging activity:

Different concentrations of extracts 125-2000 µg/mL from a stock concentration of 10 mg/mL were mixed with 500µL reaction mixture (2.8 mM 2-deoxy-2-ribose, 100 µm $FeCl_3$, 100 µm EDTA, 1 mM H_2O_2, 100 µm ascorbic acid in 20 mM KH_2PO_4 - KOH buffer of pH 7.4) and was made up to a final volume of 1 mL. The standard used was gallic acid. A control without the test compound but an equivalent amount of distilled water was taken. After incubation for 1h at 37° C, 1mL of 2.8% TCA and 1mL 1% aqueous TBA were added and the mixture was incubated at 90° C for 15 min to develop the colour. After cooling the absorbance was measured at 532 nm against blank solution[6].

$$\% \text{ inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X100$$

c) Hydrogen peroxide radical scavenging assay :

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of extracts 125-2000 µg/mL from a stock concentration of 10 mg/mL was added to 0.6 mL H_2O_2 solution. The standard used was ascorbic acid. A control without the test compound but an equivalent amount of distilled water was taken. OD was read at 230 nm after 10 minutes [7].

$$\% \text{ inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X100$$

4. Statistical analysis:

Experimental results were mean ± SD of three parallel measurements. IC_{50} values were calculated by the methods of normal regression, logarithmic regression and probit analysis.

RESULTS:

a) Super oxide free radical scavenging assay:

Combined extract of SCEL & PGEL exhibited highest activity. The percentage inhibition of superoxide radical by PGEL, SCEL and the combination was 56.71, 60.63 and 63.04 respectively at the concentration of 2000 µg/mL. The results are tabulated in table 1 and illustrated in figure 1. IC_{50} values are compared in figure 2.

Table 1: Percentage inhibition and IC_{50} values by superoxide free radical scavenging assay

Sl.No	Sample	Concentration (µg/mL)	Absorbance at 560 nm	Percentage inhibition	IC_{50} (µg/mL)
1	Ascorbic acid (standard)	Control	0.8896±0.054		286
		125	0.5231±0.099	41.19	
		250	0.4667±0.082	47.53	
		500	0.3318±0.049	62.70	
		1000	0.2106±0.086	76.32	
		2000	0.0829±0.064	90.68	
2	Ethanolic extract of *Syzygium cumini* (SCEL)	Control	0.0536±0.043	-	388.84
		125	0.0429±0.014	19.96	
		250	0.0325±0.056	39.37	
		500	0.0228±0.089	57.46	
		1000	0.0217±0.168	59.51	
		2000	0.0211±0.013	60.63	
3	Ethanolic extract of *Psidium guajava* (PGEL)	Control	0.0536±0.043	-	1000
		125	0.0453±0.009	15.48	
		250	0.0393±0.054	26.67	
		500	0.0301±0.084	43.84	
		1000	0.0268±0.094	50	
		2000	0.0232±0.074	56.71	
4	1:1 mixture of SCEL & PGEL	Control	0.0536±0.043	-	325.76
		125	0.0418±0.034	22.01	
		250	0.0312±0.094	41.79	
		500	0.0219±0.004	59.14	
		1000	0.0203±0.097	62.13	
		2000	0.0198±0.041	63.05	

Absorbance values are expressed as mean ± SD, n=3

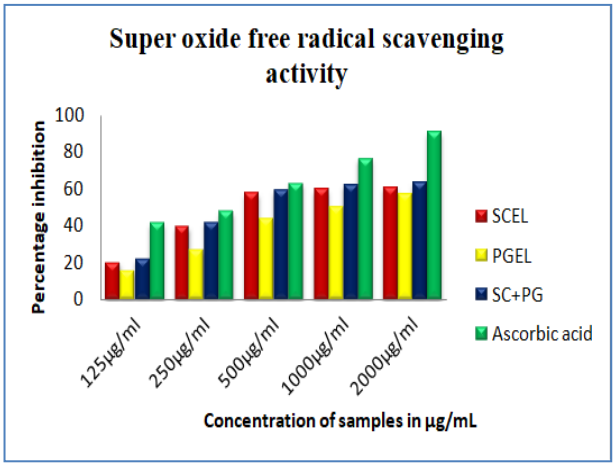

Figure 1: Comparison of super oxide free radical scavenging activity

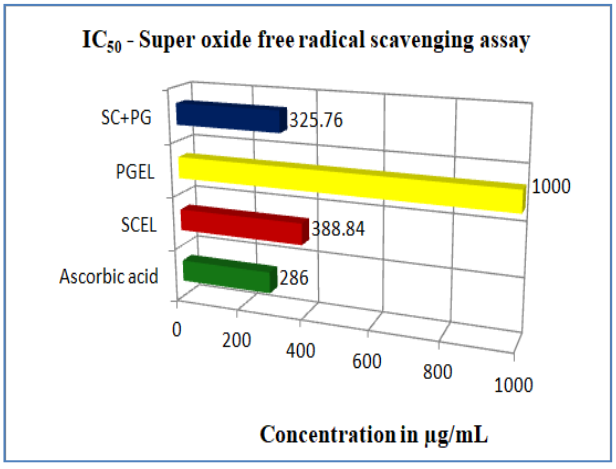

Figure 2: Comparison of IC_{50} values of super oxide free radical scavenging activity

b) Hydroxyl radical scavenging assay:

All the extracts scavenged hydroxyl radicals in a concentration dependent manner. The percentage inhibition by the combined extract of SC and PG was 72.7, closer to that of the standard ascorbic acid, 74.91 at 2000 µg/mL**.** Results are exhibited in table 2 and compared in figures 3 and 4.

Table 2: Percentage inhibition and IC_{50} values by hydroxyl radical scavenging assay

Sl.no	Sample	Concentration (µg/mL)	Absorbance at 532 nm	% Inhibition	IC_{50} (µg/mL)
1	Gallic acid (standard)	Control	0.5620± 0.021	-	205.85
		125	0.4081± 0.016	27.40	
		250	0.2479± 0.082	55.88	
		500	0.2080± 0.044	62.98	
		1000	0.1805± 0.054	67.88	
		2000	0.1410± 0.098	74.91	
2	Ethanolic extract of *Syzygium cumini* (SCEL)	Control	0.3066± 0.057	-	228.86
		125	0.2283 ± 0.045	25.54	
		250	0.1405± 0.014	54.17	
		500	0.1314± 0.076	57.14	
		1000	0.1245± 0.054	59.39	
		2000	0.1116± 0.062	63.60	
3	Ethanolic extract of *Psidium guajava* (PGEL)	Control	0.3066± 0.078	-	468.31
		125	0.2352 ± 0.091	23.29	
		250	0.1698± 0.041	44.61	
		500	0.1366± 0.089	55.45	
		1000	0.1262± 0.044	58.84	
		2000	0.1127± 0.071	63.24	
4	1:1 mixture of SCEL & PGEL	Control	0.3066± 0.094	-	215.79
		125	0.2276 ± 0.041	25.76	
		250	0.1381± 0.041	54.96	
		500	0.1239± 0.046	59.58	
		1000	0.1018± 0.033	66.79	
		2000	0.0837± 0.057	72.70	

Absorbance values are expressed as mean ± SD, n=3

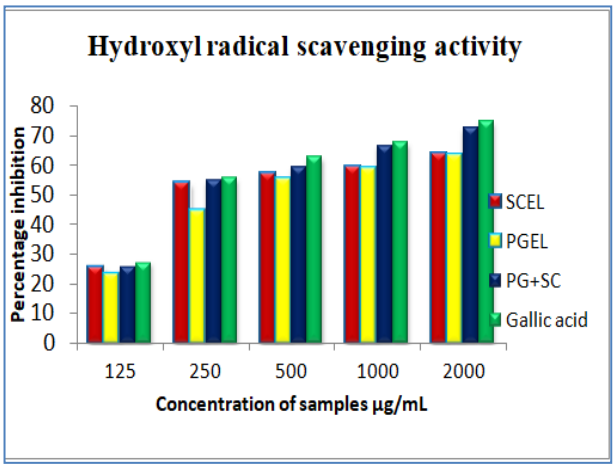

Figure 3: Comparison of hydroxyl radical scavenging activity of extracts and standard

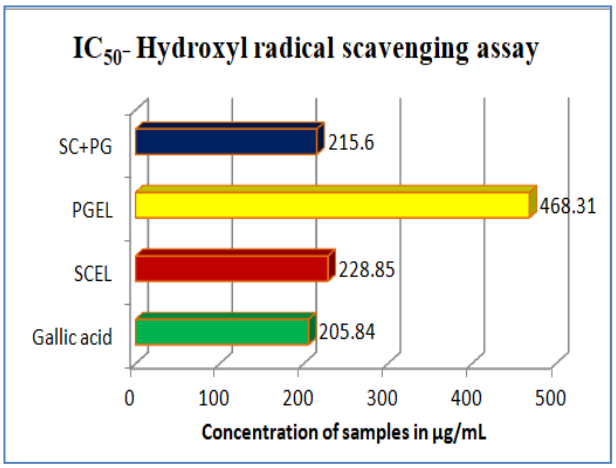

Figure 4: Comparison of the IC_{50} values of the extracts by hydroxyl radical scavenging assay.

c) **Hydrogen peroxide scavenging assay:**

Highest hydrogen peroxide scavenging activity was exhibited by the combined extract of SCEL and PGEL. Among the individual plant extracts SCEL showed more scavenging activity than PGEL. Table 3 and figures 5 and 6 shows the results of hydrogen peroxide scavenging assay.

Table 3: Percentage inhibition and IC_{50} values by hydrogen peroxide scavenging assay

Sl.No	Sample	Concentration (µg/mL)	Absorbance at 230 nm	% Inhibition	IC_{50} (µg/mL)
1	Ascorbic acid (standard)	Control	0.078± 0.041	-	194.98
		125	0.047±0.0295	39.74	
		250	0.032± 0.027	58.97	
		500	0.028± 0.01	64.10	
		1000	0.025± 0.022	67.94	
		2000	0.015± 0.075	80.76	
2	Ethanolic extract of *Syzygium cumini* (SCEL)	Control	0.7553± 0139	-	281.84
		125	0.4716± 1.28	37.56	
		250	0.3859± 1.18	48.91	
		500	0.3129± 0.93	58.57	
		1000	0.2703± 0.69	64.21	
		2000	0.1898± 0.82	74.87	
3	Ethanolic extract of *Psidium guajava* (PG EL)	Control	0.7553± 0.39	-	354.81
		125	0.4796± 0.17	36.51	
		250	0.4162±0.18	45.54	
		500	0.3358± 0.29	55.54	
		1000	0.2992±0.058	60.03	
		2000	0.2143± 1.48	71.62	
4	1:1 mixture of SCEL & PGEL	Control	0.7553± 0.39	-	244.79
		125	0.4622± 0.93	38.8	
		250	0.3679± 0.88	51.29	
		500	0.2930±0.068	61.2	
		1000	0.2499± 0.19	66.91	
		2000	0.1801±0.018	76.15	

Absorbance values are expressed as mean ± SD, n=3

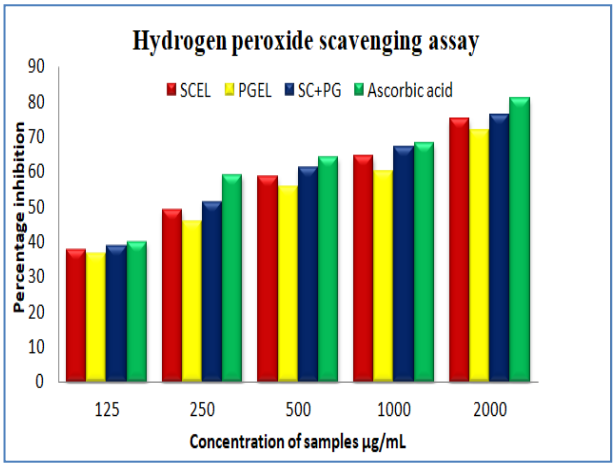

Figure 5: Comparison of hydrogen peroxide scavenging activity of the extracts

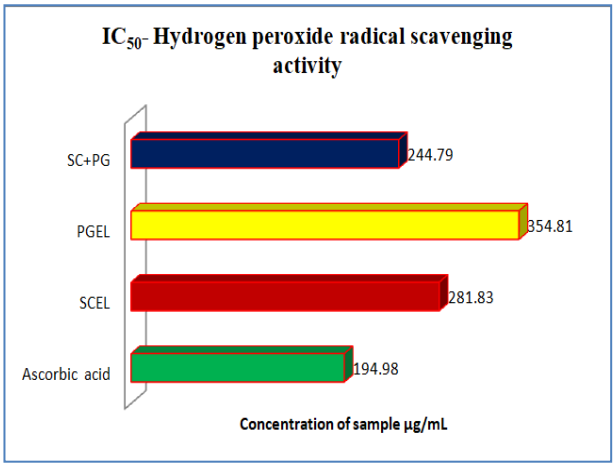

Figure 6: Comparison of the IC_{50} values of the extracts by hydrogen peroxide scavenging assay

DISCUSSION:

Superoxide free radical is biologically important as it can form singlet oxygen and hydroxyl radical damaging biomolecules leading to chronic diseases. Over production of superoxide anion radical contributes to redox imbalance leading to harmful physiological consequences. Superoxide anion is generated in riboflavin-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan product [4,8]. Both the individual extracts and the combined extracts exhibited superoxide scavenging activity showing its ability to exhibit anti-oxidant activity.

Hydroxyl radical scavenging assay is based on the formation of the degradation product of 2-deoxy-2-ribose by condensation with TBA. The hydroxyl radical scavenging ability of the extracts was determined by its ability to compete with deoxyribose for hydroxyl radical [9]. Hydroxyl radical was generated by the Fe^{3+} - ascorbate-EDTA-H_2O_2 system (The Fenton reaction). The scavenging of the hydroxyl radicals may be due to the presence of phenolic compounds in the extracts. The hydroxyl radical can induce oxidative damage to DNA, lipids and proteins[10].

The method for assessing the scavenging capacity against hydrogen peroxide radical is based on the intrinsic absorption of H_2O_2 in the UV region. Hydrogen peroxide radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves *in vitro* generation of hydroxyl radicals using Fe3+/ascorbate/EDTA/H_2O_2 system using Fenton reaction. Scavenging of this hydroxyl radical in presence of antioxidant is measured **[11]**. H_2O_2 has the ability to penetrate into biological membranes. H_2O_2 can be toxic to cell as it generates hydroxyl radicals in the cells [12].

CONCLUSION:

The comparison of antioxidant activity of ethanolic leaf extracts of *Psidium guajava* and *Syzygium cumini* revealed that *Syzygium cumini* is superior to *Psidium guajava*. But on evaluation of the activity of the combined extract of both the plants, the combination was found to exhibit more activity than either of the individual plant extracts. This suggests the synergistic or additive effects of the phytochemicals present in the plants under study. However further studies are recommended to understand the effect of this combination on other related biological activities.

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REFERENCE:

1. Maiti S, Geetha KA. Horticulture, Floriculture, Ornamental, Medicinal and Aromatic plants. Medicinal and Aromatic Plants in India , 2007;1:27
2. Shirur Dakappa Shruthi, Roshan *Adhikari, Sanjay Sharma Timilsina, Sunita Sajjekhan.* A review on the medicinal plant *Psidium Guajava* Linn.*(Myrtaceae*). *Journal of Drug Delivery & Therapeutics* 2013., 3(2): 162–168.
3. Sah A.K, Verma V.K. *Syzygium cumini:* An overview. *Journal of Chemical and Pharmaceutical Research* 2011; 3(3):108–113.
4. Valentão P, Fernandes E, Carvalho F, Andrade P.B, Seabra R.M, Lourdes Bastos M. Antioxidant activity of *Hypericum androsaemum* infusion: scavenging activity against superoxide radical, hydroxyl radical and hypochlorous acid. *Biological & Pharmaceutical Bulletin* 2002; 25(10):1320–1323.
5. Anandjiwala S, Bagul MS, Parabia M, Rajani M. Evaluation of free radical scavenging activity of Ayurvedic formulation *Panchavalkala. Indial Journal Of Pharmaceutical Sciences* 2008; 70(1) :31–35.
6. Ramakrishna H, Murthy S.S, Murthy P.G. Hydroxy radical and DPPH scavenging activity of crude protein extract of *Leucas linifolia*: A folk medicinal plant. *Asian Journal of Plant Science and Research* 2012; 2(1): 30–35.
7. Nisha et al. Antioxidant activity of ethanolic extract of *Maranta arundinacea. Asian Journal of Pharmaceutical and Clinical Research* 2012; 5(4):3–6.
8. Pratap Chandran R, Vysakhi M.V, Manju S, Kannan M, Abdul Kader S, Sreekumaran Nair. *In vitro* free radical scavenging activity of aqueous and methanolic leaf extracts of *Aegle tamilnadensis* abdul kader (*Rutaceae*). *International Journal of Pharmacy and Pharmaceutical Sciences* 2013, 5(3): 819-822.
9. Elizabeth K, Rao M.A, Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics* 1990; 58: 237-240.
10. Spencer J.P.E, Jenner A, Aruoma O.I. Intense oxidative DNA damage promoted by L-DOPA and

its metabolites, implications for neurodegenerative disease. *FEBS Letters* 1994; 353(3):246-250.

11. Boligon A. A, Machado M. M, Athayde M. L.Technical Evaluation of Antioxidant Activity. *Medicinal Chemistry* 2014; 4(7): 517–522.

12. Gulcin I, Huyut Z, Elmastas M, Aboul-Enein H.Y. Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry* 2010; 3(1): 43-53.