

# Identification of Novel Food Additive Antioxidant from *Psidium Guajava*, *Moringa Oleifera*, *Punica Granatum* Blend Leaves Extracts

Sabila R. Mirza<sup>1</sup> and Surekha B. Dabhade<sup>2</sup>

<sup>1</sup>Research Scholar, Department of Agricultural Engineering, Maharashtra Institute of Technology, Aurangabad, Maharashtra, India

<sup>2</sup>Assistant Professor, Department of Agricultural Engineering, Maharashtra Institute of Technology, Aurangabad, Maharashtra, India

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**Abstract** - Antioxidants are food additives which are added to lipid containing foods to extend its storage stability. The mechanism of antioxidant action as free radical scavenging, pro-oxidative chelating agent and quenching singlet oxygen had made antioxidant as an essential food ingredient to stop the lipid oxidations. Natural antioxidant molecules are safer than synthetic antioxidants. The leaves extract of plants can act as a potential source of antioxidant. Plant leaves exhibits a specific antioxidant components profiles with their synergistic preservative, nutritional and health benefits. Hence the study was done to formulate a food additive antioxidant blend from *Psidium Guajava*, *Moringa Oleifera* and *Punica Granatum* Leaves. The blends from three leaves formulation with different concentration as Blend A, Blend B and Blend C. The evaluation of antioxidant activity was done by DPPH radical scavenging activity assay, TLC guided Bio-autography assay and ATR-FTIR Assay and thermal stability was evaluated by TGA Assay. According to the overall evaluated results the Blend A was found to be a novel antioxidant blend. The antioxidant activity showed by DPPH assay 30.08 % inhibition. TLC Rf values 0.979 and ART-FTIR confirms the antioxidant bands regions with peaks lying between  $1097\text{ cm}^{-1}$  and  $3853.7\text{ cm}^{-1}$  respectively on graph and thermal stability was found to be 42.83 % mass loss. This study concludes that blend A (GMP-532) possess good antioxidant potential and excellent thermal stability, so thereby can consider as a novel food additive antioxidant.

**Key Words:** *Psidium guajava*, *moringa oleifera*, *punica granatum*, Leaves, Novel antioxidant, Food Additive, blend, Antioxidant activity, Thermal Stability.

## 1. INTRODUCTION

*Psidium guajava* L., popularly known as guava, is a small tree belonging to the myrtle family (Myrtaceae). leaves had been used traditionally in the treatment of various diseases. In Indonesia, Guava leaf is commonly used to treat diarrhea, gastroenteritis and other digestive complaints, while the Guava fruit has been used to increase platelets in patients with dengue fever. Many studies have been done to scientifically prove efficacy in the treatment of guava leaf.

Among them were the benefits of guava leaf as a remedy [7]. Guava leaf has great potential to be developed as functional ingredients. Firstly, they are widely available, with a guaranteed supply. Secondly, guava leaf naturally occurring compounds, and their extraction is relatively cost effective. Lastly, they contained high level of antioxidant, phenolic compound. on the measurements results of phenol total content, antioxidant [10].

*Moringa oleifera* tree (family Moringaceae) are considered as rich source of nutritionally important phytoconstituents [9]. Although the nutrient potential of *M. oleifera* has been reported, information regarding stability of phytoconstituents during dehydration is not available. Therefore, in present investigation, the effects of various drying methods on the retention of carotenoids,  $\alpha$ -tocopherol, ascorbic acid, total phenolics and antioxidant properties were evaluated [2]. A ready to use chutney powder (additive) was prepared from cabinet tray dried leaves of *M. oleifera* (retain maximum nutrients) and it was well accepted with overall high-quality score [14].

*Punica granatum* is a fruit bearing deciduous shrub or small tree in the family Lythraceae. The region extending Iran through Afghanistan and Pakistan to northern India, and has been cultivated since ancient times throughout the Mediterranean region. It grows between 5 and 8 m (16 and 26 ft) tall. [21] *P. granatum* leaves are opposite or sub opposite, glossy, narrow oblong, entire, 3–7 cm (1.2–2.8 in) long and 2 cm (0.79 in) broad. [22].

There has been considerable interest in the field of antioxidants in recent years and these efforts have led to a better understanding of the mechanisms involved and of the application areas in food. In addition, efforts are underway for devising better and more uniform methodologies for the evaluation and measurement of the oxidation and efficacy of antioxidants [17].

The latest developments about novel antioxidants particularly phenol derivatives, peptides/proteins hydrolyses, phospholipids and polysaccharides and their role in food quality preservation [5].

The antioxidant efficiency (AE) has been shown to be a more adequate parameter for selecting antioxidants than the widely used EC (50) [19].

ATR-FTIR Assay Attenuated Total Reflectance-Fourier transform Infrared spectroscopy. The ART-FTIR assay relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This Absorption corresponds specifically to the bonds present in the molecule. The frequency range are measured as wave numbers typically over the range 4000-600 cm [13].

TLC Bio-autography Assay is a combinative method using high-speed counter-current chromatography (HSCCC) and thin layer chromatography (TLC) as an antioxidant autographic assay was developed to separate antioxidant components from the fruits [6]. Thin layer chromatography (TLC) bio-autography assay is the best quick, convenience, simple and efficient method, where the TLC plates are sprayed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) as derivatizing agent and looked for yellow or pink spots for antioxidant component under UV chamber [1].

Cooking was a breakthrough for mankind that improved the flavor digestibility and quality of food. Heat treatment is an operation widely used in food processing. As heat-processed foods are much appreciated, analytical studies that can assess the changes caused by heating have been demanded from researchers [16].

Food is often a complex system including various compositions and structures. The thermal analysis information from TGA can be directly used to understand the thermal transitions that the food system may undergo during processing or storage. [16].

The knowledge of thermal stability of antioxidants is very important in food preservation. Thermogravimetric techniques continuously measure the mass of a sample as it is heated or cooled at a controlled rate or is held at a particular temperature for a period of time. It is useful for monitoring processes that involve changes in the mass of food or food component [15].

Thermogravimetry determines the mass change of sample as a function of temperature or time. In TGA a continued graph of mass change against temperature is obtained when a substance is heated at a uniform rate or kept at constant temperature. Such measurements can provide both qualitative and quantitative information concerning physical and chemical changes. [17]

## 1. MATERIAL AND METHODOLOGY

### 2.1 MATERIAL

#### 2.1.1 Plant Material

The leaves were collected from the shrub plants namely *Psidium Guajava*, *moringa oliefera* and *punica granatum* for the formulation of antioxidant novel blend from the botanical gardens of Himayat baugh, Aurangabad and was authenticated by Prof. Dhabe from the botany department of B.A.M. University with the accession numbers no. 0604, 0605, 0606.

#### 2.1.2 Chemicals and reagents

Chemical used from different reputed companies mentioned in bracket, DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich), gallic acid (SRL), ascorbic acid (SRL), HPLC grade methanol and chloroform (Deepa chemicals), All chemicals used in this study was of analytical grade and provided by the department of Agriculture Engineering, M.I.T., Aurangabad.

#### 2.1.3 Equipments

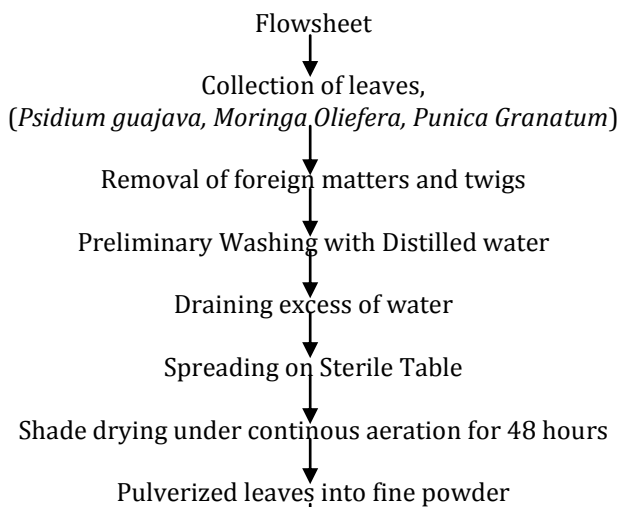
Spectrophotometer, Thermo gravimetry Analyser TGA model TGA-50, ATR-FTIR model IRAffinity-1S of Shimadzu Company, TLC silica plates (Merck, F245) was used for analysis.

## 2.2 METHODOLOGY

### 2.2.1 Preparation of extract

The leaves used for the preparation of antioxidant blend from shrub plants namely, *Psidium guajava*, *Moringa Oliefera* and *Punica Granatum*. The leaves for the formulation of antioxidant blends was harvested in the month of October from the botanical garden of Himayat baugh. The collected leaves were sorted for fresh, clean and infest free and free from any foreign matters, twigs etc. The fresh leaves were processed by multiple washing with distilled water. Then the leaves extracts were prepared by pulverizing the shade dried leaves into fine powder extracts. Each extract from different plant are packed separately in air tight PET containers and stored at room temperature. One way to preserve such plant products is to dry them in order to preserve their desirable qualities, reduce storage volume and to extend shelf-life. In addition, it is also important to retain the biological activity of important phytoconstituents, including antioxidants and nutrients as well as avoid undesirable chemical or physical changes. [14].

### 2.2.1.1 Flowsheet for Preparation of extract



**Table-1:** Optimization of blend Concentration.

S r. N o	Name of blends	Concentration in percentage			Blend codes
		<i>Guajava</i> <i>L</i>	<i>Moring</i> <i>a L</i>	<i>Punica</i> <i>L</i>	
1	Blend A	50%	30%	20%	GMP (532)
2	Blend B	20%	50%	30%	GMP (253)
3	Blend C	30%	20%	50%	GMP (325)

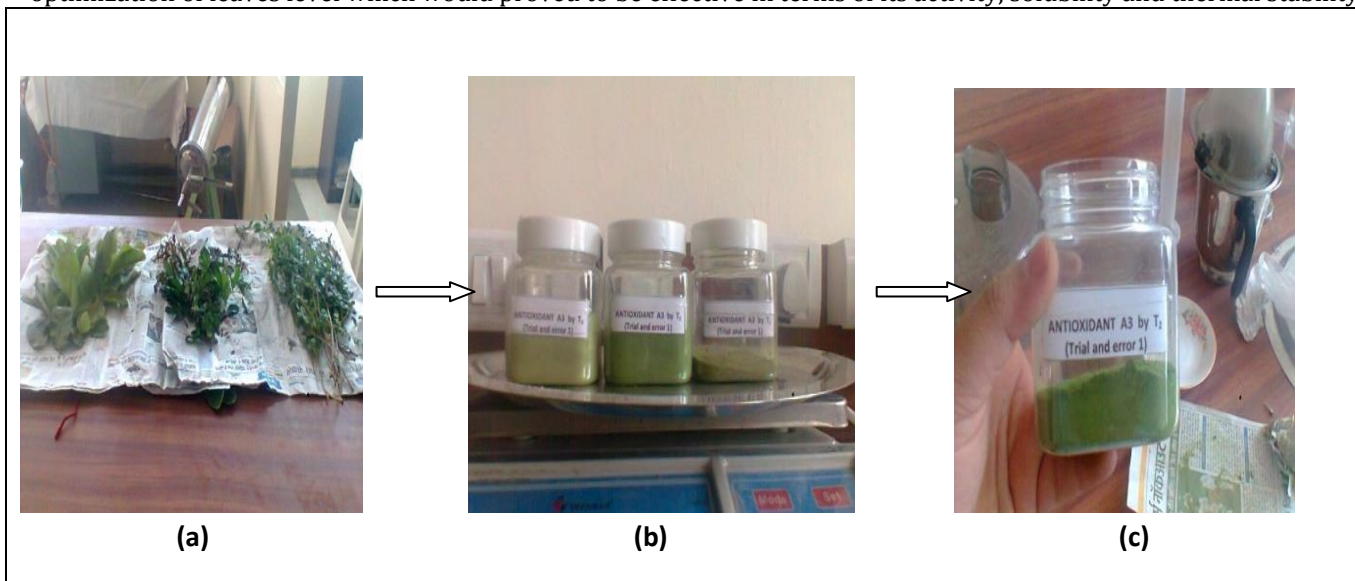
Straining of leaves extract

Packaged in air tight PET bottles,  
(Separate packing of each plant leaves extract)

Stored at Ambient temperature in cool, dry and dark place

### 2.2.2 Optimization of Blends

The antioxidant blends were formulated from the leaves of plants namely *Psidium Guajava*, *Moringa Oliefera* and *Punica Granatum* with different concentration levels of leaves extract so as to prepare the broad-spectrum antioxidant by optimization of leaves level which would proved to be effective in terms of its activity, solubility and thermal stability.



**Fig-1:** *Psidium Guajava*, *Punica Granatum* and *Moringa Oliefera* leaves (fresh) (a), Prepared leaves Extract for antioxidant blends (b) Prepared blend antioxidant from the leaves extracts (c).

### 2.2.3 DPPH radical scavenging Assay

DPPH radical scavenging activities of the selected leaves extracts from plant material *Psidium guajava*, *moringa oliefera* and *punica granatum* was first evaluated by slightly modified method reported by [4]. for its radical scavenging activity individually so as to provide antioxidant activity data to analyze blends with different concentration of leaves through its antioxidant activity profile. The scavenging activity of all leaves and their blends ascorbic acid was used as standard reference antioxidant. All reaction mixtures were kept in the dark for 15 mins and the optical density was noted at 517nm. For the control, DPPH in methanol was taken without extract and the optical density was recorded after 15 mins. The antioxidant activity of each sample was calculated using the following Eq.

$$\% \text{ Inhibition} = \left[ \frac{\text{Control (517nm)} - \text{Test absorbance(517nm)}}{\text{A control (517 nm)}} \right] \times 100$$

### 2.2.4 TLC DPPH guided Bioautography

Thin Layer Chromatography DPPH bio-autography for antioxidant activity of blend leaves extracts were chromatographed using TLC silica plate (Merck, F245) having chloroform as mobile phase. Developed plates were dried, sprayed with DPPH (0.004% w/v in 95% methanol) and observed for development of bright yellow to pink

colour under UV chamber for confirmation as antioxidant molecule. The Rf value of the samples were calculated.

$$R_f \text{ Value} = \frac{\text{Distance travelled by component}}{\text{Distance travelled by solvent}}$$

### 2.2.5 ATR- FTIR Assay

For the clear and non-interfering hydro peaks, the blend leaves extract samples were primarily moisture free till the constant moisture obtained. Then 4 to 5 mg of the sample was placed on the diamond probe and the system was run for 15 mins to show the graphs of blend leaves extracts.

ATR-FTIR follows the *Hooke's law*.

$$V = K \sqrt{f/m}$$

F=force constant  
M=mass  
K=constant

ATR-FTIR Assay Attenuated Total Reflectance-Fourier transform Infrared spectroscopy. The ART-FTIR

assay relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This Absorption corresponds specifically to the bonds present in the molecule. The frequency range are measured as wave numbers typically over the range 4000-600 cm [13].

### 2.2.6 TGA Assay

The Blend leaves extracts weighed on digital weighing balance before subjecting to thermal analysis as its function against the mass loss with respect to temperature. Approximately 4 to 5 mg of blend leaves extract was taken for thermal analysis and the system is run for several hours to reach to 0 to 400°C temperature range. These thermo analytical curves were obtained in module, simultaneously, at TG-50 Shimadzu model. The used is of aluminium, the atmosphere maintained for thermal analysis was nitrogen with flow rate of 50 ml/min with average increase in temperature by 10°C /per minute.

## 3 RESULT AND DISCUSSION

### 3.1 DPPH Assay

Antioxidant are compounds that that can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. [4]

The guava leaf extracts displayed a significant scavenging ability on the peroxy radicals. However, the scavenging effects were decreased when the extract concentration was greater than 10 µg/ml. The extracts from leaves of various guava cultivars exhibited more scavenging effects on free radicals than did commercial guava tea extracts and dried fruit extracts. [3]

DPPH Scavenging Activity was carried out to evaluate free radical scavenging activity of leaves and their formulated blends. The DPPH assay shows leaves antioxidant activity in pattern guajava > Moringa > Punica.

With 29.31 % inhibition by guajava leaves, 16.62% inhibition by punica leaves and 15.34% inhibition by moringa leaves.

DPPH Scavenging Activity was carried out for their blends shows Blend -A showed 30.08% , Blend-B 12.08%, Blend-C 18.08% inhibition and out of which Blend A has been confirmed as the most Potential Broad Spectrum Antioxidant with 30.08 % through DPPH

The antioxidant compounds found in *guajava* leaves extract was highest with 29.31% inhibition and somewhat the scavenging activity of blend A was also proved to be highest with 30.08% inhibition as the concentration of *guajava* leaves was at prominent rate of 50% in the blend A, Hence and their blends shows a linear relation in plants have different polarities; hence different solvents are used to



isolate antioxidant activity. The yield of the extract and antioxidant activity depends.

The scavenging activity of Blend B was 12.08% inhibition and moringa leaves extracts shows the least scavenging activity with 15.34% inhibition as the concentration of moringa extract was prominent in blend B with 50% so similarly Blend B showed the least scavenging activity. The scavenging activity of Blend C was 18.08% inhibition and the *punica* leaves extract showed 16.62% inhibition the blend C founds to be have an intermediate scavenging activity as the concentration of *punica* leaves extract was a prominent with 50% hence Blend C showed an intermediate. scavenging activity in between Blend A and Blend C.

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The scavenging activity of Blend C was 18.08% inhibition and the *punica* leaves extract showed 16.62% inhibition the blend C founds to be have an intermediate scavenging activity as the concentration of *punica* leaves extract was a prominent with 50% hence Blend C showed an intermediate. scavenging activity in between Blend A and Blend C.

**Table -2:** Evaluation of Antioxidant Activity of Leaves

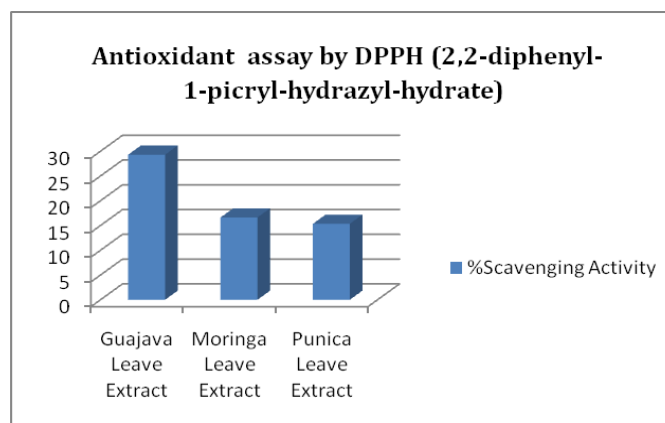
DPPH Antioxidant Scavenging Assay of Leaves			
Sample Code	Absorbance (µg/ml)	Scavenging Activity	% Scavenging Activity
<i>Psidium Guajava</i> L	0.3955	0.2931	29.31

**Chart no-1: Scavenging activities of Leaves extracts (a) and Scavenging activity of Leaves Blend Extract (b).**

### 3.2 TLC guided Bioautography

The TLC guided bio-autography was done on three shrub plant leaves to find out its antioxidant activity. The Rf values results obtained from TLC can correlate with its antioxidant activity.

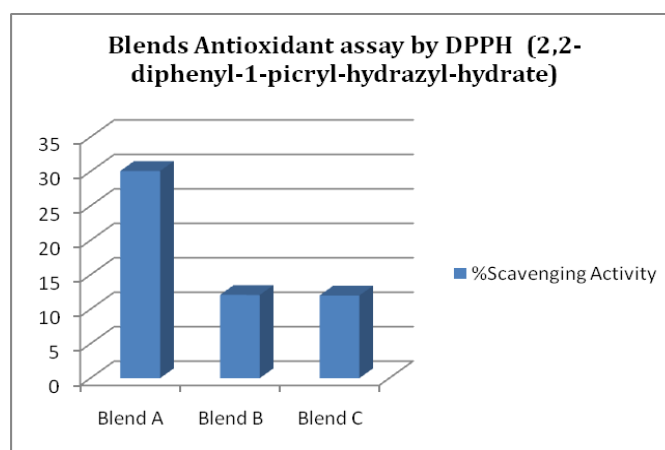
<i>Punica Granatum</i> L	0.4665	0.1662	16.62
<i>Moringa Oliefera</i> L	0.4061	0.1534	15.34



(a)

**Table -3:** Evaluation of Antioxidant Activity of Blends

DPPH Antioxidant Scavenging Assay of Blends			
Sample Code	Absorbance (µg/ml)	Scavenging Activity	% Scavenging Activity
Blend A	0.3912	0.3008	30.08
Blend B	0.4919	0.1208	12.08
Blend C	0.4583	0.1808	18.08



(b)

All the extracts possess antioxidant activity that can be evidenced through the TLC bio-autography analysis for antioxidants (Fig.6 & 7).

The leaves extracts of *psidium guajava*, showed Rf values as 0.979 and the distance travelled by the spot was 4.8 cm and distance travelled by solvent front was 4.9 cm, *moringa oliefera* shown Rf Value 0.792 and the distance

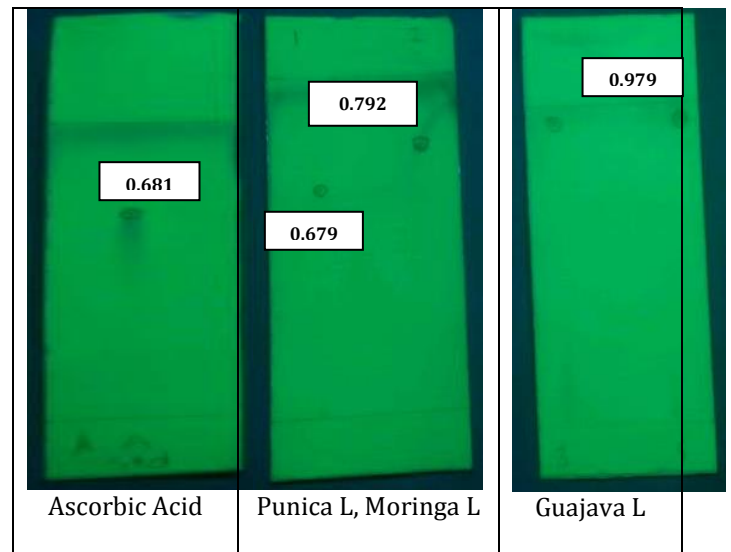
travelled by the spot was 4.2 cm and distance travelled by solvent front was 5.3 cm, *Punica granatum* shown Rf Value 0.679 developed and the distance travelled by the spot was 4.8 cm and distance travelled by solvent front was 4.9 cm. Amongst these three leaves extracts, the guajava showed highest Rf value. The Rf value of Acacia Leaves extract was highest with Rf value 0.984

The TLC guided identification Assays. The Rf values obtained by Psidium guajava 0.979, Moringa Oliefera 0.792 and punica granatum 0.679. The Rf values obtained by the blends shows, blend-A 0.979, blend-B 0.941 and blend-C 0.901. The Rf value obtained by blend -A shows higher values than synthetic antioxidant BHT [1].

The chromatogram data indicated that guava extracts contained phenolic acids, such as ferulic acid, which appeared to be responsible for their antioxidant activity. Correlation analysis indicated that there was a linear relationship between antioxidant potency, free radical-scavenging ability and the content of phenolic compounds of guava leaf extract [3].

**Table -4:** TLC Assay for Rf Values of Leaves for Blend

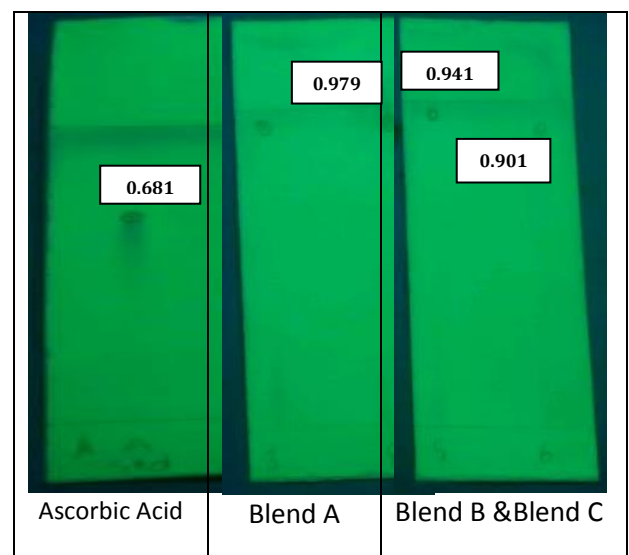
DPPH TLC bioautography of Leaves			
Sample code	Distance Traveled by the centre of the spot (cm)	Distance travelled by the solvent front (cm)	Rf Values
Std Ascorbic Acid	3.0	4.4	0.681
Punica L	3.6	5.3	0.679
Moringa L	4.2	5.3	0.792
Guajava L	4.8	4.9	0.979



(a)

**Table -5:** TLC Assay for Rf Values of Antioxidant Blends

DPPH TLC bioautography of Blends against Synthetic Antioxidant			
Sample code	Distance Traveled by the centre of the spot (cm)	Distance travelled by the solvent front (cm)	Rf Values
Std Ascorbic Acid	3.0	4.4	0.681
Blend A	4.8	4.9	0.979
Blend B	4.8	5.1	0.941
Blend C	4.6	5.1	0.901



(b)

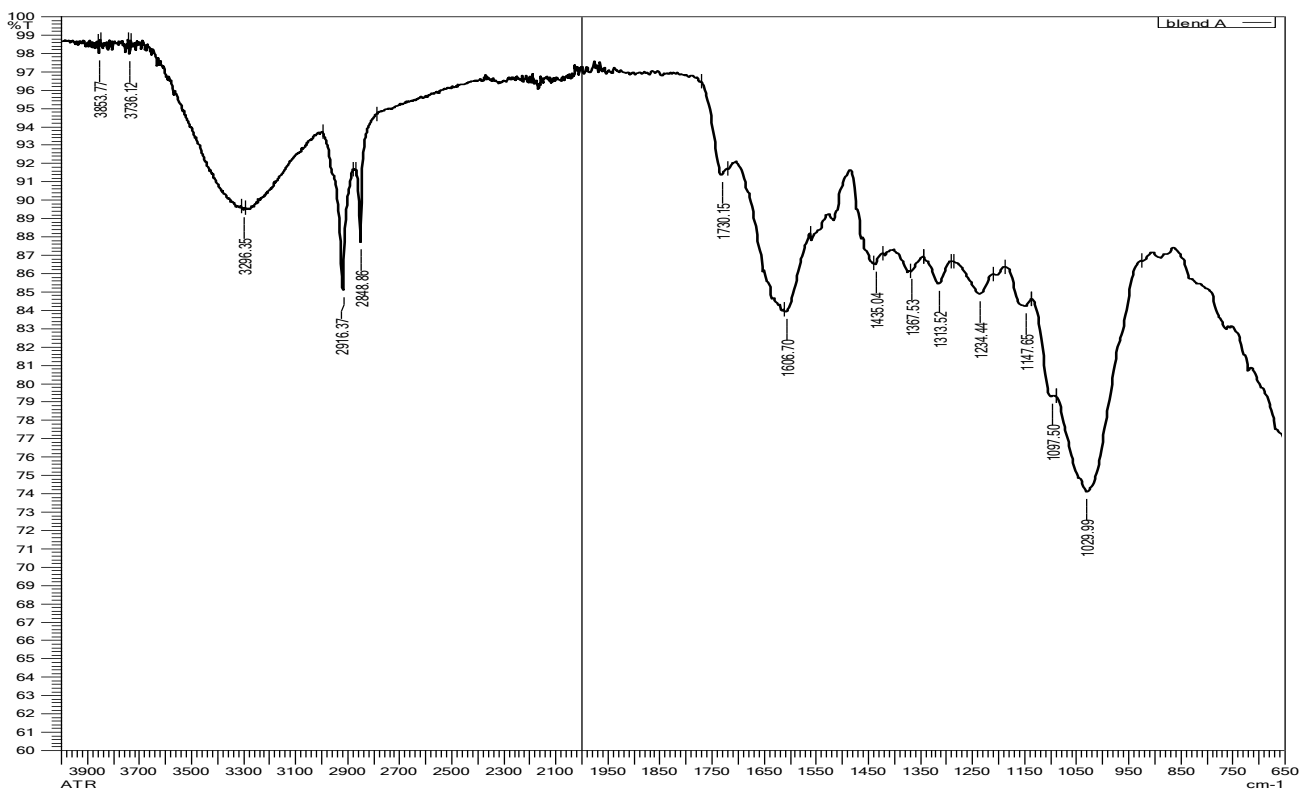
**Chart no-2:** TLC bio-autography of Leaves Extracts (a) and TLC bio-autography of Antioxidant Blends (b).

### 3.3 ATR-FTIR Assay

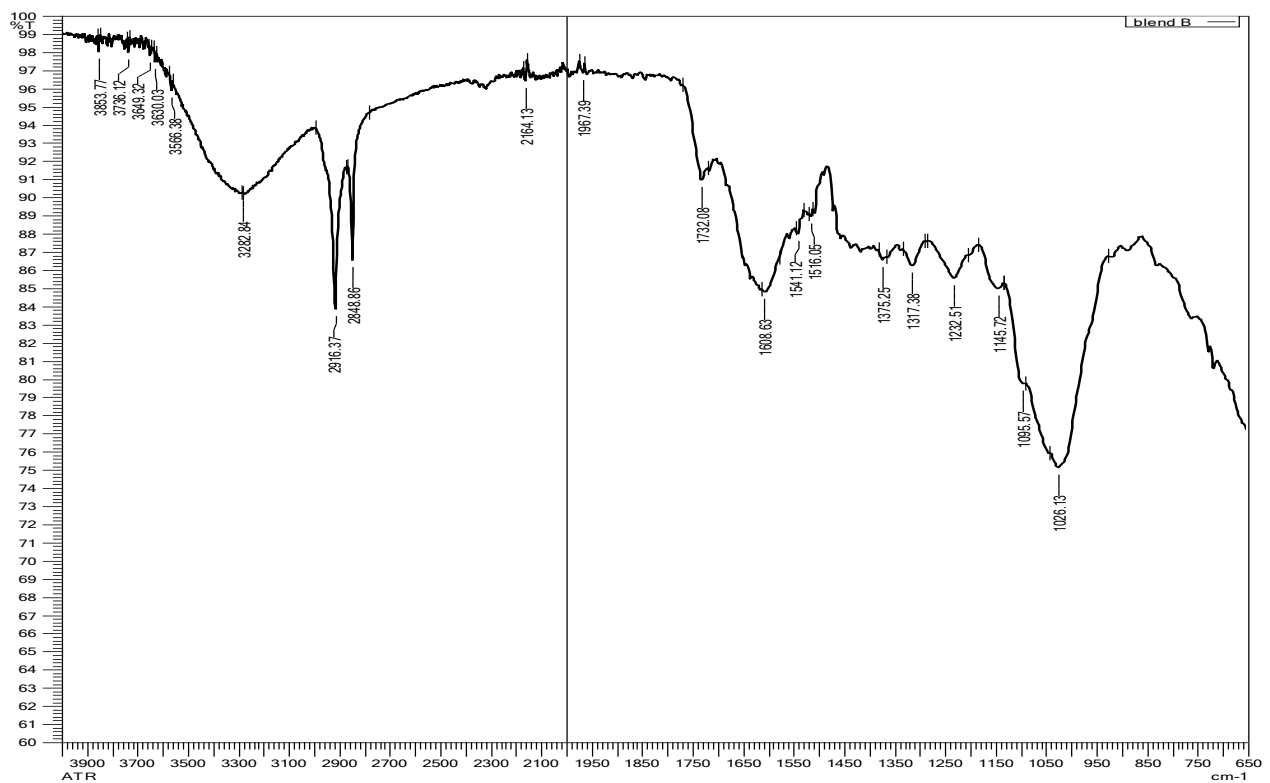
**Table-6:** FTIR Assay for Antioxidant components screening of Blend A, B & C with their comparative peak value.

ATR-FTIR Screening for Antioxidant Components of Blends			
ATR-FTIR Scale	Peak Values	Peak Values	Peak Values
(400-4000)	Blend -A	Blend- B	Blend-C
	1029.99	1026.13	763.81
	1097.5	1095.57	833.32
	1147.65	1145.72	1022.27
	1234.44	1232.51	1097.5
	1313.52	1317.38	1163.08
	1367.53	1375.25	1228.66
	1435.04	1516.05	1317.38
	1606.7	1541.12	1367.53
	1730.15	1608.63	1456.26
	2848.66	1732.08	1514.12
	2916.37	1967.39	1620.21
	3296.35	2164.13	1639.49
	3736.12	2848.86	1687.71
	3853.77	2916.37	1730.15
		3282.84	2850.79
		3566.38	2918.3
		3630.03	3305.99
		3649.37	

FTIR assay reveals that Blend A shows 14 peaks lies in between 1097  $\text{cm}^{-1}$  and 3853.7  $\text{cm}^{-1}$ . Blend B shows 18 peaks between 1026.13  $\text{cm}^{-1}$  and 3649.32  $\text{cm}^{-1}$  and Blend C shows 16 peaks that ranges between 763.81  $\text{cm}^{-1}$  and 3305.99  $\text{cm}^{-1}$ . Higher the peak values showed the presence of antioxidant components when compared with the standard gallic acid and other synthetic antioxidant peaks values 3398.98, 1448.35, 1619.90, 1448.35, 1206.31, 1363.89, 1054.90, 607.19 peak values obtained in guajava leaf extract Upendra [18]. Band at 3433.49  $\text{cm}^{-1}$  indicates phenolic compounds OH group. [8]. Blend A showed more peaks in this phenolic region where as Blend C showed peak at 763.81  $\text{cm}^{-1}$ . Band at 688.67  $\text{cm}^{-1}$  indicates C-H bonding to aromatic groups which are responsible for antioxidant activity [8]. we considered that the blend A contains highest phenolic contains showed by peak 3853.77  $\text{cm}^{-1}$ .

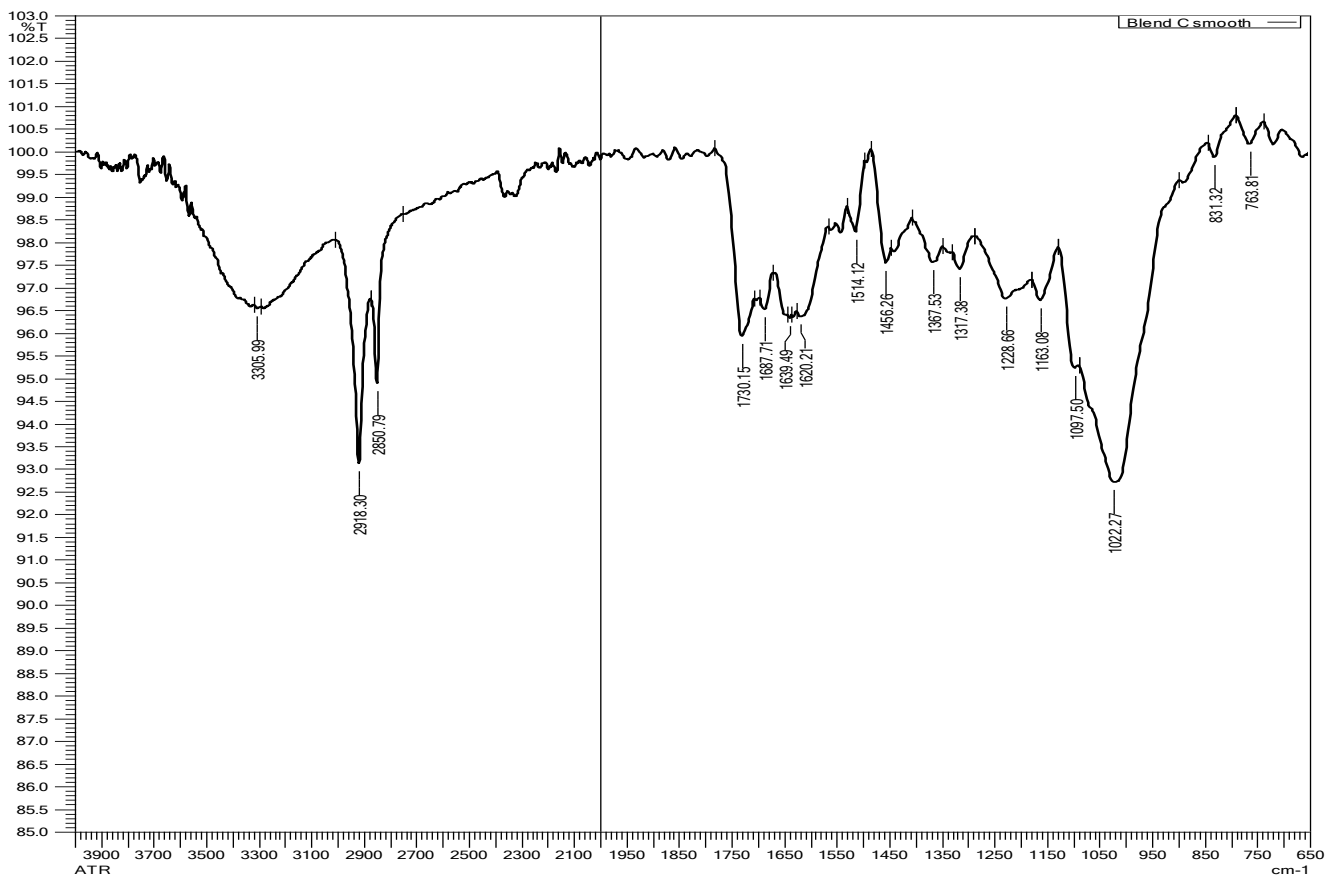


(a)



(b)





(c)

**Chart-3:** FTIR ATR graph shows the screening antioxidant groups from the specific band region with peak values of Blend A (GMP-532) (a), Blend C (GMP-235) (b) & Blend C(GMP-, (c) here peak value in the band region considered as confirmation for the antioxidant activity.

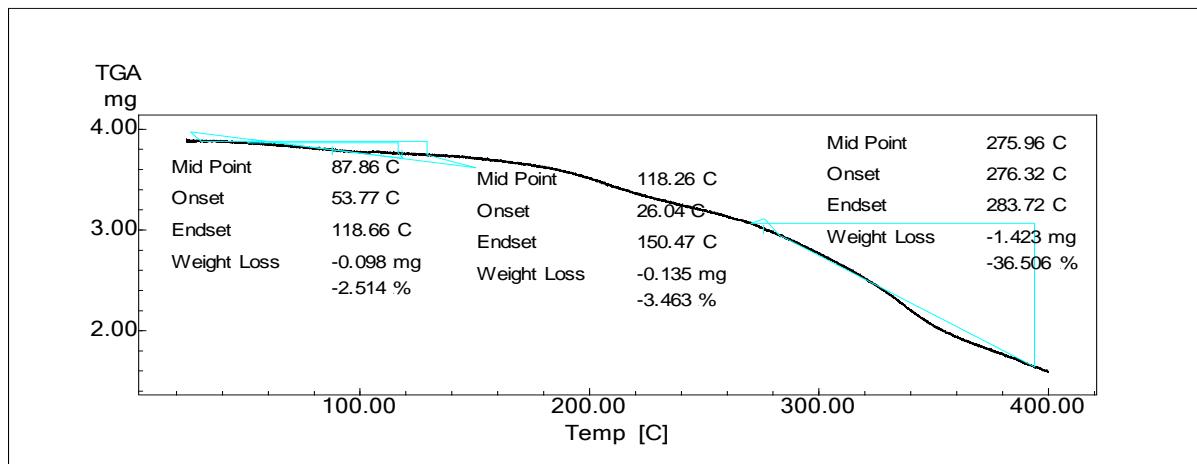
### 3.4 TGA Assay

Thermal stability of blend A at three different temperature ranges. At 118.6 °C mass loss with -2.514%, 150.47 °C mass loss with 3.463%, 283.72 °C mass loss with 36.506%. Blend B showed mass loss at different temperature at 92.63 °C with 4.549% mass loss, at 220.4 °C with 15.486% and about 349.43 °C with 23.366% mass loss. Blend C showed mass loss at different temperatures at 274.05 °C with 12.192% mass loss, at 346.33 °C with 31.164% and at 443 °C with 10.682% mass loss. The thermal stability of antioxidant blends revealed as Blend A shows 42.83% and 1.656 mg of mass loss, blend B shows 43.446% and 1.746 mg of mass loss and blend C shows 54.038% and 6.11 mg of mass loss. The higher the percentage of mass loss data blend A proved to be most thermo-stable antioxidant blend amongst blend A, B and C.

**Table -7:** Thermal Stability of Antioxidant Blends by TGA Assay

Thermal Stability of Blends by TGA						
Temp range for TGA (100 °C-400 °C)	Blend - A (GMP-532)		Blend B (GMP-)		Blend C (GMP-)	
	Temp	Mass Loss	Temp	Mass Loss	Temp	Mass Loss
Onset	53.71 °C	0.098 mg	47.87 °C	0.183 mg	206.88 °C	1.38 mg
Endset	118.26 °C		72.63 °C		274.05 °C	
Onset	118.66 °C	0.135 mg	202 °C	0.623 mg	305.45 °C	3.530 mg
Endset	150.47 °C		220 °C		346.33 °C	
Onset	276.32 °C	1.423 mg	323.14 °C	0.940 mg	438.27 °C	1.210 mg
Endset	283.72 °C		437.08 °C		443.69 °C	
Initial mass of Sample	3.898 mg		4.023 mg		11.327mg	
Mass loss (mg)	1.656 mg		1.746 mg		6.11 mg	
(%) mass loss	42.83		43.446		54.038	

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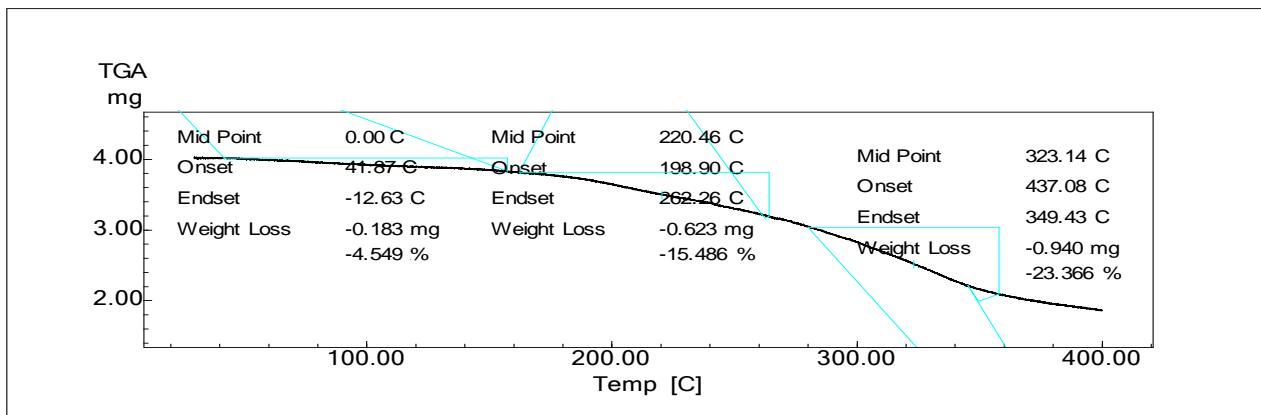


[Temp Program  
Temp RateHold TempHold Time  
[C/min] [C] [min]  
10.00 400.0 0

File Name: BMP-40 2016-04-26 14-48:  
Detector: TGA-5C  
Acquisition Date: 16/04/26  
Acquisition Time: 14:48:30(+053)  
Sample Name: BMP-40  
Sample Weight: 3.898[mg]  
Cell: Aluminum  
Atmosphere: Nitrogen  
Flow Rate: 50[ml/min]  
Operator: Pallavi Shindika  
Annotation: Food Technolog:  
Aurangabac

(a)

MIT - SHIMADZU AURANGABAD

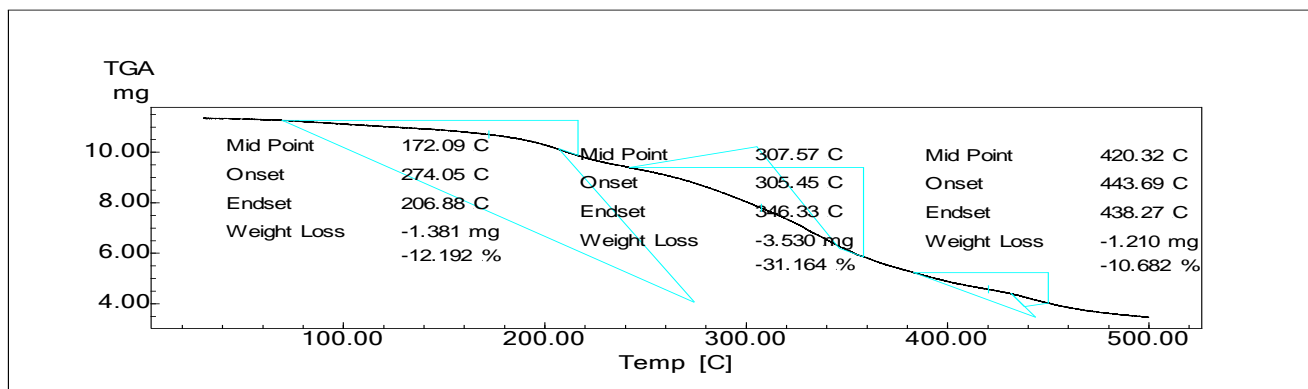


[Temp Program  
Temp RateHold TempHold Time  
[C/min] [C] [min]  
10.00 400.0 0

File Name: BMP-50 2016-04-27 10-09:  
Detector: TGA-5C  
Acquisition Date16/04/27  
Acquisition Time10:09:20(+053)  
Sample Name: BMP-5C  
Sample Weight: 4.023[mg]  
Cell: Aluminum  
Atmosphere: Nitroger  
Flow Rate: 50[ml/min]  
Operator: Pallavi Shindika  
Annotation: Food technology colleg  
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(b)

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[Temp Program  
Temp RateHold TempHold Time  
[C/min] [C] [min]  
10.00 500.0 0

File Name: BMP-60 2016-04-27 12-19:  
Detector: TGA-5C  
Acquisition Date16/04/27  
Acquisition Time12:20:16(+053)  
Sample Name: BMP-6C  
Sample Weight: 11.327[mg]  
Cell: Aluminum  
Atmosphere: Nitroger  
Flow Rate: 50[ml/min]  
Operator: Pallavi Shindika  
Annotation: Food Technology colleg  
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(c)

Chart-4: TGA graph shows the thermal stability of Blends A (GMP-532) (a), Blend C (GMP-) (b) & Blend C(GMP-) , (c) to mass change of sample as a function of temperature.

### 3. CONCLUSION

In this study, *Psidium guajava*, *Moringa Oleifera* and *Punica Granatum* Leaves was collected from the botanical garden of Himayat Baugh from Aurangabad and got authenticated by autonomous botany department of B.A.M. University, Aurangabad, Maharashtra.

The evaluation of Antioxidant Activity of the shrub plants leaves methanolic extracts was done individually and in their blends forms by Comparative Assay methods DPPH Assay, TLC-Bioautography, TGA Assay and its confirmation with FTIR Assay.

DPPH Scavenging Activity was carried out to evaluate free radical scavenging activity of leaves and their formulated blends. The DPPH assay shows leaves antioxidant activity in pattern guajava > Moringa > Punica. With 29.31 % inhibition by guajava leaves, 16.62% inhibition by punica leaves and 15.34% inhibition by moringa leaves. DPPH Scavenging Activity was carried out for their blends shows Blend -A showed 30.08%, Blend-B 12.08%, Blend-C 18.08% inhibition and out of which Blend A has been confirmed as the most Potential Broad-spectrum Antioxidant with 30.08 % through DPPH.

The TLC guided identification Assays. The Rf value obtained by *Psidium guajava* 0.979, *Moringa Oleifera* 0.792 and *punica granatum* 0.679. The Rf values obtained by the blends shows, blend-A 0.979, blend-B 0.941 and blend-C 0.901. The Rf value obtained by blend -A shows higher values than synthetic antioxidant BHT with 0.941.

FTIR assay reveals that Blend A shows 14 peaks lies in the region responsible for antioxidant components that ranges between 1097  $\text{cm}^{-1}$  and 3853.7  $\text{cm}^{-1}$  and Blend B shows 18 peaks between 1026.13  $\text{cm}^{-1}$  and 3649.32  $\text{cm}^{-1}$  and Blend C shows 16 peaks that ranges between 763.81  $\text{cm}^{-1}$  and 3305.99  $\text{cm}^{-1}$ . Higher the peak values showed the presence of antioxidant components when compared with the standard gallic acid and other synthetic antioxidant peaks values.

The thermal stability revealed by TGA assay shows the thermal of the antioxidant blends as Blend A shows 42.83% and 1.656 mg of mass loss, blend B shows 43.446% and 1.746 mg of mass loss and blend C shows 54.038% and 6.11 mg of mass loss. The higher the percentage of mass loss data blend A proved to be most thermo-stable antioxidant blend shows less thermal stability.

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### REFERENCES

- [1] Antony V. Samrot, Sahiti K., Raji P., Bennet Rohan., Divya Kumar and Kripu Sharma, "TLC Bio-autography guided identification of antioxidant and antibacterial activity of *Acacia senega*," Der Pharmacia Letter. Vol-8, 2016, pp41-47.
- [2] Anwar F, Latir S, Ashraf M, Gilani AH. "Moringa oleifera: a food plant with multiple medicinal uses,". *Phytother Res* Vol 21(2007), pp 17-25.
- [3] Chen Hui-Yin, Gow-Chin Yen, "Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves," *Article in Food Chemistry* 101(2):686-694 (2007).
- [4] Dai I and Mumper R.I "Plant Phenolics: Extraction, analysis and their antioxidant and anticancer properties," *Molecules*, (2010).
- [5] Fereidoon. Shahidi, Ying Zhong "Novel antioxidants in food quality preservation and health promotion" *European journal of lipid science and technology*, Vol 112, Issue 9 (2010).
- [6] Guodong Xiao, Guowen Li, Lian Chen, Zijia Zhang, Jun Yin, Tao Wu, Zhihong. Chena "Isolation of antioxidants from *Psoralea corylifolia* fruits using high-speed counter-current chromatography guided by thin layer chromatography antioxidant autographic assay" *Journals of chromatography A* 1217, (2010) 5470-5476.
- [7] Jahagirdar K, Ghosh P, Adil SM, Ziyaurrahman AR. Effect of hydroalcoholic extract of *Psidium guajava* Linnon complete Freund's adjuvant induced arthritis in laboratory animals. *Pharmacologyonline* (2010).
- [8] M.Haniff, Nisha.R, Tamileswari, S.Jesurani, S.Kanagesan, M.Hashim, "Greensynthesis of silver nano particles from pomegranate (*punica granatum*) leaves and analysis of anti-bacterial activity," *International Journal of Advanced Technology in Engineering and Science*, Volume No.03, Issue No. 06, (2015).
- [9] Nambiar VS, Seshadri S. A study on  $\beta$ -carotene content of some green leafy vegetables of Western India by high performance liquid chromatography. *J Food Sci Tech.* (1998).

- [10] Noer Lailya, Retno Windya Kusum. aningtyasa, Iim Sukartia, Maria Rosari, Devi Kartika Rinia "The Potency of Guava *Psidium guajava* (L.) Leaves as a Functional Immunostimulatory Ingredient" *Procedia Chemistry* 14 (2015), pp- 301 – 307.
- [11] Padayachee B, Baijnath H. "An overview of the medicinal importance of Moringaceae". *Journals of medicinal plants*. 2012;6:5831–5839
- [12] Panel. Lu, wang. Yanan, Wu Jia, Xie Sheng.Wu, Zhengiang Wu "Characterization, antioxidant and antimicrobial activities of green synthesized silver nanoparticles from *Psidium guajava* L. leaf aqueous extracts," *Journals of material science and engineering: C*, Vol 86,(2018),pp 1-8.
- [13] P. Heussen, Peng.Ye, K. Menard, P. Courtney, K. Elissa, "Differential Scanning Calorimetry" Application note. Uniliver Research & Development, Vlaardingen, The Netherlands
- [14] R. K. Saini, N. P. Shetty, Maya Prakash, and P. Giridhar "Effect of dehydration methods on retention of carotenoids, tocopherols, ascorbic acid and antioxidant activity in *Moringa oleifera* leaves and preparation of a RTE product," *Journal of Food Science and Technology*. Vol 9, pp 51.
- [15] ROOS, Y. H., "Thermal analysis, state transitions and food quality" *Journal of Thermal Analysis and Calorimetry*, v. 71, (2003).
- [16] Seme Youssef REDA<sup>1</sup>, "Evaluation of antioxidants stability by thermal analysis and its protective effect in heated edible vegetable oil" 4748, CEP 84030-900, *Ciencia e Tecnologia de Alimentos* (2009) ISSN 0101-2061.
- [17] Shahidi, F. Antioxidants: Extraction, Identification, Application and Efficacy Measurement. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, v. 7, n. 8, p. 3325-3330, 2008.
- [18] Upendra K. Parashar, Vinod Kumar, Tanmay Bera and Anchal Srivastava "Study of mechanism of enhanced antibacterial activity by green synthesis of silver nanoparticles," *Article on nanotechnology*. Vol 22, (2011), pp-41.
- [19] Venant. Nihorimbere and He. Qian "Antioxidant power of phytochemicals from *Psidium guajava*," *Journal of Zhejiang University Science*. Vol-5, issue 6, (2004).
- [20] R.K. Pal, K. Dinesh Babu, N.V. Singh, Nilesh Gaikwad "Pomegranate research in India-Status and future challenges" *Progressive Horticulture*, Vol 46,(2014).
- [21] Morton J.F." Pomegranate, *Punica granatum*L" *Fruits of warm climates*.Purdue New crops profile,pp. 352-5(2012)