A study on hepatoprotective activity and antioxidant properties of Musa balbisianacolla Seeds

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Research Article

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Abstract

This study aims to determine and characterize the antioxidant properties of *Musa balbasiana* colla flower and seeds and *Musa Paradisiaca*. Hepatoprotective potential of *Musa balbisiana* colla seeds was also assessed. The ethanolic extract of flower and seeds of the samples were evaluated for antioxidant assay using 1,1-diphenyl-2-picrl-hydrazyl (DPPH), ferric reducing antioxidant power (FRAP), 2,2-azino-bis (3-ethlbenzothiazoline-6-sulfonic acid) (ABTS), Nitric oxide (NO) assays. The seed extract of *Musa balbisiana* exhibited the highest scavenging activity. Also, the highest total phenolic content (195.62µg/mg) was obtained from the seed extract. Thus, having high antioxidant activity, the seed was further evaluated for *in- vitro* hepatoprotective effect in paracetamol- induced hepatotoxicity in HepG2 cell line. Dose dependent effect of MbS (25, 50, 100, 200 µM) were tested on HepG2 cell line. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used for the determination of toxicity which resulted in 80% recovery with 200µg/ml concentration in a dose dependent manner. The result indicated that the high IC50 was the important factor ensuring to posses powerful radical scavenging activity. The research conducted contributes data to validate the hepatoprotective effect of *Musa balbisiana colla* seeds on paracetamol -induced liver toxicity.

Introduction

Liver being one of the important organs of gastrointestinal system is vulnerable to develop chronic condition such as hepatic fibrosis, liver cirrhosis and hepatocellular carcinoma. Acute liver injury induced by hepatotoxin such as CCl4, toxic level of paracetamol is one of the major causes of hepatotoxicity (Parthasarathy *et al.*, 2021). Multiple diseases are caused in liver. Several factors such as viral infections, alcohol abuse, toxic substance and drugs have potential harmful effect on liver. To tackle these conditions multidisciplinary approach is required. Modern medicine has come a long way in its management, but there exist scope for other form of treatment for holistic approach. Such approach focuses on new therapeutic approaches that can contribute in the management of liver conditions. Acetaminophen (APAP; Paracetamol) is widely used as analgesic and antipyretic drug and one of cause of dose related toxicity (Zur *et al.*, 2018). Higher dose of paracetamol is one of the pharmaceutical product causing heaptotixicity and nephrotoxicity. When in overdose condition, paracetamol is metabolized by cytochrome P450 enzymes to N-acetyl-p-benzoquinone imine(NAPQI). Excessive production of NAPQI can cause hepatic necrosis and unlikely generation of free radicals subsequently causing hepatic cell death (Tejo *et al.*, 2021).

Banana is the oldest cultivated source of food which stands as the fourth most important dietary staple (Aurore et al., 2009). Assam is native to different varieties of banana of which a variety *Musa balbisiana* colla BB is widely consumed and also its various parts such as fruit, seeds, flower, peel are used as ethnic therapeutic medicine(Das et al., 2016; Deka et al., 2019). *Musa balisiana* belongs to the family *Musaceae* and typically grows in rain forest habit and characterizes as an exception of having seeds (Gosh and Dash, 2018; Minh et al., 2019). *Musa balbisiana* colla is locally known as Bhimkol or Athiyakol(Borborah et al., 2016). This variety is usually consumed by the people of Assam and other parts of North East India

as a dietary food as it contain rich amount of carbohydrate, proteins, minerals and vitamins(Basumatary and Nath, 2018). *Musa balbasiana* benefits not only being used as food but also its medicinal and therapeutic potential has made it a super food. It is considered to be high in bioactive compounds polyphenols, flavonoid, phenols, tannins, saponins, dieterpens, triterpenes(Borah and Das, 2017). Various studies showed the role of phytoconstituents having beneficial hepatoprotective effect. Flower and peel constitute ability as antioxidant properties which can prevent the oxidative stress and related diseases (Basumatary and Nath, 2018; Nofianti et al., 2021). Inflorescence of *Musa balbisiana* also posses antioxidant activity and cytotoxic attributes(Revadigar et al., 2017). Apiforol found in the seeds of *Musa balbisiana* colla can cause reduction in postprandial hyperglycemia therefore is an antidiabetic agent(Gopalan *et al.*,2019). The objective of the present study is to identify the unrecognized potential characteristic of *Musa balbisiana* colla seed and evaluate its hepatoprotective effect on paracetamol – induced hepatoxicity.

Materials and methods

1. Collection, Drying and extraction of different parts of banana: Bulk numbers of samples *Musa balbisiana* seed (MbS) and flower (MbF) and *Musa Paradisiaca* (Mp) were procured from local sellers of Guwahati. Drying of the samples was done in a hot air oven at the temperature of 40ºC. After drying, the samples were ground to homogeneous powder. Cold extraction was performed by placing the powdered samples in a stopper container with the solvent and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter of the samples had dissolved .The ethanol solvents were used in the extraction process.

2. Evaluation of the *In Vitro* Antioxidant Activity: Different *In Vitro* Antioxidant Assays includes

1. DPPH radical scavenging Activity

2. ABTS radical scavenging Activity

3. NO radical scavenging Activity

4. Ferric reducing antioxidant capacity

All the *In Vitro* Antioxidant Activities were performed by using standard protocol with some minor modifications. NO radical scavenging activity was carried out by using griess reagent method. UV visible spectrophotometer was used for the analysis purposes for all assays. IC50 (µg/ml) value was calculated by plotting the standard graph using graph pad.

3. Hepatoprotective activity of extract on Paracetamol induced hepatotoxicity: HepG2 cells were treated with paracetamol (5 mM, 10 mM, or 15 mM and 25 mM) and incubated with medium only for 24 hours. The evaluation assays were performed using standard methods. After standardizing the model, *in vitro* hepatoprotective activity of seed methanolic extract was performed on paracetamol- induced hepatotoxicity in HepG2 cell line.

4. Statistical analysis

Duncan multiple range test(DMRT) were performed to test post hoc differences between groups means. Values were considered significant at $p<0.05$ level. Statistical analysis was conducted using SPSS 22.0.

Results

Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) is depicted in Fig. 1 .A significant difference was observed among the three samples. TPC was in the range of 76.65µg/mg- 195.62 µg/mg.TPC of ethanolic extract of MbS (*Musa balbasiana* seed) was significantly high in seeds compared to MbF (*Musa balbasiana* flower) and Mp (*Musa Paradisiaca)*. The MbS extract depicted a high phenolic content of 195.62µg/mg.

Antioxidant assays

DPPH radical scavenging activity

DPPH assays were used to assess the free radical scavenging activities of MbS, MbF, Mp ethanolic extract. Table 1 illustrates the significant reduction in the concentration of DPPH radical due to scavenging activity of banana. The study showed MbS has highest DPPH scavenging activity with an IC50 value of 784.77 ± 1.2 µg/ml .IC50 values of MbF and Mp were 1.1560 ± 1.01, 1.9928 ± 0.8 mg/ml.The positive control ascorbic acid IC 50 value was123.1 ± 0.05µg/ml. The result demonstrated that scavenging activity was different for flower and seeds which could be due to difference of the phenolic content. The correlation between total phenolic content and DPPH IC50 value was found with regression coefficient R^2 value of 0.962 shown in Fig. 2. Reports suggest extracts having more total phenolic content showed better free radical scavenging activity (less IC50 value). When DPPH IC50 values were plotted against total phenol content a linear regression curve was established.

Table 1
Antioxidant assays of *Musa balbisiana* seed and flower, *Musa paradisiaca* ethanolic extract

Sl. No.	Name of Extract	IC50 Values			
		DPPH	ABTS	FRAP	NO
1	Banana Seeds Ethanolic Extract	784.77 ± 1.2 µg/ml	592.08 ± 1.28 µg/ml	156.76 ± 2.21 µg/ml	931.36 ± 1.16µg/ml
2	Banana Flower Ethanolic Extracts	1.1560 ± 1.01 mg/ml	922.5 ± 1.07 µg/ml	426.33 ± 1.19 µg/ml	1.92 ± 2.13 mg/ml
3	Musa paradisiaca Ethanolic Extract	1.9928 ± 0.8 mg/ml	4.418 ± 1.23 mg/ml	2.63 ± 2.11 mg/ml	5.81 ± 3.31 mg/ml
4	Ascorbic Acid	123.1 ± 0.05µg/ml	141 ± 0.06 µg/ml	30.23 ± 0.07 µg/ml	115.31 ± 0.08 µg/ml
Values represent means of triplicate determination ± S.D.					
*Antioxidant assays performed for MbS, MbF and Mp. The table represents MbS ethanolic extract has high radical scavenging power(IC50).					

Table 2
The result of Duncan Multiple Range Test (DMRT) analysis on antioxidant activities of ascorbic acid, *Musa paradisiaca Musa balbasiana* flower, seed.

Treatment Group	DPPH	FRAP	ABTS	NO
Group 1	64.9851^{b}	25.2011^{a}	48.2507^{b}	51.0501^{b}
Group 2	27.7343^{a}	71.8094^{c}	50.7454^{b}	43.5728^{b}
Group 3	33.6729^{a}	56.5260^{bc}	44.2132^{ab}	32.2346^{ab}
Group 4	41.5471^{ab}	29.2767^{ab}	16.4119^{a}	13.8563^{a}
Different letters in the same column show the significant difference at the level of $p < 0.05$.				

ABTS radical scavenging activity

Musa balbisiana seed showed the highest ABTS scavenging activity compared to other two samples with an IC50 value of 592.08 ± 1.28µg/ml. IC50 values of MbF and Mp was922.5 ± 1.07µg/ml, 4.418 ± 1.23 mg/ml .The IC50 value of ascorbic acid was141 ± 0.06µg/ml.

Nitric oxide (NO) scavenging

The result of ABTS assay is presented in Table 1.The values of ABTS radical scavenging activities were observed to be highest for *Musa balbisiana* seed 931.36 ± 1.16 µg/ml followed by MbF and Mp with IC50

value of 1.92 ± 2.13, 5.81 ± 3.31 mg/ml. Ascorbic acid was used as a reference compound with an IC50 value of 115.31 ± 0.08 µg/ml.

FRAP scavenging activity

The MbS ethanolic extract showed the highest FRAP scavenging activity compared to MbF and Mp with inhibition of FRAP radical with an IC50 value of 156.76 ± 2.21µg/ml followed by IC50 value of MbF and Mp 426.33 ± 1.19 µg/ml, 2.63 ± 2.11 mg/ml respectively .The IC50 value of ascorbic acid which was used as reference is 30.23 ± 0.07 µg/ml.

The result of DMRT analysis showed that group 1(ascorbic acid) has the highest DPPH scavenging activity. Meanwhile group 2(Mp) and group 3(MbF) is not significantly different from group 4(MbS). Analysis of antioxidant activity is measured using reference compound ascorbic acid. Group 4(MbS) is significant to group 1 in all the activities DPPH,ABTS.FRAP,NO.

Hepatoprotective effect of Musa balbisiana seed:

The cell cytotoxicity of ethanolic extract of banana seeds was performed against HePG2 cell lines. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used for the determination of toxicity further treated with different concentration of banana seed extract for 24h. After 24h MTT was added to the walls of the cell and incubated for 4h and solubilized the MTT crystals in DMSO. Then absorbance of each well measured at 570nm using multimode reader. MTT test showed hepatoprotective effect of *Musa balbisiana* seed ethanolic extract in a dose dependent manner against paracetamol induced toxicity. The result showed that addition of 15mM paracetamol caused significant decrease in viability of HepG2 cells. Meanwhile addition of *Musa balbasiana* seed extract at concentrations 25, 50,100,200 µM caused significant elevation in cell viability. It was observed that paracetamol induced toxicated cells were recovered to about 60% after supplementation with 100 µg/ml of MbS extract, further initiating the dose by double 200 µg/ml better resulted in liver cells damaged recovery by 80%. Figure 3 illustrates the hepatoprotective effect of *Musa balbisiana* colla seed.

Discussion

DPPH radical scavenging activity is an *in -vitro* method of assessing antioxidant capacity studies in a short period of time. Antioxidant compound in the extracts react with DPPH and become stable diamagnetic molecule resulting in color change from purple to yellow by accepting an electron which could be an indicator of hydrogen donating ability of the samples (Adijimani and Asare, 2015). The DPPH radical scavenging activity of the MbS was significantly higher than ascorbic acid. The MbS showed good antioxidant activity in all the four assays. IC50 value is the inhibitory concentration where extract exhibits 50% of inhibition. Lower IC50 indicates greater antioxidant activity (Basumatary and Nath, 2018). IC50 value of MbS indicates high proton donating ability than other two samples. The proton donating ability of the extracts can serve as free radical scavengers possessing antioxidant potential (Abdul Qadir et al., 2017). The study illustrates the MbS extract inhibit nitrite formation. Therefore our study showed

the MbS extract has the highest nitric oxide scavenging activity when compared to MbF and Mp. Nitric oxide when in excessive production can lead to septic shock and cardiogenic shock(Cauwels 2007). As reported phenolic compounds can have a high nitric oxide scavenging activity(Kandasamy and Aradhya, 2014) which is exhibited by MbS.

Our study showed that the MbS contains a good amount of phenols. Phenols are plant compounds possessing antioxidant activity. Antioxidant play an important role in acute liver injury by normalizing the oxidative damage. Phenols act as antioxidants by transferring H atoms to free radicals(Vuolo et al., 2019).This antioxidant activity of phenolic compounds has ability to chelate metal ions involved in the generation of free radicals(Perron and Brumaghim, 2007). Our study also indicated the high phenol and its potential use in free radical scavenging activity. The results of determination of correlation between total phenolic and antioxidant indicated phenolic compounds possess high radical scavenging ability as demonstrated by good correlation between total phenolic and DPPH radical scavenging assay. Some reports have also depicted the strong correlation between total phenols and antioxidant activity(Baskaret al., 2011). Oxidative stress generated during paracetamol toxicity causing hepatic injury can be prevented which can be attributed to antioxidant activity of phenolic compound (Wu et al., 2017)

Paracetamol, used as antipyretic has been known to cause liver toxicity when in excessive overdose (Park et al., 2021). Paracetamol when in above the therapeutic level, exerts toxicity by the activity of hepatocytes metabolizing paracetamol through microsomal cytochrome P450 (CYP450) into toxic byproducts. The enzyme cytochrome P450 converts paracetamol to its active metabolite NAPQI. NAPQI in order to be excreted in urine has to be bound with cell glutathione. During toxicity cell glutathione is depleted and excess NAPQI binds to critical mitochondrial proteins causes thereby resulting in generation of reactive oxygen species (ROS). Such ROS causes damage to hepatocytes (Naquib *et al.*, 2014; Jaeschke et al., 2012). ROS occurs during oxidative stress in the cells therefore MbS having high antioxidant properties can be an element for treating paracetamol induced cytotoxicity and liver damage (Rao *et al.*, 2011). (Arumugam et al., 2021) also identified the hepatoprotective activity of *Musa balbisiana* unripe fruit extract which they explained the activity might be due to antioxidant potential and also presence of phenolic compound. HepG2 cell lines have been selected for toxicity studies related to hepatotoxin injury. HepG2 cells are susceptible to paracetamol exposure that can cause decrease cell viability (Palabiyik et al., 2016). *Musa balbisiana* seed effect in cell viability was assessed using *in- vitro* model. The study resulted in hepatoprotective effect of MbS extract in HepG2 cell line. Cell treated with MbS extract showed significant increase in cell viability when compared with APAP group.

Conclusion

The results of our study presents that *Musa balbasiana* seed can be a promising source of antioxidative and hepatoprotective property against paracetamol induced liver damage. Maximum beneficial effect was identified at a dose of 200µg/ml. This result suggests that the hepatoprotective activity can be related to potent antioxidative activity. Our study therefore defines the therapeutic potential of *Musa balbisiana* seeds in multiple liver diseases. The findings of antioxidant potential and total phenol content

showed good content of total phenols which can be correlated with its high DPPH scavenging activity. Further investigations are required to establish its other therapeutic potential of different parts of the plant.

Declarations

Conflict of interest:

No conflict of interest to disclose

Author Contribution

Manuscript writing and lab work- Dr Daisy SharmaIdea of work, critical review, revision of the manuscript and supervision - Dr Manash Sarma

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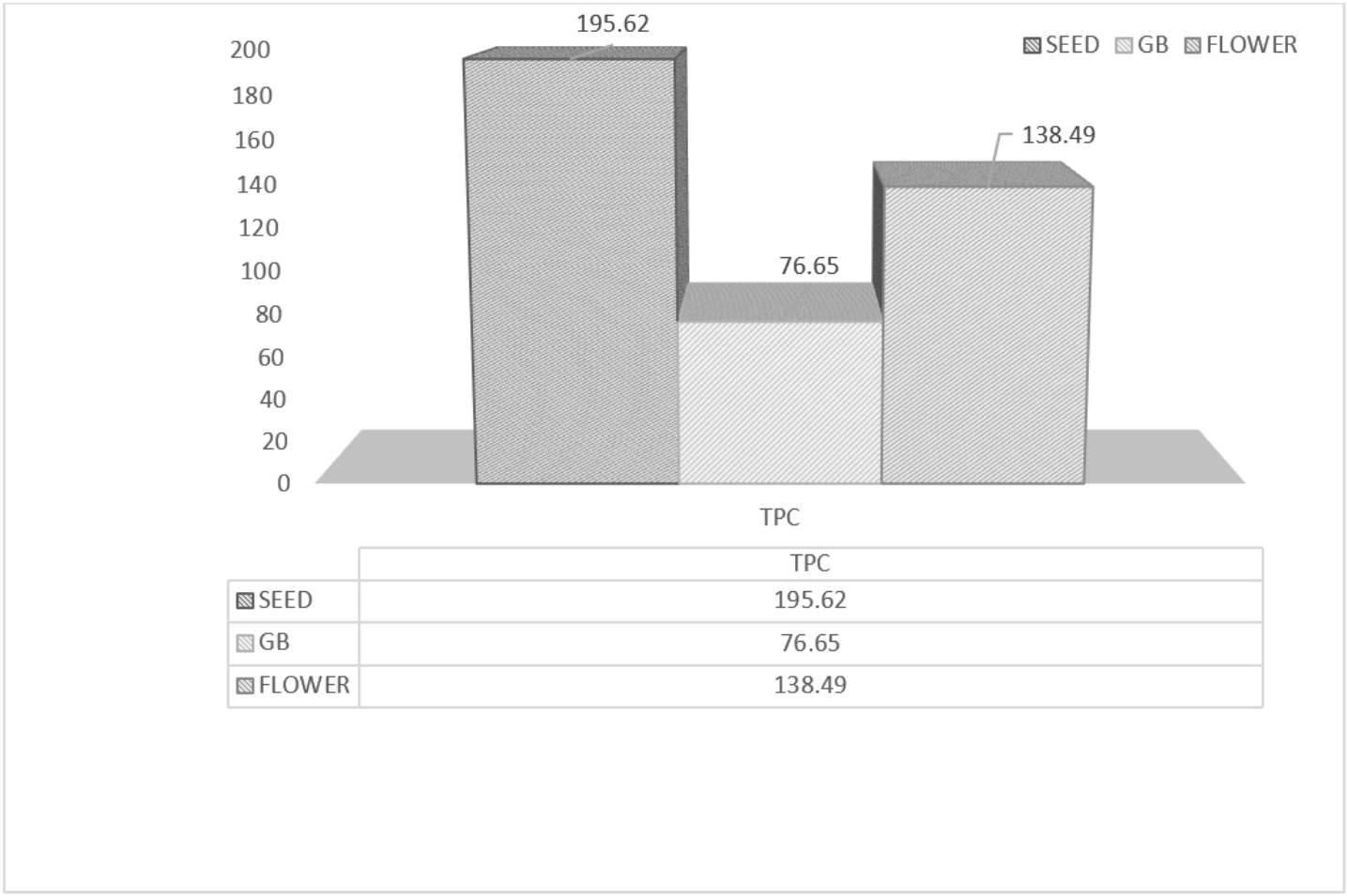
Figures



	TPC
SEED	195.62
GB	76.65
FLOWER	138.49

Figure 1

Total Phenolic Content (TPC) of *Musa balbisiana* colla seed, flower and *Musa Paradisiaca*(GB)

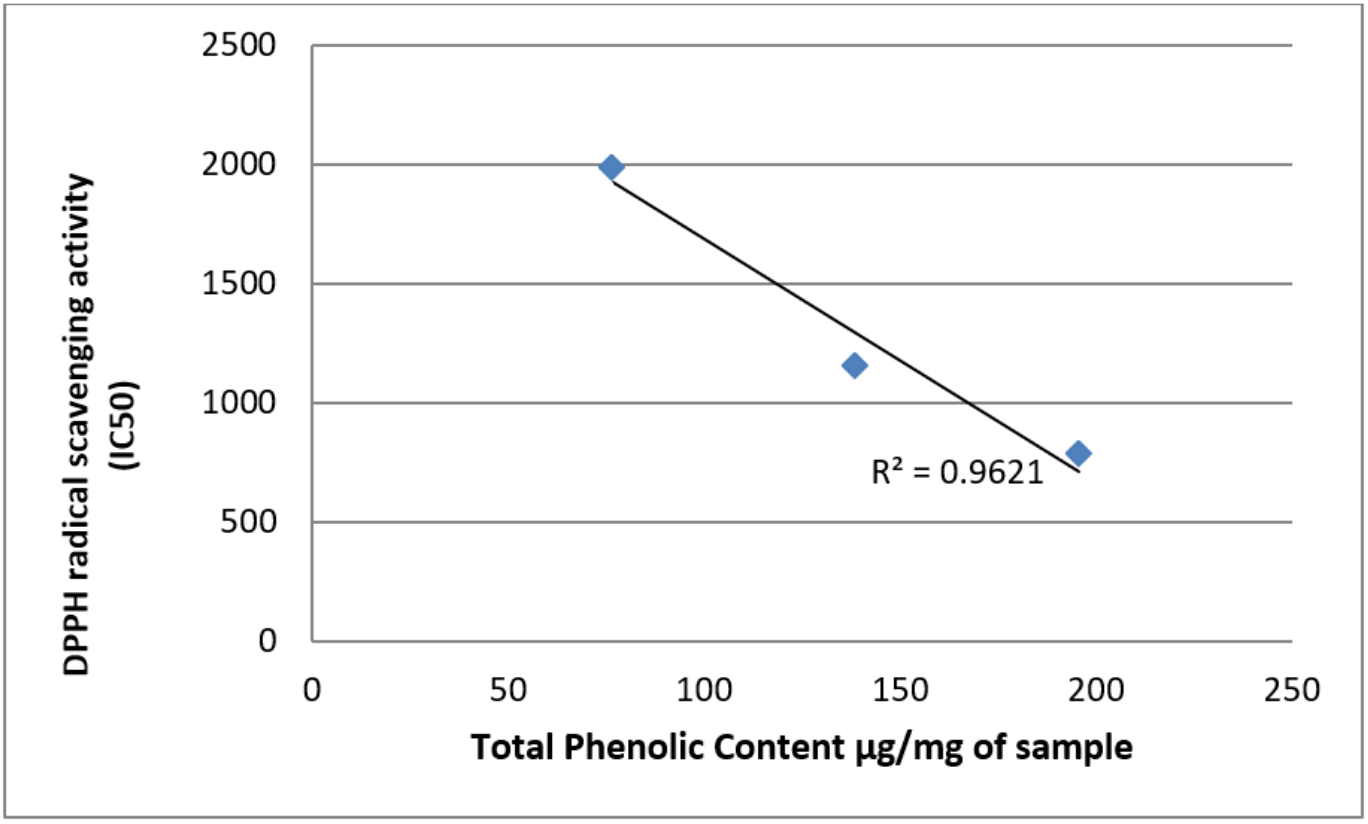


Figure 2

Linear regression between total phenolic content (TPC) and antioxidant activity(DPPH) of *Musa balbisisna* colla seed.

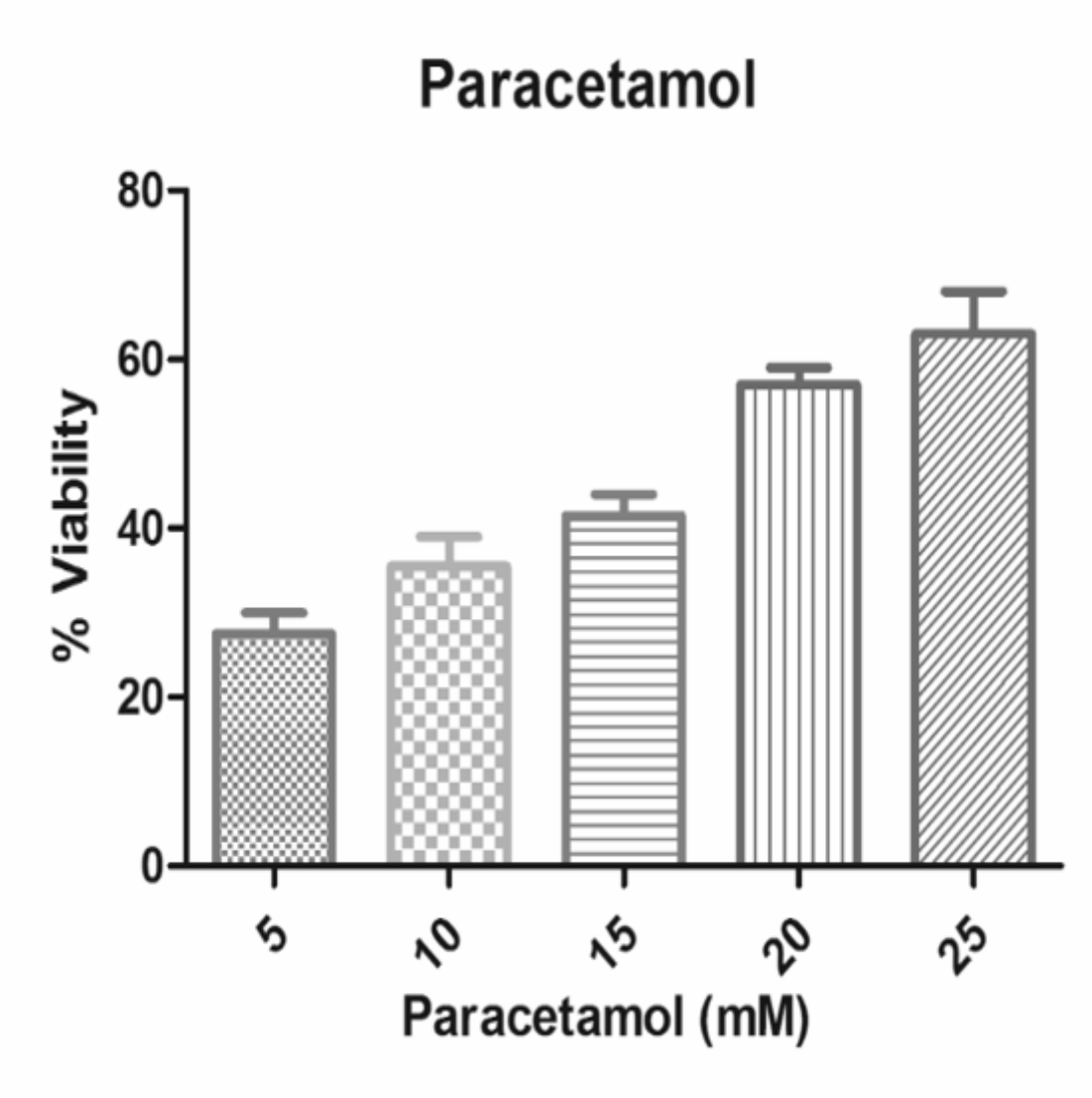


Figure 3

Fig 3 Hepatoprotective effect of *Musa balbisiana* colla seed against damage induced by 15mM paracetamol for 24hr in HepG2 cells.

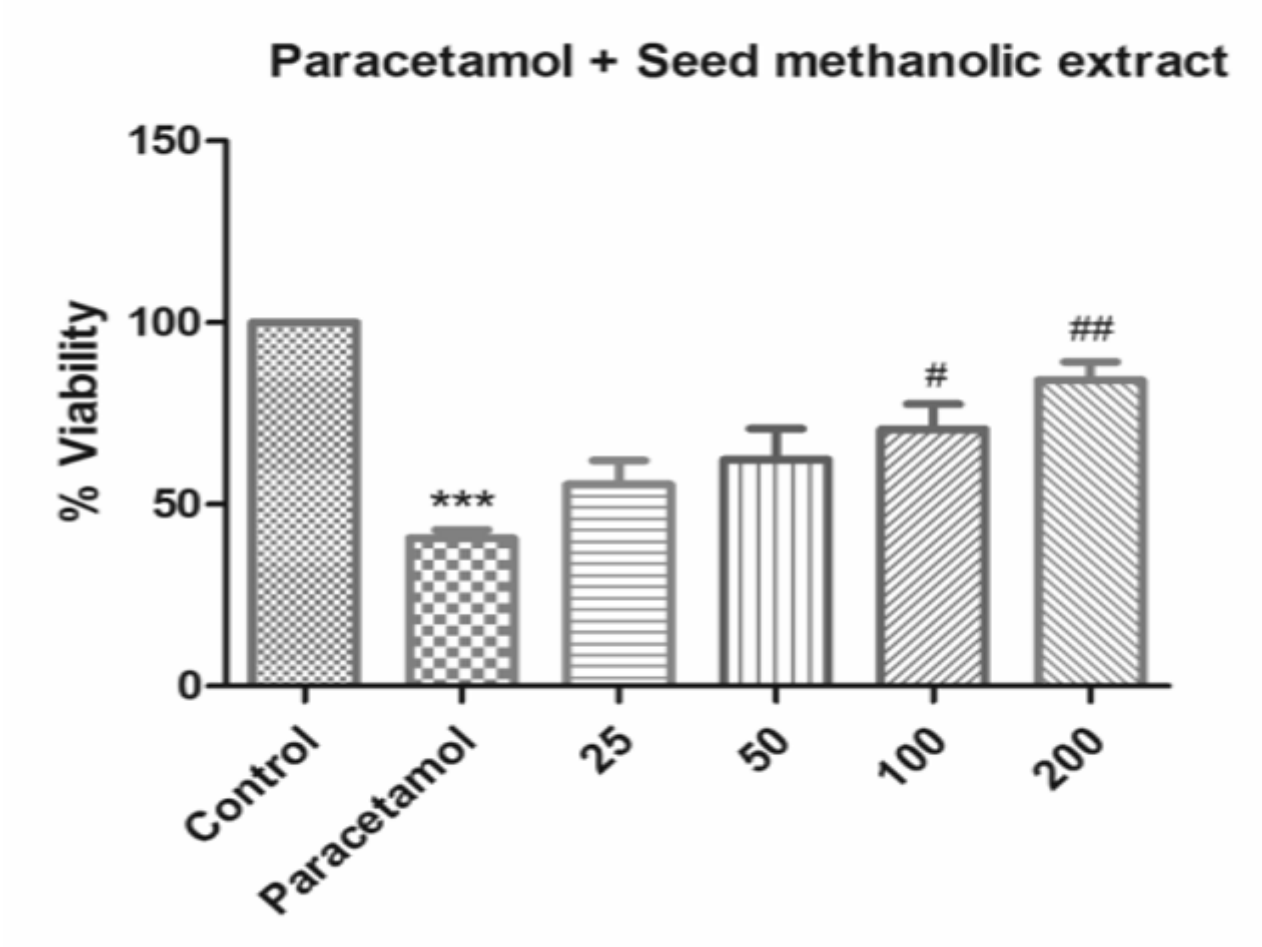
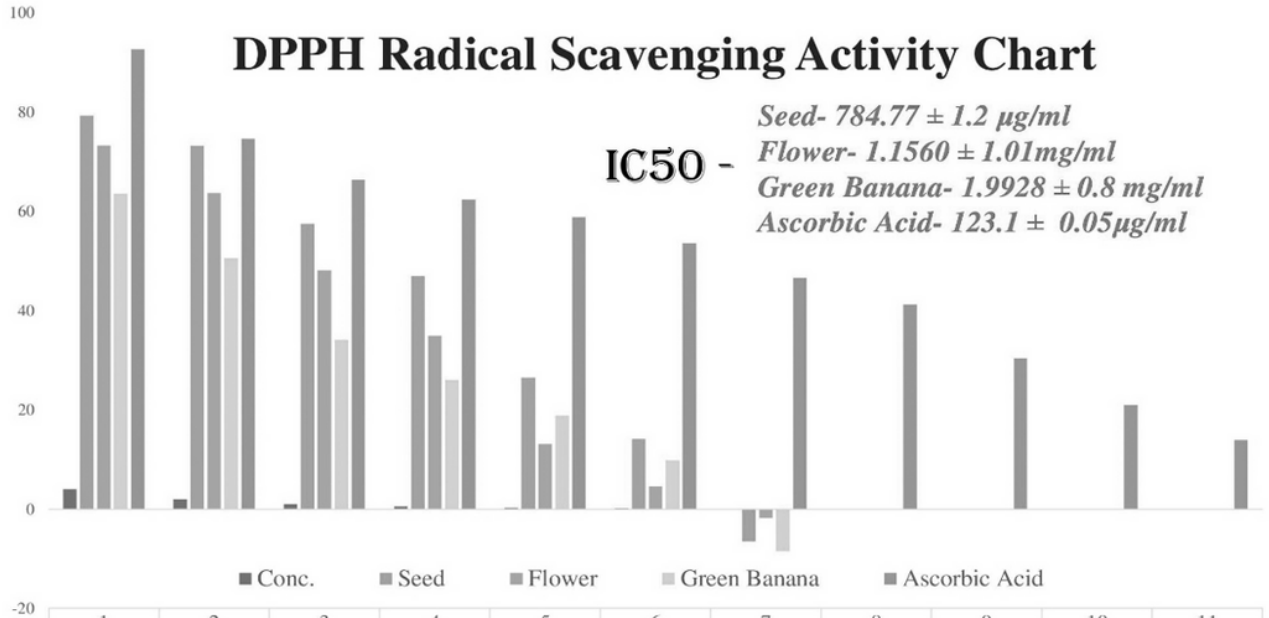


Figure 4

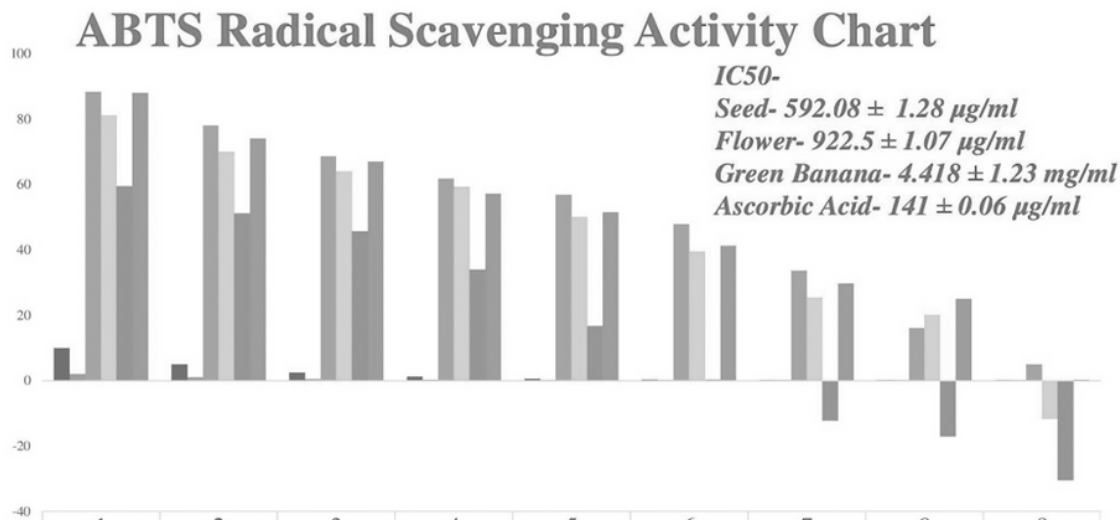
Fig 4 Hepatoprotective effect of *Musa balbisiana* colla seed against damage induced by 15mM paracetamol for 24hr in HepG2 cells.



	1	2	3	4	5	6	7	8	9	10	11
Conc.	4	2	1	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125	0.00390625
Seed	79.22	73.16	57.45	46.92	26.47	14.12	-6.51				
Flower	73.23	63.63	48.08	34.90	13.08	4.56	-1.77				
Green Banana	63.50	50.48	34.02	26.00	18.82	9.82	-8.50				
Ascorbic Acid	92.65	74.58	66.40	62.36	58.76	53.56	46.58	41.17	30.32	20.94	13.91

Figure 5

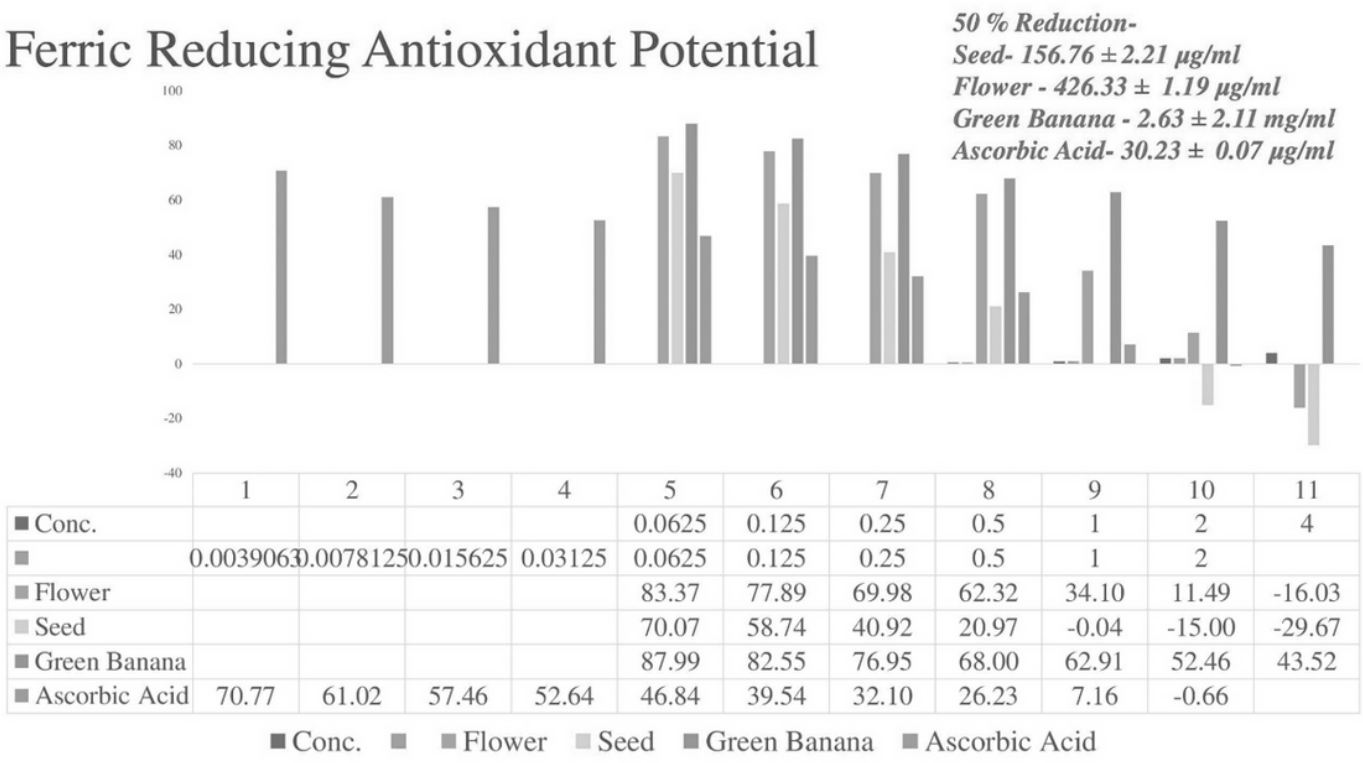
DPPH Radical scavenging activity chart of banana samples



	1	2	3	4	5	6	7	8	9
Concentration (Extract)	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.0390625
Concentration (Ascorbic Acid)	2	1	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125
Seed	88.38	78.12	68.64	61.80	56.93	47.93	33.76	16.20	4.95
Flower	81.13	70.01	64.05	59.32	50.13	39.44	25.43	20.14	-11.73
Green Banana	59.49	51.17	45.65	34.09	16.74	0.37	-12.21	-17.07	-30.52
Ascorbic Acid	88.01	74.10	67.06	57.23	51.52	41.22	29.74	25.12	0.23

Figure 6

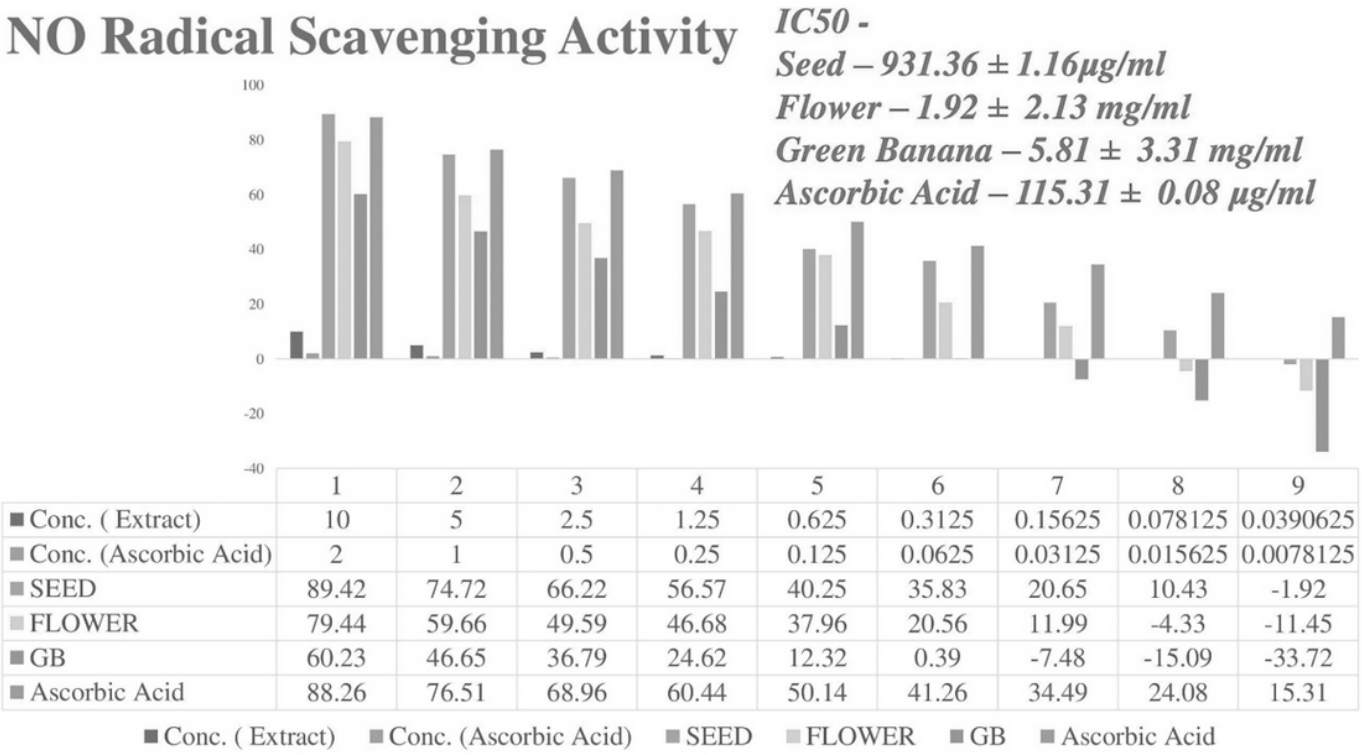
ABTS Radical scavenging activity chart of banana samples



	1	2	3	4	5	6	7	8	9	10	11
Conc.					0.0625	0.125	0.25	0.5	1	2	4
	0.0039063	0.0078125	0.015625	0.03125	0.0625	0.125	0.25	0.5	1	2	
Flower					83.37	77.89	69.98	62.32	34.10	11.49	-16.03
Seed					70.07	58.74	40.92	20.97	-0.04	-15.00	-29.67
Green Banana					87.99	82.55	76.95	68.00	62.91	52.46	43.52
Ascorbic Acid	70.77	61.02	57.46	52.64	46.84	39.54	32.10	26.23	7.16	-0.66	

Figure 7

FRAP chart of banana samples



	1	2	3	4	5	6	7	8	9
Conc. (Extract)	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.0390625
Conc. (Ascorbic Acid)	2	1	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125
SEED	89.42	74.72	66.22	56.57	40.25	35.83	20.65	10.43	-1.92
FLOWER	79.44	59.66	49.59	46.68	37.96	20.56	11.99	-4.33	-11.45
GB	60.23	46.65	36.79	24.62	12.32	0.39	-7.48	-15.09	-33.72
Ascorbic Acid	88.26	76.51	68.96	60.44	50.14	41.26	34.49	24.08	15.31

Conc. (Extract) Conc. (Ascorbic Acid) SEED FLOWER GB Ascorbic Acid

Figure 8

NO Radical Scavenging activity chart of banana samples

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FIG.pptx