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COMPARATIVE ANALYSIS OF ANTIOXIDANT PROFILES OF BARK, LEAVES AND SEEDS OF SYZYGIUM CUMINI (INDIAN BLACKBERRY)

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Abstract:
Syzygium cumini plant is well known for its medicinal values. This study focuses on the comparative antioxidative capacity of bark, leaves and seeds of Syzygium cumini plant. Several fractions were made from the crude methanolic extract of bark, leaves and seeds in n-hexane, chloroform, ethyl acetate and butanol and aqueous. Leaves aqueous fraction showed highest phenolic content of 253.6 ± 0.025mg/mL while seed aqueous showed maximum flavonoid content of 0.732 ± 0.0005 mg/mL among all extracts. Bark Ethyl acetate fraction via reducing power assay showed absorbance 3.27 ± 0.19 abs and methanolic and Ethyl acetate fraction of leaves via phosphomolybdate assay showed maximum reducing strength of 15.97 ± 0.008 and 15.97 ± 0.037 mg/mL respectively among all. Seed Chloroform fraction showed maximum TEAC value of 6.28 whereas, Ethyl acetate leaves fraction has shown maximum DPPH inhibition. Where, Hexane fraction of bark showed maximum antioxidant consumption against peroxyl radical at 1.24 at 500 nm among all. The results of the assays showed that, in general, all the extracts of Syzygium cumini posses considerable antioxidant and radical scavenging properties, however, polar fractions of all three samples i.e., aqueous and ethyl acetate have exhibited a remarkable antioxidant behavior in all the antioxidant assays.

Keywords:
Antioxidant, Syzygium cumini, bark, leaves, seeds, phenolic, flavonoid.

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1. INTRODUCTION

In recent years, due to the toxicological concerns associated with synthetic substances used in food, there is an increasing interest in the use of natural substance as food preservatives and antioxidants [1]

Natural product chemistry plays an irrefutable role in drug discovery due to therapeutic effects of medicinal plants. For, research in cancer and infectious diseases, majority of drugs have natural origin, or based on some natural product pharmacophore [2]. Contemporary study talks about the comparative analysis of antioxidant profiles of bark, leaves and seeds of *Syzygium cumini* L. colloquially known as Indian blackberry and Java plum.

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Syzygium cumini is an evergreen tropical tree, highly domesticated to Pakistan, India, Bangladesh and Indonesia. All parts of this plant are used for medicinal purposes. Preclinical studies done over decades on various parts of this plant have emphasized on its pharmacological dimension. *Syzygium cumini* bark being astringent and sour is reported to possess antioxidant [3] [4], anti inflammatory [5] and antithelmintic [6] [7], anticancer and hypoglycemic properties [8]. Its elliptic and leathery leaves were observed of antioxidant [9], antibacterial [10] anti-inflammatory [11] and antidiabetic [12] are used in the treatment of gums and teeth. The bark and leaves of the subject plant work against diarrhea. Seeds of *Syzygium cumini* claimed to have antioxidant activity[13] [14] antidiabetic[15] [16] [17] [18] [19] [20] , enzymatic inhibition[21], and works against inflammation too [22] [23] [24].

This study talks about the comparative quantitative analysis of antioxidant profiles of bark, leaves and seeds of *Syzygium cumini*, (colloquially known as Indian blackberry or black plum). The antioxidant activity is gauged on the basis of radical scavenging capacity of the extracts and the presence of phenolics.

Abbreviations

Crude methanolic Extract (CME), Phosphomolybdate Reagent (PMR), Rutin equivalent (RE), Ascorbic acid equivalent (AAE), Gallic acid Equivalent (GAE) Standard error of measurement (SEM), Folin - Ciocalteau Reagent (FCR), Crude methanolic extract (CME), 2, 2'-Azinobis (3-Ethylbenzo Thiazoline)-6Sulphonic acid (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Butylated Hydroxyanisole (BHA), Trolox equivalent of Antioxidant values (TEAC), Hexane fraction (*Hf*), Chloroform fraction (*Cf*), Ethyl acetate fraction (*Ef*), Butanol fraction (*Bf*) and Aqueous fraction (*Af*).

2. MATERIALS AND METHODS

2.1. CHEMICALS, REAGENTS AND SAMPLES

Methanol, Chloroform, Ethanol, N-Hexane, Ethyl acetate, Gallic acid, Hydrochloric acid, Sulfuric acid, Aluminum Chloride, Ammonium thiocyanate, Ammonium molybdate, Folin – Cioacalteau Reagent (FC Reagent), Iron (II) chloride. Tetra hydrate, Iron (III) chloride. Hexa hydrate, Mono potassium phosphate, Di-potassium phosphate, Potassium persulfate, Potassium ferricyanide, Potassium chloride, Sodium nitrite, Sodium acetate, Sodium chloride, Disodium hydrogen phosphate were bought from Riedel-deHaën, Germany, Ascorbic Acid (Fisher Scientific), Tween 20 (Fisher Scientific), n-Butanol (Merck, Germany), Rutin (Alfa Aesar GmbH & Co.), Linoleic acid (BioPLUS fine research chemicals), Trichloroacetic acid (Uni-Chem Chemical Reagents), Trolox, BHA, Glacial Acetic acid, ABTS and DPPH radical were bought from Sigma-Aldrich. The samples of the study are the **leaves, bark** and **seeds** of *Syzygium cumini***.**

The samples were collected from Garden Town Lahore, Pakistan, Dated: 20-6-2012.

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2.2. PREPARATION OF PLANT EXTRACT AND ITS FRACTIONS

The leaves and fruits were washed. The fruit pulp, seed coat and outer layer of the stem bark were removed. Samples were shadow dried for 14 days and ground to fine powder. 200g grounded samples of bark, leaves and seeds were soaked in 1000mL volumetric flasks for 15 days in 1L methanol and filtered. The filtrates were concentrated under reduced pressure on Rotavap (30^0C) to give respective CMEs. These CMEs was dissolved in 200mL distilled water and successively fractionated in a separating funnel with solvents of varying polarity: n-hexane, chloroform, ethyl acetate and n-butanol to produce their respective fractions

2.3. ANTIOXIDANT STUDIES

2.3.1. TOTAL PHENOLIC CONTENT

The phenolic compounds estimation was done using Folin Ciocalteu colorimetric method [25]. 40 µl plant extract (or standard rutin solution) was taken to which 3.16 mL distilled water and 200 µl Folin Ciocalteu reagent was added and mixed. *Af*ter 8 minutes, 600 µl sodium carbonate solution (20%) was added, mixed and incubated at 40 centigrade for 30 minutes. The blank contained methanol in place of sample. The absorbance was measures at 765nm. The total phenolic content was expressed as mg/mL of GAE.

2.3.2. TOTAL FLAVONOID CONTENT

The estimation of flavonoid content was done using Aluminum Colorimetric method of Sahreen *et al.,* 2010 [26]. To 300 µL plant extract (or standard rutin solution), 3.14 mL of 30% aqueous methanol was added. To the same solution, 150 µL of 0.5 M sodium nitrite, 150µL of 0.3 M aluminum chloride and 1 mL of 1M NaOH was added with 5 minutes intervals *after* each addition. The absorbance was measured at 506nm. Control contained methanol in place of sample. The total flavonoid content was expressed in mg/mL of RE.

2.3.3. DPPH Assay

The radical scavenging potential was done using DPPH assay[27]. 3mL of 0.004% DPPH working solution (prepared using DPPH stock solution and methanol in correct proportions to give 0.899 abs) was added per every 100 µL of different concentrations of the extract and incubated at 37^0 centigrade for 30 minutes in dark. Then absorbance was taken at 517nm wavelength in UV spectrophotometer. Negative control contained 100 µL of methanol in place of sample solution.

The percentage antioxidant inhibition was obtained by equation: Control (Abs) -Sample (Abs) / Control (Abs) * 100.
Ascorbic acid was used as positive control. Inhibition curves were made and EC_{50} value per sample was calculated.

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2.3.4. ABTS Assay

The radical scavenging potential was done using ABTS assay[28]. 9.5 mL ABTS solution (7mM) was reacted with 245 µl of potassium persulfate (100mM). The volume was made up to 10 mL by distilled water. The stock solution was kept in dark for 18 hours. The ABTS stock solution was diluted with 0.1M potassium buffer solution pH (7.4) to an absorbance of 0.7 (±0.02). 0.91g of di-potassium hydrogen phosphate, 0.16 g mono-potassium dihydrogen phosphate and 4.35 g NaCl was dissolved in 490 mL distilled water. The volume was made up to 500mL. In a cuvette, 2.99 mL ABTS working solution was added and mixed with 10µl plant extract solution (or standard Trolox solution). Control was prepared by adding 10 µl methanol in place of sample. The absorbance was measured at 734 nm till first eight minutes with the interval of 30 seconds.

The percentage inhibition was calculated by using the equation: Percentage Inhibition = [1 - Sample Absorbance / Control Absorbance] *100.

2.3.5. LIPID PEROXIDATION Assay

100 µl plant extract (or standard BHA solution) was mixed with 2.4 mL 0.04 M potassium phosphate buffer and 2.5mL Linoleic acid emulsion. Linoleic acid is insoluble in water and to make it soluble Tween 20 (anionic surfactant is added).The buffer was prepared by using 6.9 g di-potassium hydrogen phosphate was dissolved in 980 mL distilled water. The pH was adjusted by mono-potassium dihydrogen phosphate and diluted to 1L. The mixture was incubated at 37^0C for 25 minutes.100 µl plant sample was regularly taken at 24 hours interval, dissolved in 3.7 mL ethanol. Then it was reacted with 100 µl ferrous chloride (20mM) and then 100 µl 20% ammonium thiocyanate was added. Negative control contained 2.4 mL potassium phosphate buffer and 2.5mL Linoleic acid emulsion and methanol in place of sample. BHA was used as positive control. Absorbance was measured at 500nm.

2.3.6. REDUCING POWER Assay

The protocol of Oyaziu *et al.,* 1986 was followed [29]. 2.5 mL plant extract (or standard gallic acid sol.) was mixed with 2.5 mL of 0.2M sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide solution (2.5 mL) and incubated at 50^0C for 20 minutes. Sodium phosphate buffer was prepared by dissolving 8.9g disodium hydrogen phosphate in 200 mL distilled water, while the pH at 6.6 was acclimatized with monosodium dihydrogen phosphate. *Af*ter incubation 10 % trichloroacetic acid solution (2.5 mL) was added and centrifuged at 650 rpm for 10 minutes. 5 mL supernatant was mixed with 5 mL distilled water and 0.1 % ferric chloride solution (1 mL). The control had methanol instead of plant sample.
The absorbance was measured at 700nm.

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2.3.7. PHOSPHOMOLYBDATE Assay

The protocol of Umamaheswari and Chatterjee *et al.,* 2008 was followed [30]. PMR was prepared with 0.6 M Sulfuric acid (100 mL), 4 mM ammonium molybdate solution (100 mL) and 28mM sodium phosphate solution (100 mL). 3 mL PMR was added to 300 µL plant extract solution and incubated in water bath for 90 min at 95^0 C in dark.

The absorbance was measured at 765nm. For control, in place of plant extract sample 300 µL methanol was added. The antioxidant activity of extract and fractions were expressed in mg/mL of AAE.

3. RESULTS AND DISCUSSIONS

3.1. TOTAL PHENOLIC CONTENT

Estimation of phenolic compounds is the most important and the initial step to set the foundation that whether the subject possess antioxidant properties of not. Phenolics have the ability to stabilize radicals [31], they stabilize free radicals by using their hydroxyl groups as an agent to donate hydrogen bonds to prevent radical chain reaction. The present study indicated that the *Af* of leaves contained highest content of Phenolic compounds.

Table1: Total phenolic content of various fractions of bark, leaves and seeds extracts

No.	Fraction	mg/mL of gallic acid equivalent (GE)		
		Bark	Leaves	Seeds
1	Methanol	124.94 ± 0.01	137.27 ± 0.039	40.27 ± 0.0168
2	n-Hexane	38.67 ± 0.002	32.94 ± 0.0165	19.27 ±0.012
3	Chloroform	46.94 ± 0.011	39.94 ± 0.065	46.94 ± 0.004
4	Ethyl acetate	150.67 ±0.066	29.27 ± 0.015	42.27 ± 0.0025
5	n-Butanol	105.67 ± 0.042	29.94 ± 0.026	55.94 ± 0.004
6	Aqueous	87.94 ± 0.048	253.6 ± 0.025	69.94 ± 0.0034

*3.2.*TOTAL FLAVONOID CONTENT

Not all flavonoids can be targeted with Aluminum calorimetric method only flavones and flavonols forms stable complex with aluminum chloride[32]. The present study indicated that the *Af* of leaves contained highest content of flavonoid compounds.

Table 2: Total flavonoid content of various fractions of bark, leaves and seeds extracts.

No.	Fraction	mg/mL of rutin equivalent (RE) ±SEM		
		Bark	Leaves	Seeds
1	Methanol	0.65 ± 0.01	0.65 ± 0.01	0.51 ± 0.006
2	n-Hexane	0.58 ± 0.011	0.54 ± 0.029	0.47 ± 0.007

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3	**Chloroform**	0.57 ± 0.0179	0.57± 0.003	0.66 ± 0.001
4	**Ethyl acetate**	0.62 ± 0.027	0.62 ± 0.015	0.65 ± 0.001
5	**n-Butanol**	0.59 ± 0.018	0.59 ± 0.007	0.61 ± 0.001
6	**Aqueous**	0.53 ± 0.0072	0.46 ± 0.004	0.73 ± 0.0005

The flavonoid content of all CMEs and fractions expressed as RE are:

For bark: CME> *Ef*> *Bf*> *Hf*> *Cf* > *Af*.

For leaves: CME> *Ef*> *Bf*> *Cf* >*Hf* > *Af*.

For seeds: *Af*> *Cf*> *Ef*> *Bf*> CME> *Hf*.

3.3.DPPH Assay

DPPH is an organic N-free radical stable at room temperature that gives absorbance at 516nm. The principle of DPPH decolorization assay is based on the ability of the radical to accept an electron or Hydrogen atom from a reducing agent to form a stable non radical diamagnetic molecule [33].

When the DPPH radical reduces, it shows a decrease in absorbance at 517nm (Purple to yellow).The degree of color change tells you about the antioxidant concentration, lower the absorbance, greater the scavenging potential of the compounds in the Sample.

At first 10 mg/10mL methanol for all samples was experimented. All the extracts showed maximum scavenging except *Hf* and *Cf* of all the three parts (leaves, bark and seeds).

Table 3: A comparison of percentage inhibition of DPPH free radical by fractions of methanolic extract of bark, leaves and seeds (10mg/10mL).

No.	Fraction	Percentage Inhibition		
		Bark	Leaves	Seeds
1	**Methanolic**	84.03	76.82	90.24
2	**n-Hexane**	2.1	28.04	26.94
3	**Chloroform**	28.93	26.3	68.62
4	**Ethyl acetate**	84.81	75.27	90.7
5	**n-Butanol**	87.69	89.35	86.15
6	**Aqueous**	82.48	89.92	90.83

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Table 4: Percentage inhibition of Ascorbic acid, a standard antioxidant

No	Standard Antioxidant	Percentage inhibition
	Ascorbic acid	99.9176

The *Ef* of leaves showed lowest EC_{50} among all extracts and fractions whereas *Ef* of bark showed minimum EC_{50} among all bark fractions and *Bf* of seed showed lowest EC_{50} value among all seed extracts. The *Ef* of all parts (Bark, leaves and seeds) has produced lowest EC_{50} value indicating greater scavenging potential against DPPH.

Order of Scavenging per solvent
Bark: *Ef*> *Af* > CME > *Bf* > *Cf* > *Hf*
Leaves: *Ef* > CME > *Bf* > *Af* > *Hf* > *Cf*
Seeds: *Bf* > *Ef* > *Af* >CME > *Cf* > *Hf*.

Table 5: A comparison of Antioxidant effect (EC_{50} values) on DPPH radical of different fractions and CMEs of bark, leaves and seeds.

No.	Fractions	EC_{50} (mg/mL)		
		Bark	**Leaves**	**Seeds**
1	**Methanol**	0.632	0.186	0.173
2	**Ethyl acetate**	0.060	0.048	0.144
3	**n-Butanol**	0.960	0.198	0.097
4	**Aqueous**	0.570	0.522	0.152

3.4. ABTS Assay

In this decolorization assay, ABTS radical is formed by oxidation by persulphate and undergoes reduction by H-bonding antioxidants. The extent of reduction by any scavenging moiety can be seen by the decrease in absorbance and increase in percentage inhibition at 734 nm.

It was observed, the entire reaction mixture loses its color within 24 hours. It was also observed that ABTS radical reacts readily within 30 seconds of reaction initiation and can be used over a wide range of pH with any antioxidant substance [34]. The results are expressed as TEAC. Higher TEAC values indicate higher antioxidant potential [35]. The TEAC values have shown the highest content of antioxidant potential was present in *Cf* of seeds and bark of 6.28 and 5.95 respectively.

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Table 6: Comparison of percentage scavenging of ABTS $^{\cdot+}$ by CMEs (1mg/mL) and various fractions of bark, leaves and seeds extract *After* 8 minutes.

No.	Fraction	Percentage Inhibition		
		Bark	Leaves	Seeds
1	Methanolic	19.32	20.70	23.83
2	n-Hexane	7.52	18.44	21.58
3	Chloroform	6.27	18.82	2.88
4	Ethyl acetate	26.22	21.95	22.45
5	n-Butanol	11.91	36.63	7.77
6	Aqueous	25.09	25.59	19.5

Table 7: TEAC value of different fractions of bark, leaves and seeds After 8 minutes.

No.	Fraction	TEAC (mmol)		
		Bark	Leaves	Seeds
1	Methanolic	4.68	4.54	4.24
2	n-Hexane	5.82	4.76	4.46
3	Chloroform	5.95	4.73	6.28
4	Ethyl acetate	4.01	4.42	4.37
5	n-Butanol	5.40	3	5.80
6	Aqueous	4.12	4.07	4.65

Table 8: Absorbance and TEAC value of Positive controls seeds *After* 8 minutes.

No.	Standards	Absorbance	TEAC Value
1	Gallic Acid	0.067	5.71
2	Ascorbic Acid	0.045	5.98

3.5. LIPID PEROXIDATION Assay

Peroxyl radicals are formed because of peroxidation of lipids by degradation of processed food. These radicals may attack polyunsaturated fatty acids causing cancer cells. Upon these peroxyl radical scavenging, peroxidation of fatty acids can be stopped [36]. The mechanism of the assay is based on the oxidation of Linoleic acid to generate peroxyl radicals during the oxidation process because of their thermal and catalytic destruction [37]. The extent of scavenging indicates the consumption of the antioxidant present. Peroxide radicals not scavenged by this process oxidize ferrous to ferric, which forms a complex ammonium thiocyanate. Redder the color, more are the peroxide radicals present in the reaction mixture[38].

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The decrease in absorbance indicates the presence of antioxidants in the extract, i.e., increase in lipid peroxidation value indicates decrease in antioxidant potential and vice versa.

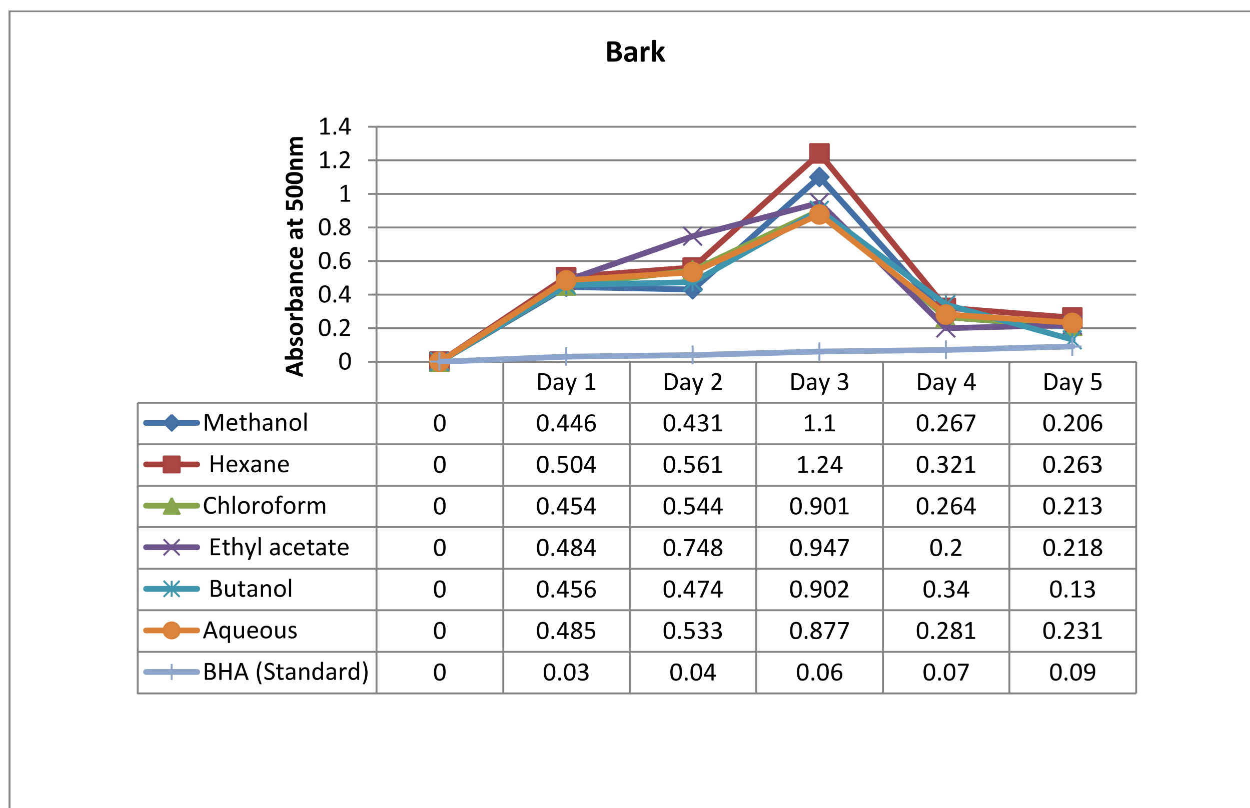
The lipid peroxidation value of different fractions was observed in Linoleic acid emulsion system, the absorbance of the samples was determined after every 24 hours for 5 days, while BHA was used as a standard. Results showed *Hf*s of bark, and seeds and also CME of leaves gave highest lipid peroxidation value among all other fractions.

Order of Scavenging per solvent

Bark: *Hf* > CME> *Ef* > *Bf* > *Af* > *Cf* (Figure 1)

Leaves: *Ef* > *Cf* > *Hf* > CME > *Bf* > *Af* (Figure 2)

Seeds: *Hf*>CME>*Ef* > *Af* = *Bf* > *Cf* (Figure 3)



		Day 1	Day 2	Day 3	Day 4	Day 5
Methanol	0	0.446	0.431	1.1	0.267	0.206
Hexane	0	0.504	0.561	1.24	0.321	0.263
Chloroform	0	0.454	0.544	0.901	0.264	0.213
Ethyl acetate	0	0.484	0.748	0.947	0.2	0.218
Butanol	0	0.456	0.474	0.902	0.34	0.13
Aqueous	0	0.485	0.533	0.877	0.281	0.231
BHA (Standard)	0	0.03	0.04	0.06	0.07	0.09

Fig 1: Determination of antioxidant activity over a time period of various fractions of Bark along with its CME by ferric thiocyanate method (Linoleic acid emulsion assay).

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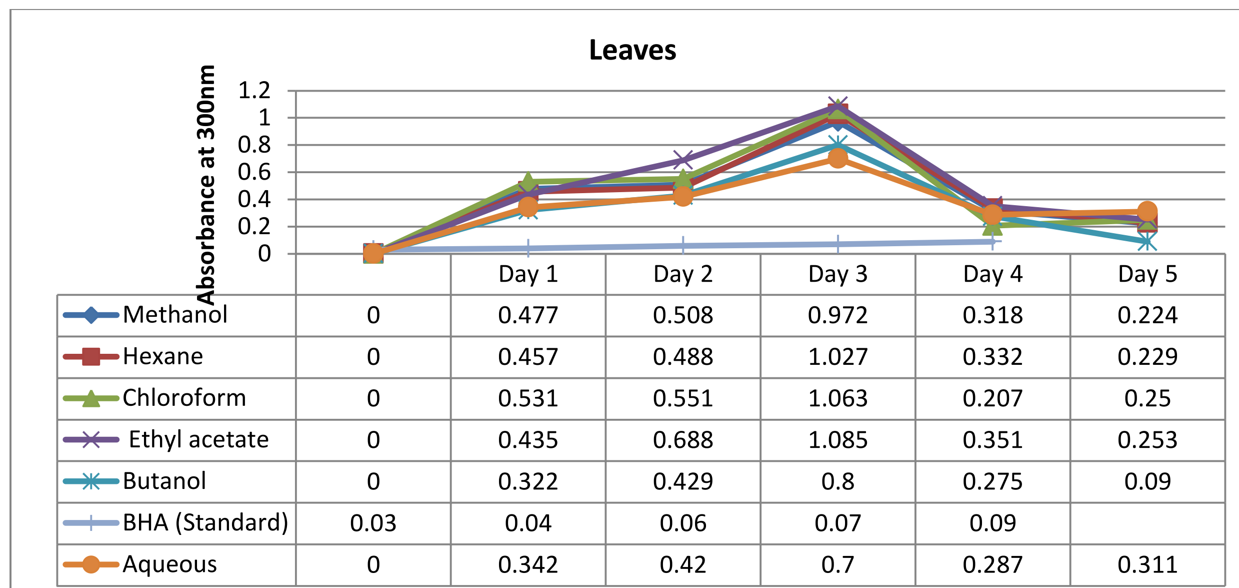
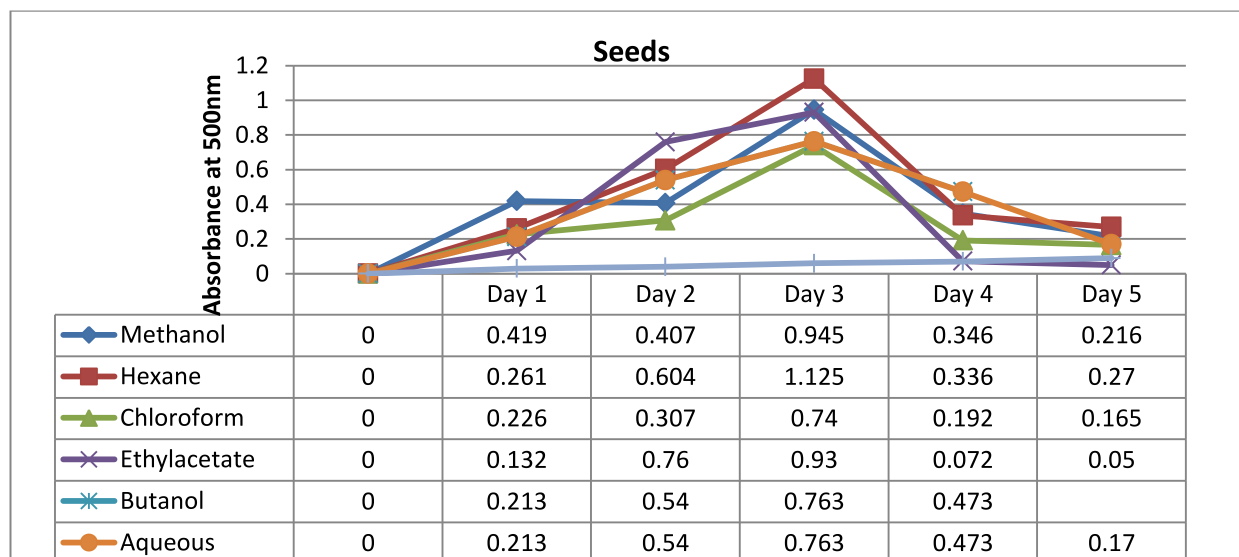


		Day 1	Day 2	Day 3	Day 4	Day 5
Methanol	0	0.477	0.508	0.972	0.318	0.224
Hexane	0	0.457	0.488	1.027	0.332	0.229
Chloroform	0	0.531	0.551	1.063	0.207	0.25
Ethyl acetate	0	0.435	0.688	1.085	0.351	0.253
Butanol	0	0.322	0.429	0.8	0.275	0.09
BHA (Standard)	0.03	0.04	0.06	0.07	0.09	
Aqueous	0	0.342	0.42	0.7	0.287	0.311

Fig 2: Determination of antioxidant activity over a time period of various fractions of leaves along with its CME by ferric thiocyanate method (Linoleic acid emulsion assay).



		Day 1	Day 2	Day 3	Day 4	Day 5
Methanol	0	0.419	0.407	0.945	0.346	0.216
Hexane	0	0.261	0.604	1.125	0.336	0.27
Chloroform	0	0.226	0.307	0.74	0.192	0.165
Ethylacetate	0	0.132	0.76	0.93	0.072	0.05
Butanol	0	0.213	0.54	0.763	0.473	
Aqueous	0	0.213	0.54	0.763	0.473	0.17

Fig 3: Determination of antioxidant activity over a time period of various fractions of seeds along with its CME by ferric thiocyanate method (Linoleic acid emulsion assay).

3.6. REDUCING POWER Assay

Reductones are associated with reducing capacity as they break the free radical chain reaction by donating a hydrogen atom [39]. The reduction from ferric to ferrous is determined by a change in

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color from pale yellow to green at 700 nm. Gallic acid was used as a standard giving absorbance at 3.385±0.011. Where, absorbance is directly proportional to the reducing power of the sample.

Table 9: The reducing power of gallic acid

No	Standard Antioxidant	Mean absorbance (700nm) ± SEM
	Gallic acid	3.38±0.011

Table 10: Mean Absorbance of various fractions of bark, leaves and seeds.

No.	Fraction	Mean absorbance (700nm) ±SEM		
		Bark	Leaves	Seeds
1	Methanol	2.99 ± 0.23	3.01 ± 0.11	2.98 ± 0.005
2	n-Hexane	0.52 ± 0.04	0.45 ± 0.08	1.31 ± 0.304
3	Chloroform	1.48 ± 0.07	0.82 ± 0.02	1.99 ± 0.01
4	Ethyl acetate	3.27 ± 0.19	3.12 ± 0.18	2.67 ± 0.25
5	n-Butanol	3.05 ± 0.55	2.85 ± 0.13	2.20 ± 0.24
6	Aqueous	3.13 ± 0.46	2.92 ± 0.02	2.60 ± 0.15

The reducing capacity of all methanolic extracts and fractions in reducing power assay are expressed in antioxidant potential.

For bark: *Ef*> *Af*> *Bf*> CME >*Cf*> *Hf*.

For leaves: *Ef*> CME> *Af*> *Bf*> *Cf*>*Hf*.

For seeds: CME> *Ef*> *Af*> *Bf*> *Cf*> *Hf*

3.7. PHOSPHOMOLYBDATE Assay

It is the quantification of reducing agent that reduces Mo (VI) to Mo (V) and giving green phosphomolybdenum (VI) complex at 700nm [40]. The results were expressed in mg/mL AAE.

Table 11: Reducing power of various fractions of bark, leaves and seeds

No.	Fraction	mg/mL of ascorbic acid equivalent ±SEM		
		Bark	Leaves	Seeds
1	Methanolic	15.91 ± 0.073	15.97 ± 0.008	15.82 ± 0.045
2	n-Hexane	15.67 ± 0.014	15.74 ± 0.012	15.67 ± 0.01
3	Chloroform	15.64 ± 0.012	15.57 ± 0.002	15.73 ± 0.013
4	Ethyl acetate	15.76± 0.013	15.97 ± 0.037	15.92 ± 0.003
5	n-Butanol	15.69 ± 0.009	15.9 ± 0.031	15.72 ± 0.008
6	Aqueous	15.56 ± 0.023	15.79 ± 0.017	15.89 ± 0.005

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The reducing capacity of all methanol extracts and fractions in phosphomolybdate assay expressed as ascorbic acid equivalent are written in descending antioxidant potential.
For bark: CME> *Ef*> *Bf*> *Hf*, *Cf*> *Af*.

For leaves: CME> *Ef*> *Bf*> *Af*> *Hf*> *Cf*.

For seeds: *Ef*> *Af*> CME> *Cf Bf*> *Hf*.

4. CONCLUSIONS & RECOMMENDATIONS

The variation in results of different fractions in various assays owes to the presence of different compounds in extracts of varying polarity solvents. The isolation of the active compounds from the most active fraction in any particular assay can be processed for drug discovery against reactive oxygen species.

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