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OPTIMIZING EXTRACTION METHOD FOR THE STUDY OF SYNERGISTIC EFFECT ON ANTIOXIDANT PROPERTY IN *ALOE VERA* LEAF SKIN AND *MANGO SEED KERNEL* EXTRACT

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ABSTARCT
The agricultural food waste and industrial by products of food processing industries are potential bioactive compound sources like antioxidants, flavonoids and phenolics. These by products can be exploited to formulate a low cost rich antioxidant food supplement which will enhance the nutritional value and shelf life of lipid containing food products. Fruits and vegetables are a very good source of Antioxidant compounds including polyphenolics, flavanoids, anthocyanins ect. Fruit peels and seeds are rich in these compounds and being a byproduct of food industries they act as a promising raw material for producing antioxidants.[1,2] The high nutritional value and medicinal properties of mango can be associated with high antioxidant content. The phytochemicals like ascorbic acid, polyphenols and carotenoids are mainly responsible for antioxidant properties. The various extracts of peel from mango processing industries have shown anti-inflammatory and anti aging properties which will be utilized to enhance the nutritional values different food products.[3] Aloe vera is very well known for its therapeutic effects and is extensively used in the treatment of wounds, inflammation, antibacterial, antioxidant and burns. The bioactive compounds present in the gel comprise of hydroxyanthracenic derivatives, ferulic acid, chlorogenic acid, caffeic acid etc. which are mainly responsible for the therapeutic properties. The antioxidant properties shown by Aloe vera are widely exploited by the food industries to enhance the nutritional values of their product. It can be utilized to enhance the shelf life of lipid containing food products by reducing the oxidative rancidity and quenching the free oxygen radicals.[4] The current study is based on the optimization of extraction method as the amount of phytochemical extracted by different extraction method varies. Further attempts are made to study the synergistic effects of Aloe vera leaf skin and Mango seed kernel extract on antioxidant activity. The results showed that the extraction method involving shade drying of sample has shown better results in terms of total phenolics, total flavonoids and reducing power of the combination extract.

INTRODUCTION
Extraction as the term medically used, can be explained as the method used for separation of therapeutically required active constituent(s) and removal of unwanted insoluble material by treatment with selective solvents. Extraction generally includes the separation of complex plant compounds and solubilisation of secondary metabolites from the complex, followed by separation of soluble target components from the crude extract via selective utilization of solvents.[5]

Screening the crude plant extracts for the required bioactivity is considered as the most important step in medicinal plant research, and extraction is the initial critical step of the process. To get a complete picture of bioactivity of crude extracts, it becomes compulsory to optimize the extraction process, in order to achieve maximum possible efficiency of extraction. The necessity for selection of most appropriate extraction process is justified from the fact that when various methods are implicated on same plant material and with same solvent, extraction efficiency may vary significantly. Moreover, the method chosen as the best one also requires to be optimized in order to achieve acceptable limits of reproducibility.[6]

Mango is the tropical fruit and stands second in global production. These mangoes are processed for different food products like syrup, pickle, jam etc. and thus produce a large amount of waste in the form of peel and seed kernel. Kernel accounts for approximately 20% of the fruit weight. Studies have shown that mango seed kernel has been used to reduce oxidative rancidity of ghee, thus it can be used as a significant source of antioxidant.[7]

Aloe vera is a xerophytic succulent known for its therapeutic properties. The medicinal properties present in aloe vera gel are reported to be due to the synergistic effect of many chemical compounds in the parenchyma cells of leaf. Aloe vera is used as a health drink supplement in food industry as well as a base for the formulation of formulation of shampoo, creams, ointment and capsules.[4]

5.2 MATERIALS AND METHODS

5.2.1 Sample Preparations

Method I: 20 gm of leaf gel of Aloe vera and 20 gm of Mango kernel were weighed and mascinerated in morter and pestle with the help of water. The mascinerated sample was boiled in 200ml of distilled water for half an hour. The residue was filtered and concentrated in rotary vacuum evaporator. It was resuspended in distilled water to get the final concentration of 0.2 mg/ml. Sample was kept in the capped bottle in refrigerator till further use.

Method II: Fresh leaf gel of Aloe vera sand Mango kernel were shade dried and coarsely powdered in grinder. 40 gm of this powder was soaked in 200ml distilled water and kept for extraction for 48 hours at room temperature. This residue was filtered and concentrated in rotary vacuum evaporator. It was resuspended in distilled water to get the final concentration of 0.2 mg/ml.

Method III: Similar to method II but the solvent used was ethanol instead of distilled water.

5.2.2 Determination of Total Phenolic Content

The determination of TPC of the combination extract was performed by using Folin-Ciocalteu reagent. Briefly, 1ml of extract was prepared with 1.8 ml of Folin-Ciocalteu reagent (10 fold diluted) and kept for 5 min at 25 degree Celsius. Later 1.2 ml of 15% Sodium Carbonate was added to the reaction mixture and kept for 90 min at RT and the absorbance was measured at 765nm. The concentration of the TPC was determined as mg of Gallic acid equivalents (GAE) per gm FW.[8]

5.2.3 Determination of Total Flavonoid Content

TFC of combination extract was determined by using the aluminium chloride colorimetric method. Briefly, 0.5 ml of the extract, 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water were mixed for 5 min by vortexing. Reaction mixture was kept at RT for 30 min and the absorbance was measured at 415 nm. The results were expressed as mg of rutin equivalents (RE) per gm FW.[9]

5.2.4 Determination of Reducing Power

The reducing power was determined by the method Athukorala et al (2006). 1 ml sample of different concentration were taken. 1ml 0.2M sodium phosphate buffer pH 6.6 was added to each sample. 1ml of 1% potassium ferricyanide was added and incubated at 50^0C for 20 minutes. 1ml of 10% TCA (w/v) was added and then the samples were centrifuged at 2000 rpm for 10 minutes, 2.5ml of upper layer was taken and mixed with 2.5 ml DW. 0.5 ml of 0.1% fresh ferric chloride was added and the readings were taken at 700 nm.[10]

5.2.5 Determination of Antioxidant Activity

Different concentration of ascorbic acid (25-125µg/ml) in distilled water were prepared as standard. 0.3ml of extracts was taken in test tubes. Reagent was prepared by mixing 10ml of 0.6M sulphuric acid, 10ml of 28mM sodium phosphate, 10ml of 4mM ammonium molybdate into a beaker. 3ml reagent solution was added to the tubes. 0.3 ml reagent of methanol served as blank. All the tubes were incubated at 95ºC for 90 min. Tubes were cooled to room temperature. Optical density was measurement at 695nm using UV Spectrophotometer.

5.2.6 Free Radical Scavenging Assay or Dpph Assay

Diphenyl-picryl-hydrazyl radical scavenging (DPPH) Assay involves use of DPPH which is a stable free radical and is mostly used to estimate the radical scavenging activity of antioxidant compounds. This method is based on the principle of reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant because of the formation of the non radical form DPPH-H (Blois MS (1958). This conversion results in a color change from purple to yellow, which is measured by spectrophotometer. The disappearance of the purple color is measured at 517 nm. The inhibition percentage can be calculated using the formula:

$$\text{Inhibition (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of sample.

5.3 RESULTS

5.3.1 TOTAL PHENOLIC CONTENT

5.3.1.1 Comparative analysis of Extraction method

The variation of the total phenolic content over time for various concentrations of combination sample extracted by both the methods is presented in Table 7. TPC of the extract was expressed as mg/g GAE of dry weight of sample. Phenolic content is increasing with increasing concentration in both the samples and it is observed that the content is high in the sample extracted by the Method II. The maximum concentration of 32.2 mg/gm GAE of dry weight is observed at 0.2 mg/ml concentration.

Table 7: Total phenolic content of combination extract of Aloe vera and Mango kernel by both the methods.

S. No.	Sample conc. (mg/ml)	Phenolic content GAE (mg/gm)	
		Method I	Method II
1	0.08	11.5±0.01	14.5±0.01
2	0.12	15.7±0.04	18.7±0.02
3	0.16	22.4 ±0.03	25.4 ±0.02
4	0.20	29.2±0.04	32.2±0.04

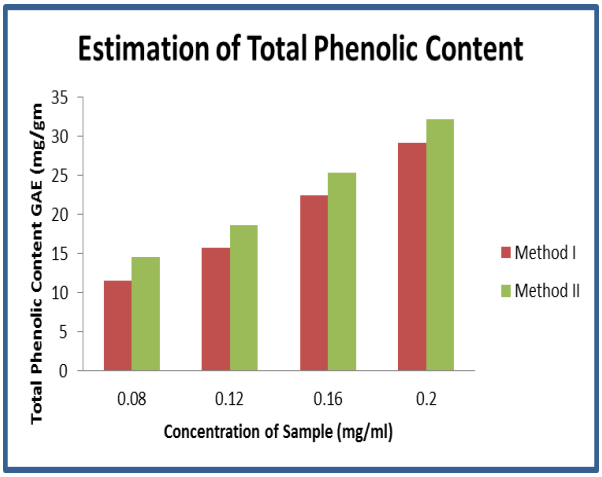

Figure 26: Total Phenolic Content of Combination Extract of Aloe Vera and Mango Kernel By Both The Methods.

5.3.1.2 Comparative Analysis of Extraction Solvent

The variation of the total phenolic content over time for various concentrations of both the solvent extracts is presented in Table 8.TPC of the extract was expressed as mg/g GAE of dry weight of sample. Phenolic content is increasing with increasing concentration in both the samples and it is observed that the content is high in the sample extracted in ethanol. The maximum concentration of 37.6mg/gm GAE of dry weight is observed at 0.2 mg/ml concentration.

Table 8: Total Phenolic Content of Combination Extract of Aloe Vera and Mango Kernel By Both The Solvents.

S. No.	Sample conc. (mg/ml)	Phenolic content GAE (mg/gm)	
		Water Extract	Ethanol Extract
1	0.08	14.5±0.01	16.3 ±0.05
2	0.12	18.7±0.02	28.62±0.04
3	0.16	25.4 ±0.02	30.93±0.01
4	0.20	32.2±0.04	37. 6 ±0.05

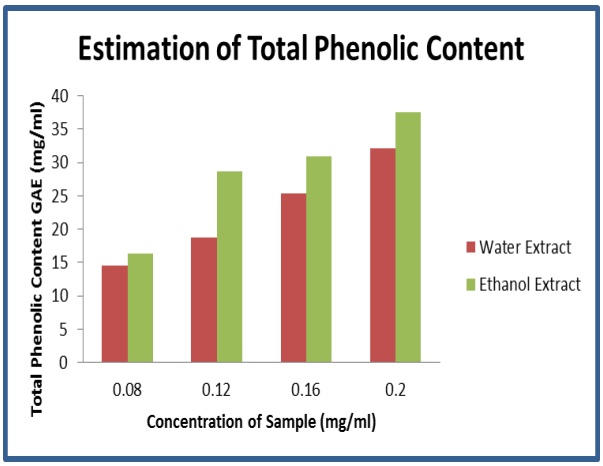

Figure 27: Total Phenolic Content of Combination Extract of Aloe Vera And Mango Kernel By Both The Solvents.

5.3.2 TOTAL FLAVONOID CONTENT

5.3.2.1 Comparative analysis of Extraction method

The variation of the flavonoid content over time for various concentrations of sample extracted by both the methods is presented in Table 9. Flavonoid content of the extract was expressed as mg RE/gm of sample. Total Flavonoid content (TFC) is increasing with increasing concentration in both the samples and it is observed that the amount of flavonoid is high in the sample extracted by the Method II.Rutin was used as standard and the maximum concentration of 36.22mg/gm RE of dry weight is observed at 0.2 mg/ml concentration.

Table 9: Total Flavonoid Content of Combination Extract of Aloe Vera and Mango Kernel By Both The Methods.

S. No.	Sample Conc. (mg/ml)	Flavonoid Content RE (mg/gm)	
		Method I	Method II
1	0.08	17.8±0.03	19.39±0.02
2	0.12	19.14±0.06	22.43±0.01
3	0.16	24.29±0.01	29.40±0.03
4	0.20	28.66±0.02	36.22±0.04

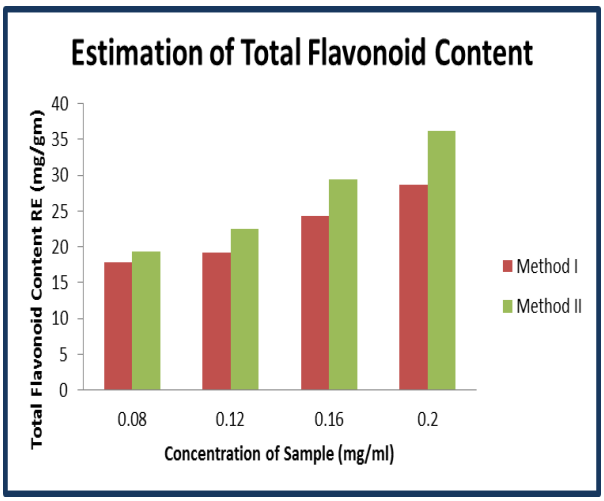

Figure 28: Total Flavonoid Content of Combination Extract of Aloe Vera and Mango Kernel By Both The Methods.

5.3.2.2 Comparative analysis of Extraction Solvent

The variation of the flavonoid content over time for different concentrations of both the solvent extracts is presented in Table 10. It was observed that the Flavonoid content of the ethanol extract was higher as compared to water extract. Total Flavonoid content (TFC) is increasing with increasing concentration in both the samples. As Rutin was used as the standard the maximum concentration of 49.02mg/gm RE of dry weight is observed at 0.2 mg/ml concentration of ethanol extract.

Table 10: Total Flavonoid Content of Combination Extract of Aloe Vera and Mango Kernel By Both The Solvents.

S. No.	Sample conc. (mg/ml)	Flavonoid content RE (mg/gm)	
		Water Extract	Ethanol Extract
1	0.08	19.39±0.02	20.43±0.03
2	0.12	22.43±0.01	28.05±0.02
3	0.16	29.40±0.03	41.2±0.04
4	0.2	36.22±0.04	49.02±0.02

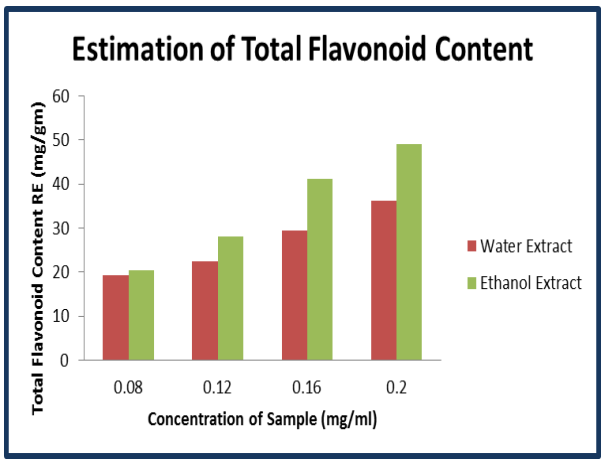

Figure 29: Total Flavonoid Content of Combination Extract of Aloe Vera and Mango Kernel By Both The Solvents.

5.3.3 REDUCING POWER

5.3.3.1 Comparative analysis of Extraction method

The variation of the reducing activity over time for various concentrations of both the samples is presented in Table 11. It is observed that the reducing power is concentration dependent in both the samples and it is comparatively high in sample extracted by Method II. The maximum activity is observed at 0.2 mg/ml concentration of sample extracted by Method II.

Table 11: Reducing Activity of Combination Extract of Aloe Vera and Mango Kernel By Both The Methods.

S. No.	Sample conc. (mg/ml)	Reducing power BHAE (mg/gm)	
		Method I	Method II
1	0.08	0.376±0.05	0..498±0.03
2	0.12	0.492±0.03	0.568±0.05
3	0.16	0.526±0.05	0.652±0.03
4	0.20	0.588±0.04	0.733±0.02

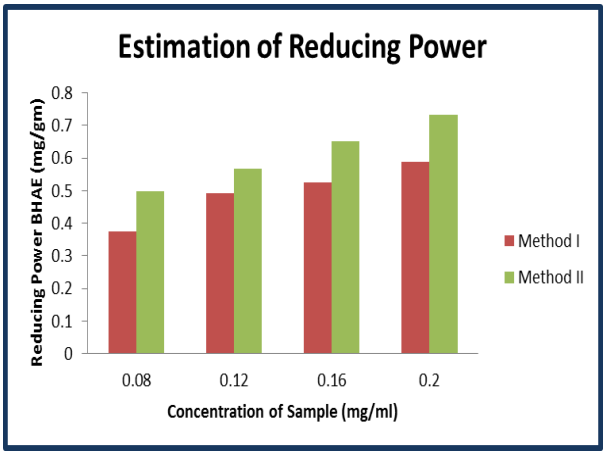

Figure 30: Reducing Power of Combination Extract of Aloe Vera and Mango Kernel By Both The Methods

5.3.3.2 Comparative analysis of Extraction solvent

The variation of the reducing activity over time for both the solvents at various concentrations is presented in Table 12. BHA is used as a standard. It is observed that the reducing power is concentration dependent in both the solvents but in ethanol extract it has shown high reducing capacity as compared with water extract. The maximum activity is observed at 0.2 mg/ml concentration of ethanol extract.

Table 12: Reducing Activity of Combination Extract of Aloe Vera and Mango Kernel By Both The Solvents.

S. No.	Sample conc. (mg/ml)	Reducing power BHAE (gm/gm)	
		Water Extract	Ethanol Extract
1	0.08	0..498±0.03	0.587±0.03
2	0.12	0.568±0.05	0.724±0.06
3	0.16	0.652±0.03	0.865±0.02
4	0.2	0.733±0.02	0.921±0.04

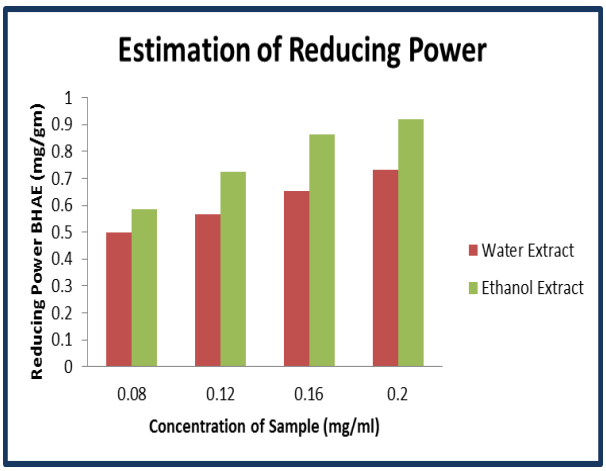

Figure 31: Reducing Power of Combination Extract of Aloe Vera and Mango Kernel By Both The Solvents.

5.3.4 TOTAL ANTIOXIDANT ACTIVITY

The variation of the antioxidant activity over time for various concentrations of both the samples is presented in Table 13. Total antioxidant activity of the extract was expressed as Ascorbic acid equivalents (AAE). Total antioxidant activity is concentration dependent and is high in sample extracted by Method II. The maximum concentration of 8.31 mg/gm of AAE of dry weight is observed at 0.2 mg/ml concentration in sample extracted by Method II.

Table 13: Total Antioxidant Activity of Combination Extract of Aloe Vera and Mango Kernel By Both The Methods.

S. No.	Sample conc.(mg/ml)	Antioxidant Capacity AAE (mg/gm)	
		Method I	Method II
1	0.08	3.01±0.04	3.24 ±0.05
2	0.12	3.92±0.05	4.87±0.07
3	0.16	4.56±0.08	6.56±0.02
4	0.20	5.83 ±0.03	8.31±0.06

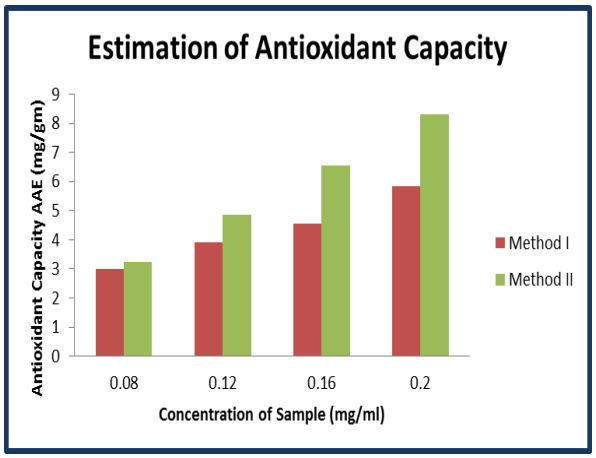

Figure 32: Total Antioxidant Activity of Combination Extract of Aloe Vera and Mango Kernel By Both The Methods.

5.3.5 Radical Scavenging Activity

The result of radical scavenging property of combination extract of Aloe vera and Mango kernel is shown in Table 14. It states that the maximum % inhibition of 96.5% was shown by the ethanol extract at the concentration of 20 µg/ml. The % inhibition in both the samples increases with increase in concentration. The high amount of radical scavenging property shown by both the extracts makes them a great candidate with natural antioxidant capacity.

Table 14: Radical Scavenging Activity of Combination Extract of Aloe Vera and Mango Kernel By Both The Solvents.

S. No.	Sample conc. (µg/ml)	Radical scavenging % inhibition	
		Water Extract	Ethanol Extract
1	08	83.5±0.05	87.4±0.01
2	12	86.8±0.07	91.9 ±0.05
3	16	90.6±0.04	94.2 ±0.04
4	20	91.9±0.02	96.5 ±0.08

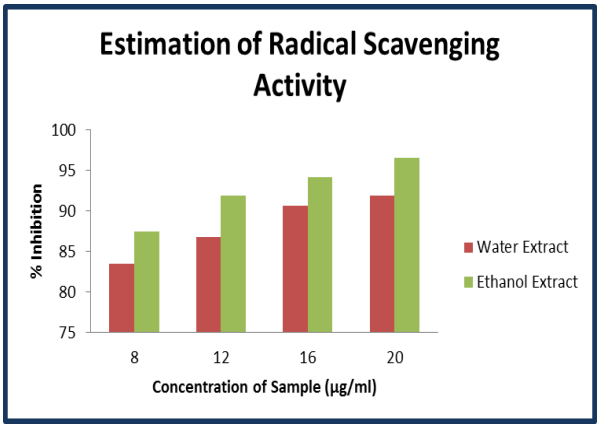

Figure 33: Radical Scavenging Activity of Combination Extract of Aloe Vera and Mango Kernel By Both The Solvents.

5.4 DISCUSSION

Selective isolation of the desired compounds from the sample with maximum yield and removal of undesired components are the main objectives of the extraction processes. To have the full image of the bioactivity of crude extracts, it is very important to standardize the extraction process to get all the possible varieties of phytochemicals. With the increasing demand for natural medicinal products, and herbal products for health care all over the world, herbal drug producers target at using the most apparent extraction techniques to generate extracts of desired quality with minimum batch to batch variation, which can also help in scale-up of extraction. Quality of an extract is depended on several factors like, plant parts utilized as starting material, solvent utilized for extraction, extraction procedure, and plant material: solvent ratio, etc.

5.5 Statistical Analysis

Total Phenolic content, Total Flavonoid content, Reducing power, Antioxidant activity and DPPH assays were performed in triplicate. Mean values for different parameters were calculated and compared by analysis of variance (one-way ANOVA) using online software. Moreover, statistical differences between mean values were identified at confidence level $p<0.001$.

5.6 CONCLUSION

The sample extracted by Method II showed high polyphenolic and flavonoid content as well as strong

antioxidant potential when compared with method I of extraction. Similarly, the ethanol extract of combination of Aloe vera and Mango kernel shows better antioxidant properties as compared to water extract. The extract contains lower amount of phenolics as compared to the flavonoids. The raw material being inexpensive and easily available should be regarded as potential nutraceutical resource, capable of offering significant nutritional dietary supplements. Also the natural antioxidants in the form of phenols and flavonoids can be easily extracted and thus it offers opportunities to formulate value added products in nutraceutical and food applications to enhance health benefits.

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