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Antioxidant Potential and Secondary Metabolites in the Fruits of Spondia Pinnata (L. F) Kurz

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ABSTRACT

Spondias pinnata L.(Family- Anacardiaceae) is often known as Indian hog plum. This plant is using traditionally in the treatment of infectious diseases like bronchitis, ulcer, dysentery and skin diseases. The aim of the present study was to evaluate the antioxidant potential and secondary metabolites of the pulp extracted from the fruits S. pinnata L. In the estimation of antioxidant analysis the highest antioxidant activity was found in reducing power assay in methanol extract of ripe fruit at 4 mg concentration (1853.44±0.1mg/100g) than the other antioxidant assays. In secondary metabolite assessment more activity was found in alkaloid at 4mg concentration (2094.47±0.88mg/100g) of methanol extract of ripe fruit than the flavonoid content. The present study demonstrates that the fruit pulp of S. pinnata can be considered as a valuable source of an antioxidant activity and secondary metabolite. In brief, all concentration manifests good antioxidant and secondary metabolite activity.

Key words- Antioxidant, secondary metabolite, ripe fruit, unripe fruit, S. pinnata

I. INTRODUCTION

Spondias pinnata L. belonging to family Anacardiaceae is commonly known as Indian hog or Ambada in Marathi. It is a deciduous, medium-sized glabrous plum and tree up to 10.5 m tall with a straight trunk. The fruit is drupe, ovate, elliptical in shape and more than an inch long, acidic, fragrant, greenish yellow when ripe (Angami et al., 2020). In various languages, plants are known by many names, such as hog-plum, wild mango (English), amra (Bengali), mambulichi (Tamil), jangali aam (Hindi) etc (Manik M. et al., 2013). Its fruits are highly nutritious and rich in vitamin A and C, minerals and iron (Raju et al., 2017). Literature has shown that fruits are astringent and antiscorbutic, as well as sedation by bilious dyspepsia (Rao and Raju, 2010). Plants of the genus Spondias, made up of as a traditional medicine, 18 species were included, to treat multiple illnesses (Sri Laksemi, 2019). Oxygen is the crucial part of aerobic life. Under certain condition, it can seriously affect our well living system by the formation of reactive oxygen species such as free radical and non-free radical species as a result form harmful effect like atherosclerosis, ischemic heart diseases, ageing, inflammation, diabetes, immunosuppressant, neurodegenerative diseases, cancer and others (Das et al., 2011). This plant is using traditionally in the treatment of infectious diseases like bronchitis, ulcer, dysentery and skin diseases (Grasvenor et al., 1995) and (Hout et al., 2006). The S. pinnata plant is important for medicinal, nutritional and

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economical purposes. The fruit as well as root used as an anti-thirst remedy (Bora et al., 2014). Studies of plant extracts are essential in the search for molecules of antioxidants and anti-radical compounds. The ability to scavenge radicals and endogenous reactive oxygen species from plant extracts and isolated compounds and to act as enzyme inhibitors participating in their generation (such as peroxidases and oxidases), may be useful for the treatment of different types of drugs radical species-mediated diseases (Vellosa et al., 2008). In the living system, free radicals are sometimes formed and it may be responsible for damage to cells and tissues. As a result, research is focused on exploring stable and healthy efficient antioxidants and the enhancement of intake natural antioxidants from food supplements and traditional dietary supplements (Yazdanparast and Ardestani, 2007). Flavones are a class of flavonoids and a number of beneficial effects have been associated with their intake, including increased erythrocyte superoxide dismutase activity, decreased lymphocyte DNA damage, decreased urinary 8-hydroxy-2-deoxyguanosine (an oxidative damage marker) and increased plasma antioxidant ability (Williamson and Manach, 2005). Flavonoids also have strong antioxidant activity due to their ability to reduce the formation of free radicals and scavenge free radicals (Pietta, 2000). In antioxidants, secondary metabolites have a significant function any plants capacity (Kudale et al., 2016). Vitro antioxidant action has shown by Quercetin and rutin (Araújo da Silva et al, 2005). Flavonoid involve in oxidation of lipids and other molecules because they immediately donate hydrogen atom to free radicals (Schroeter et al., 2002). The present study deals with antioxidant activity and secondary metabolites in the fruit pulp of S. pinnata.

II. MATERIAL AND METHODOLOGY

2.1 Collection and Preparation of Plant Material

Fresh fruits of Spondias pinnata were collected from Amba (Kolhapur) in the month of July 2017, air dried and ground to a fine powder. Methanol, ethanol, acetone and aqueous extracts were then prepared for further analysis. The plant extract had been prepared mg/ml. The specimen was identified by using Flora of Kolhapur. The voucher specimen was submitted to SUK Herbarium. The plant extract was prepared through ultrasonic machine (Rivotek).

2.2 DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Wang et al., (1998).100 μl of plant extract (mg / ml) was combined with 2.9 ml of DPPH methanol solution for each extract at various concentrations (1, 2, 3, 4 mg/ml). After an incubation time of 30 min, the absorbance against blank methanol was measured at 517 nm using a UV-visible spectrophotometer. Based on the following formula, the DPPH radical scavenging activity (%) was calculated: DPPH scavenging activity (%) = [(AB-AT) / AB] X 100 where AB and AT are respectively the absorbance of blank and plant content. The percentage of scavenging activity of each extract was compared with the positive control, L-Ascorbic acid. The initial free radical concentration of DPPH is reduced by 50 %. The methanol was used as a blank

2.3 Ferric Ion Reducing Antioxidant Power (FRAP Assay)

Ferric Ion Reducing Antioxidant Power was analyzed following Benzie and Strain (1996). The FRAP reagent was prepared in 0.3 mM Acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM of HCl and 20 mM of $FeCl_3$ is combined in a 10: 1: 1 ratio. The plant extract (100 μl) was Mixed with 2.9 ml of FRAP reagent and absorbance it was measured at 593 nm.

2.4 Reducing Power

Anti-oxidative activity (reducing power) was determined as described by Oyaizu, (1986). Each extract (1 ml) of various concentrations (1,2 3, 4 mg/ml), 2.5 ml of phosphate buffer and 2.5 ml of 1 % potassium ferricyanide were combined with double-distilled water. The mixture was incubated for 20 minutes at 50 °C, after which 2.5 ml of 10% trichloroacetic acid (TCA) was added and centrifugated for 10 min at 3000 rpm. Then add 2.5 ml of top layer of supernatant was combined with 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride while L-Ascorbic acid was used as a positive control. Using a UV-visible spectrophotometer, the absorbance was measured at 700 nm.

2.5 Total Antioxidant Capacity

Total antioxidant capacity (TAC) by Prieto et al., (1999). Each 0.2 ml extract of methanol, ethanol, acetone and aqueous with a concentration of 0.2 mg/ml was combined with 2ml (600 mM sulfuric acid, 28 mM sodium sulphate and 4 mM ammonium molybdate) of reagent solution, the reaction mixtures were then incubated at 950C with 90 min. The absorbance has been calculated Using a UV-visible spectrophotometer at 695 nm against a blank that includes 3 ml of reagent solution. The overall antioxidant activity of the crude extract was expressed in mg/100g of dry weight as an L-ascorbic acid equivalent.

2.6 Chelation Power on Ferrous (Fe2+) Ions

Chelation Power on Ferrous (Fe2+) Ions was determined with the method suggested by Decker and Welch (1990). The plant extracts mixed with 0.1 ml of 2 mM $FeCl_2$ was 0.5 ml of methanol plant extract (100 µg / ml), 0.2 ml of 5 mM ferrozine solutions were able to react at room temperature for 10 min. The absorbance was measured by a spectrophotometer at wavelength 562 nm. The percentage of ferrous ion inhibition was determined by comparing the results of the L-Ascorbic acid (100 µg / ml).

2.7 Total Flavonoid

The total flavonoid content was calculated using the (Luximon-Ramma et al., 2002). The each extract (1 ml) or std. solution of Quiercitine (1,2 3, 4 mg/ml), 5% Sodium nitrate and keep in 5 min, then add 0.3 ml of 10 % $AlCl_3$. After 5 min 2 ml of 1 M NaOH was added. The reaction mixture was measured against the blank and absorbance taken at 510 nm.

2.8 Total Alkaloids

The total alkaloid content calculated using the 1, 10 Phenanthroline method was determined by (Singh et al., 2004) with minor modification. The extracts were preparing in 70 % methanol, ethanol, acetone and aqueous. There was 100 µl of plant extract in the reaction mixture. The reaction mixture was 1 ml of 0.05 M solution of 1, 10- Phenanthroline in methanol solvents and 1 ml of 0.025 M solution of $FeCl_3$ in 0.5 M solution of HCL. The reaction mixture was incubated for 30 min. in water bath at 70±20C. The absorption of the red-colored substance against the blank reagent was measured at 510 nm. The contents of alkaloids were measured and determined using the typical colchicine curve.

2.9 Statistical Analysis

Statistical data was calculated using MS Excel. Pearson's correlation analysis was used for the correlation study.

III. RESULTS AND DISCUSSION

The values for DPPH radical scavenging activity, Ferric Ion Reducing Antioxidant Power (FRAP), Reducing power, Total Antioxidant Capacity, Chelation Power on Ferrous Ion, Total flavonoids and Total Alkaloids, in the extracts of various solvents, at various concentrations have been presented in Table 3.

3.1 DPPH radical scavenging activity

The DPPH activity was high in methanol as compared to other solvents such as ethanol, acetone and aqueous. The lowest activity was showing in aqueous. The inhibition percentage also increases with increased concentration (mg/ml). The ripe fruit was observed the highest scavenging activity than the unripe fruits. The highest activity in both ripe and unripe fruit was shown by the methanol extract result. More activity was seen at 4 mg/ml (85.05±0.09) concentrations in ripe fruit of methanol extract. The lowest activity was observed at 1mg/ml (20.95±0.4) concentrations in unripe fruit of aqueous extract. The unripe fruit demonstrated the lowest activity when comparing between both ripe and unripe fruits. The unripe acetone extract displayed the highest activity relative to ripe fruit. According to (Jain et al., 2014) highest DPPH activity was found in ethanol extract of Spondias pinnata (85.3±3.05%). In present study ethanol extract result show in 4 mg/ml (83.87±0.1%) of unripe fruit approximately similar activity was showed by Jain et. al.

3.2 Ferric Ion Reducing Antioxidant Power (FRAP Assay)

The reducing potential of antioxidants was estimated through FRAP assay to react with ferric tripyridyl triazine (Fe3+-TPTZ) complex and produce the blue color of ferrous form which can be measured at absorbance 593 nm (Benzie and Strain, 1996). In present study taken crude pulp extract of Spondias pinnata and result showed better FRAP activity. The FRAP activity is highest as compared to other methods. The highest activity was observed in the ripe fruits of methanol extract in 1mg/ml (553.62±0.6) and lowest activity was observed in aqueous extract of 1mg/ml (188.02±0.6) of unripe fruit. The concentrations of mg/ml increases activity also increases. Acetone extract showed FRAP activity as like methanol and ethanol extract. The values are depicted in table no.I. The antioxidant capacity was calculated in FRAP based on the ability to decrease Fe3 + to Fe2 + ions. (De Almeida et al., 2017). The FRAP activity in crude fruit juice of S. mombin (11.8 ± 0.2 µmol) studied by (Coolborn et al. 2016) and S. pinnata observed FRAP activity in crude ripe fruit of 1 (mg/ml) (553.62±0.6) of methanol extract. Hence compare between both fruits S. mombin showed less FRAP activity than the S. pinnata. The amount of extract increases the reducing power activity was increases reported by (Hafiz et al., 2010)

3.3 Reducing power activity

In present study various extracts for different concentrations used and observed that reducing power was increased with increasing amounts of extract. In reducing power best activity was observed in methanol extract of ripe fruit of 4 mg/ml (1853.44±0.1) and less activity in aqueous extract of unripe in 1mg/ml (220.34±5.1). In reducing power showed best activity in all solvents. So result showed all solvents are good for reducing activity. Antioxidant study showed better activity in methanol extract of S. purpurea Uchoa et al., (2015). The results are given in table no 3.

3.4 Total Antioxidant Capacity

The assay is depending on the reduction of Mo (VI) to Mo (V) when sample was analyzed, it has a reduction potential after formation of stable green Mo (V) phosphate complex (Prieto et al., 1999). The S. pinnata extract was decreased in the following order: methanol>ethanol>acetone>water. The greater activity was found in methanol extract of 4mg/ml in ripe fruit (146.98±0.4) and lesser activity was observed in 1mg/ml (29.20±0.4) of aqueous extract of unripe fruit. When compare with all antioxidant activity Phosphomolybdenum antioxidant activity is less than other antioxidant activity. The results are showing in table no 3.

3.5 Chelation Power on Ferrous (Fe2+) Ion

The presence of chelating agent ferrozine produces a violet complex with Fe2+ complex formation is interrupted and as a result the violet color of the complex is decreased Hazara et al.,(2008). Hafiz et al. 2010 was also observed in his experiment 70% methanol of S. pinnata bark extract has well potent of iron chelation. Iron-chelating activity is an antioxidant molecule which prevents oxyradical generation and oxidative damage (De Angelis da Costa Barros Gomes et al., 2018).The highest activity was observed in methanol extract of ripe of 4mg/ml (53.33±0.6%) and low activity of 1mg/ml (20.78±0.3%) of ripe fruit of aqueous extract. The negligible difference observed between all solvents. The Ferrous (Fe2+) Ion chelating ability result also showed all solvents are good for extract preparation for antioxidant activity. The values are showing in table no 3.

3.6 Total flavonoid

In the present study for the estimation of flavonoids taken four solvents such as methanol, ethanol, acetone and aqueous. In unripe fruits of acetone extract observed highest activity and lowest activity in the aqueous extract. Next to aqueous extract lowest activity was found in the acetone and methanol solvent respectively. Compare to ripe and unripe fruit highest flavonoid activity was observed in unripe fruits. Little difference observed between ripe and unripe fruits. The highest activity observed in 4mg/ml in unripe fruits of acetone extract (793.9±2mg/100g) as well as lowest activity was observing in 1 mg/ml in ripe fruits of aqueous extract (157.23±2.93mg/100g). Uddin et al., 2016 examined aerial components such as leaves. They were prepared different extracts and found the highest flavonoid activity (132.27 ± 0.25 mg/gm) in ethyl solvent. In present study in ethyl solvent was showed (340.01±0.3 mg/100g) in 4mg/ml concentrations of unripe fruit. The results are showed in Table No 5 and 6

3.7 Total Alkaloids

For the evaluation of alkaloids were taken four solvents such as methanol, ethanol, acetone and aqueous. The alkaloid content is highest in the methanol extract of ripe fruits (2094.47±0.88 mg/100g). Highest range show in series such as Methanol>Acetone>Ethanol>Aqueous. Alkaloid content high than the flavonoids in fruit pulp of Spondias pinnata. The results are depicted in Table No 7 and 8.

3.8 Correlation of antioxidant activity with total flavonoids

In several plants were observed high antioxidant capacity and show direct linear correlation with high phenol content and antioxidant activity (Cai et al., 2004); (Djeridane et al., 2006), (Kolar et al., 2014). In some plant extract has been established good correlation between antioxidant activity and reducing power. The reducing power is used as a good indicator of potential activity (Yen et al., 2001). The highly positive relationship between Flavonoid/FRAP was observed dominantly in ripe fruit of aqueous extract and in unripe fruit of

acetone extract (R^2 is 0.985 and 0.996 $P<0.05$). In reducing power ripe fruit showed significant correlation between flavonoid and reducing power in ethanolic extract R^2 is 0.957 $P<0.05$. In unripe fruit also observed significant correlation in acetone extract R^2 is 0.946 $P<0.05$. The highest correlation of Flavonoid with phosphomolybdenum was observed in aqueous extract of ripe fruit R^2 is 0.909 and in unripe fruit showed strong correlation in acetone extract R^2 is 0.915 $P<0.05$.
The stronger correlation of ferrous ion chelating ability with Flavonoid was observed in ripe fruit of acetone extract which is significant R^2 is 0.975 ($P<0.05$). In unripe fruit highest correlation was observed in methanol extract which is significant R^2 is 0.994 ($P<0.05$). The values are depicted in Table No 1 and 2.
The correlation studies showed that Flavonoids had significant correlation with antioxidant activity.

Table NO. 1 Correlation between secondary metabolites and antioxidants of ripe fruits of Spondias pinnata

Solvents	Correlation between secondary metabolites and antioxidants			
	FRAP	Reducing power	PMO	FICA
Methanol	0.847	0.9114	0.9169	0.9225
Ethanol	0.7855	0.9571	0.9287	0.9675
Acetone	0.9543	0.9027	0.9227	0.975
Aqueous	0.985	0.8379	0.8261	0.9527

Table NO. 2 Correlation between secondary metabolites and antioxidants of unripe fruits of Spondias pinnata

Solvents	Correlation between secondary metabolites and antioxidants			
	FRAP	Reducing power	PMO	FICA
Methanol	0.865	0.8898	0.8837	0.9942
Ethanol	0.7981	0.8284	0.873	0.9251
Acetone	0.996	0.9463	0.9152	0.9925
Aqueous	0.7691	0.5923	0.6379	0.7458

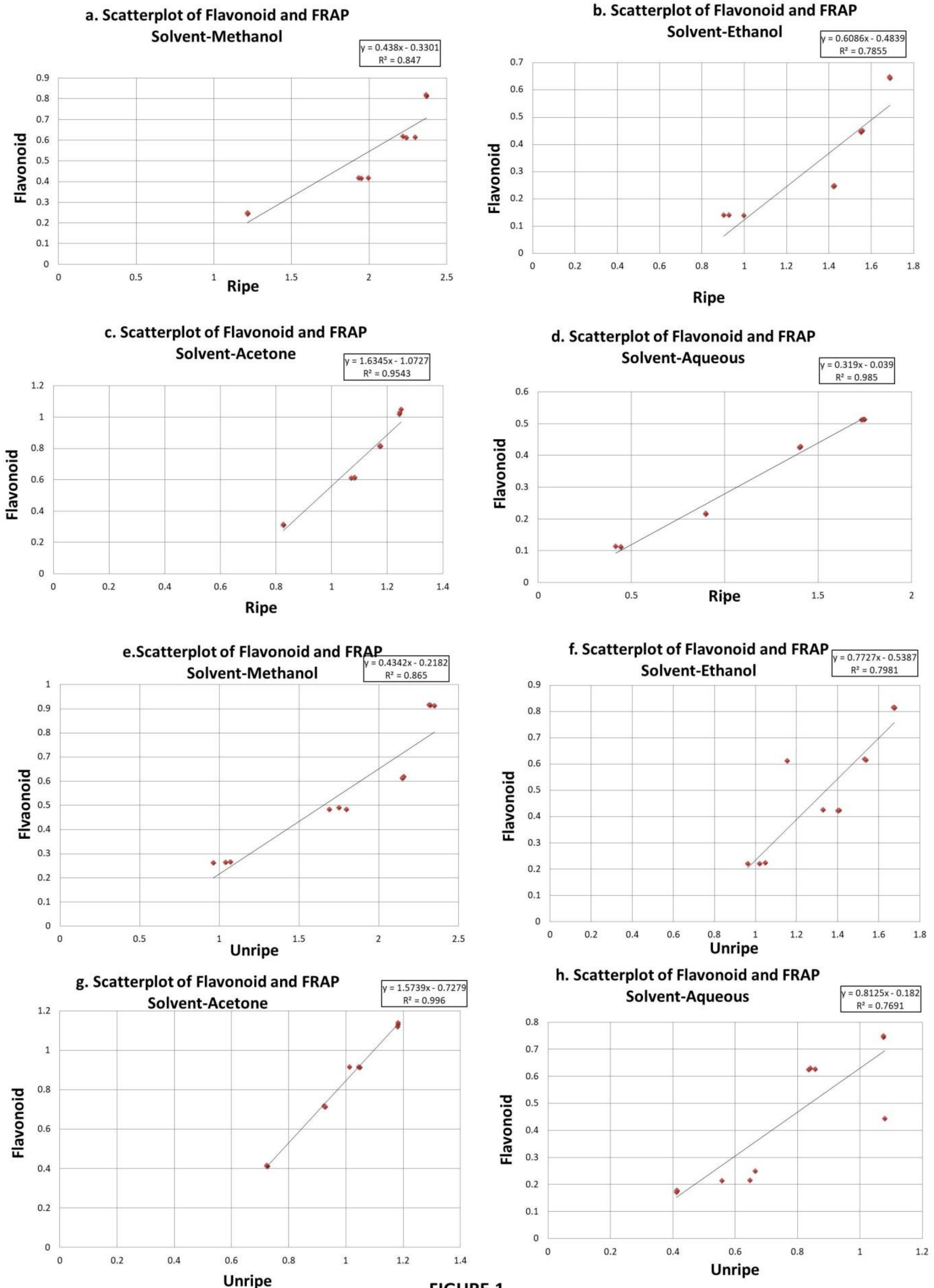


FIGURE.1

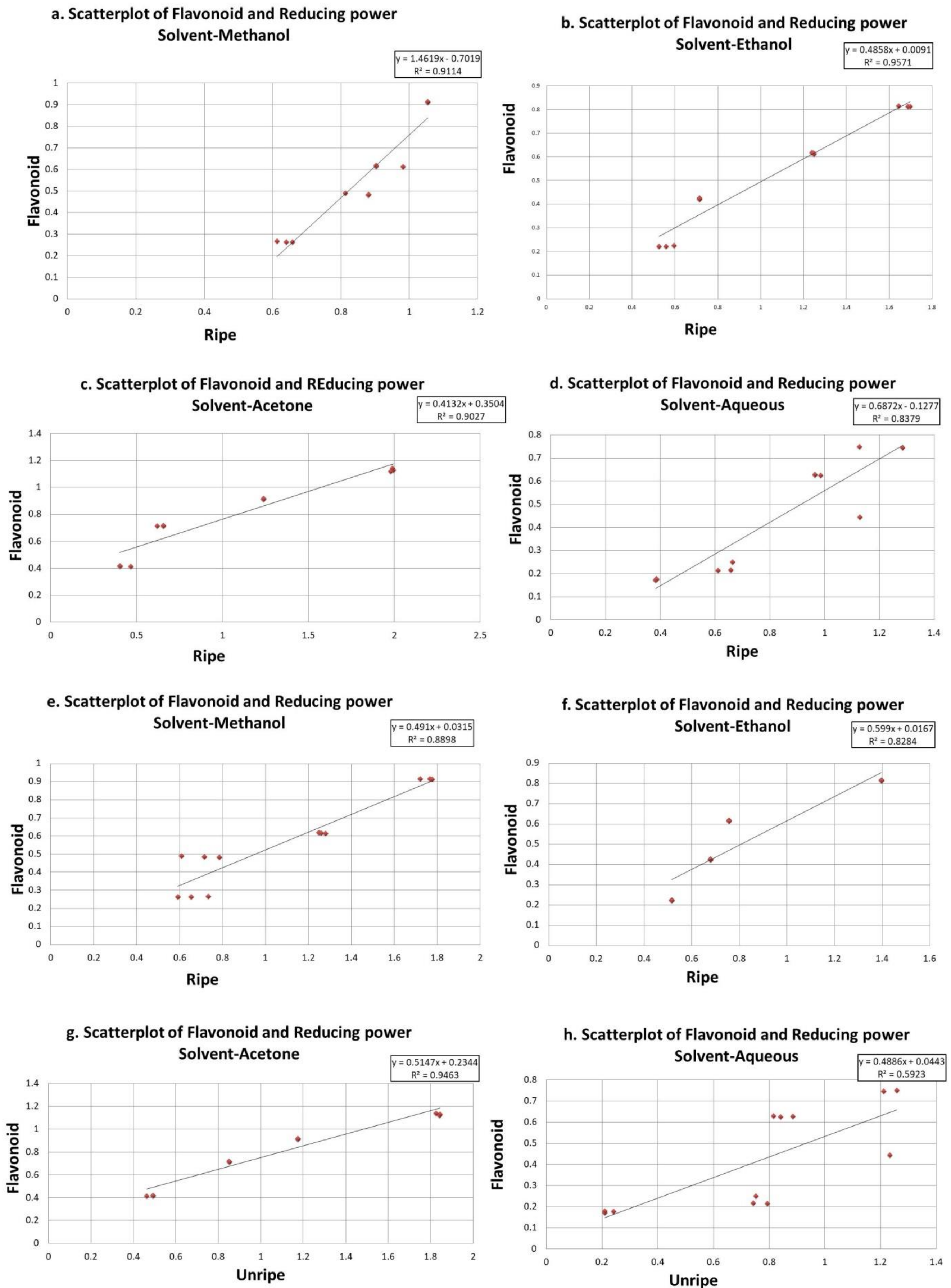


FIGURE.2

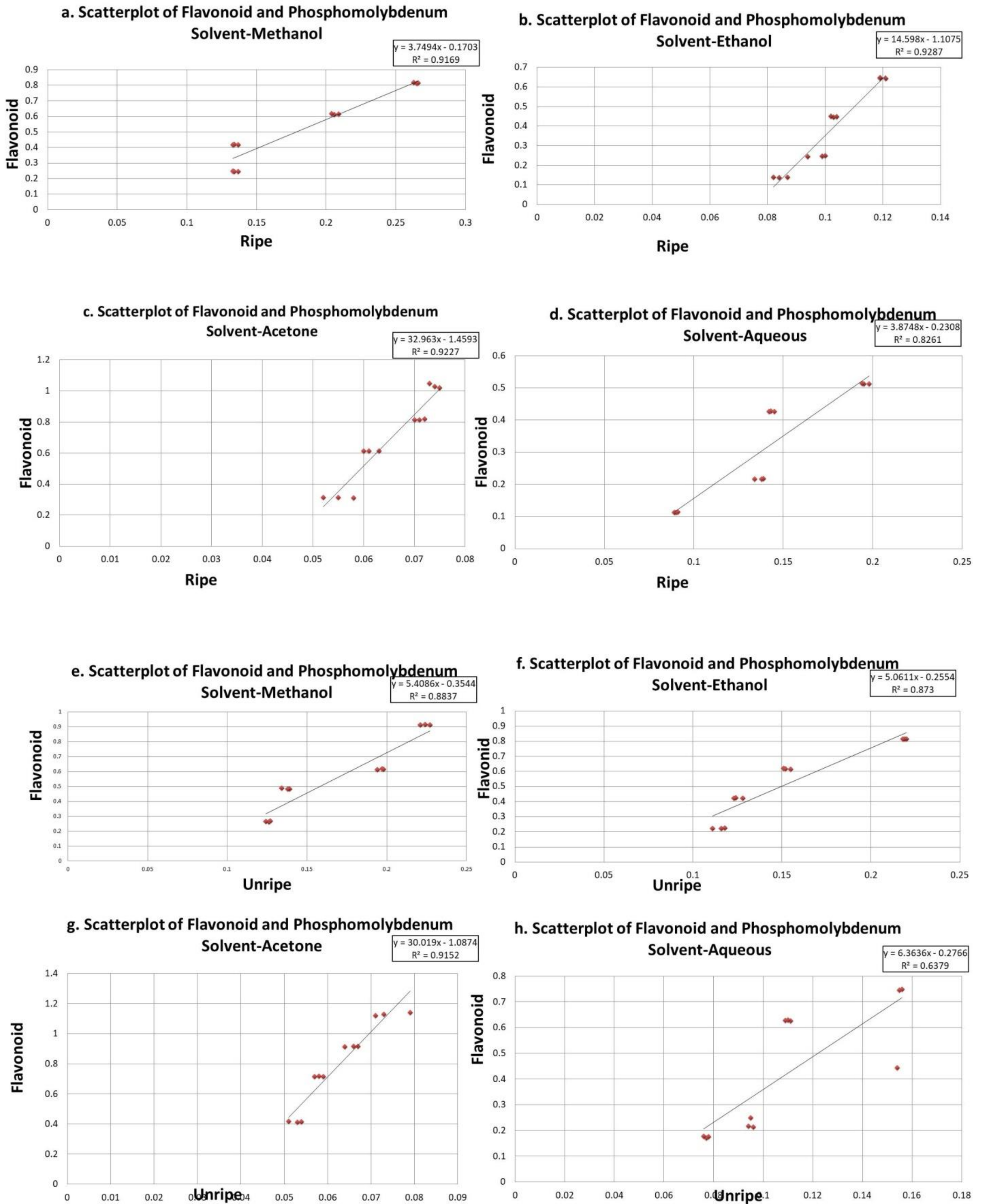


FIGURE.3

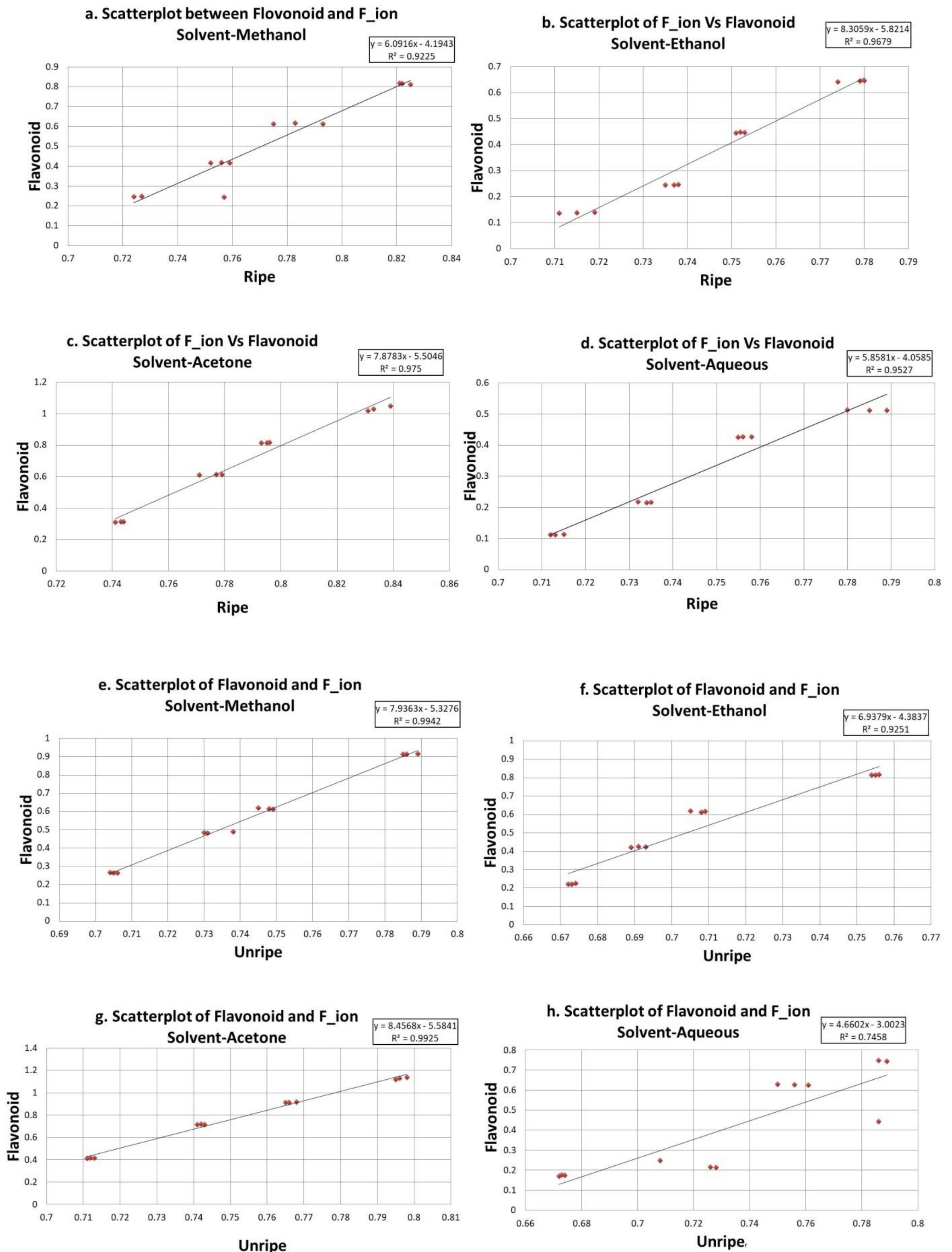


FIGURE.4

Table No.3Antioxidant potential of ripe and unripe fruits of Spondias pinnata

Solvent	Conc.	DPPH inhibition (%)		FRAP (mg/100g)		Reducing power (mg/100g)		PMO (mg/100g)		FICA mg/100g	
	Mg/ml	R	UR	R	UR	R	UR	R	UR	R	UR
Methanol	1	32.18 ±0.1	42.52± 0.1	553.62± 0.6	472.87 ±2.8	637.15± 0.9	593.01± 0.3	74.80±0.6	69.75± 0.4	41.3± 5.6	53.18 ±0.3
	2	54.97 ±0.1	64.41± 0.1	879.99± 0.7	792.34 ±1.0	914.34± 1.3	786.07± 0.6	97.90±0.4	76.05± 0.7	53.33 ±1.0	61.79 ±1.3
	3	76.69 ±0.1	66.94± 0.1	1044.53 ±0.02	979.68 ±0.7	1316.04 ±0.1	1263.11 ±4.4	114.57± 0.7	109.01 ±0.6	65.47 ±2.8	68.6± 0.6
	4	84.66 ±0.1	85.05± 0.2	1076.81 ±0.25	1059.38±4.	1853.44 ±0.1	1754.47 ±8.4	146.98± 0.4	124.38 ±0.8	72.75 ±0.6	75.64 ±0.6
Ethanol	1	28.13 ±2.5	30.83± 2.4	452.72± 0.78	329.38 ±0.1	725.50± 0.4	516.0.1 ±0.1	46.79±0.7	63.83± 1.1	30.98 ±1.2	42.9± 0.3
	2	45.50 ±0.1	55.77± 0.2	647.40± 0.9	420.90 ±0.7	824.67± 0.09	679.54± 0.03	54.20±0.9	69.38± 0.8	43.31 ±0.5	49.64 ±3.4
	3	51.68 ±0.1	72.45± 0.2	706.35± 1.13	470.64 ±5.5	1244.43 ±1.6	757.81± 0.04	57.16±0.2	84.76± 0.6	54.41 ±0.3	57.43 ±1.7
	4	66.00 ±0.5	83.87± 0.1	767.42± 0.3	537.41 ±0.5	1696.6± 0.52	1396.74 ±0.2	66.42±0.3	121.61 ±0.3	65.55 ±1.0	66.85 ±0.5
Acetone	1	33.09 ±0.1	45.00± 0.1	375.90± 0.2	438.47 ±0.9	763.80± 0.32	482.31± 4.7	49.94±0.3	42.72± 0.3	53.33 ±0.4	55.57 ±0.3
	2	45.54 ±0.3	53.97± 0.03	490.14± 2.12	638.02 ±1.0	920.67± 0.07	851.64± 0.08	76.05±0.8	52.72± 0.3	65.47 ±1.3	65.55 ±0.3
	3	53.90 ±0.1	64.86± 0.1	533.77± 0.5	698.47 ±1.3	1238.5± 0.13	1175.34 ±0.1	79.57±0.4	61.05± 0.29	76.76 ±0.5	76.53 ±0.5
	4	65.55 ±0.1	77.52± 0.1	566.35± 1.03	761.65 ±0.4	1986.8± 2.2	1837.64 ±2.8	108.64± 0.61	86.05± 0.2	77.87 ±1.3	77.65 ±0.4
Aqueous	1	22.89 ±0.1	20.95± 0.4	197.26± 3.8	188.02 ±0.6	385.23± 0.6	220.34± 5.1	30.49±0.8	29.20± 0.4	20.78 ±0.4	32.06 ±0.3

	2	34.92 ±1.3	24.28± 0.1	408.32± 0.5	302.11 ±0.7	894.43± 0.8	762.91± 7.7	34.01±0 .4	32.16± 0.3	32.06 ±0.5	38.58 ±0.6
	3	46.30 ±0.6	36.83± 0.1	638.02± 1.2	383.93 ±2.9	984.90± 0.02	815.78± 0.04	39.38±0 .29	36.42± 0.4	37.54 ±0.8	53.22 ±0.6
	4	55.80 ±0.1	40.92± 0.1	791.96± 2.24	489.53 ±0.7	1528±0. 17	1214.81 ±0.8	41.05±0 .29	41.24± 1.23	42.16 ±1.4	65.36 ±0.3

± SE (Standard Deviation), FRAP- Ferric Reducing Antioxidant activity, PMO-Phosphomolybdenum, FICA-Ferrous ion chelating ability, R-Ripe, UR-Unripe. (%) - Percentage.

Figure No.5 Flavonoid content of ripe fruits of Spondias pinnata

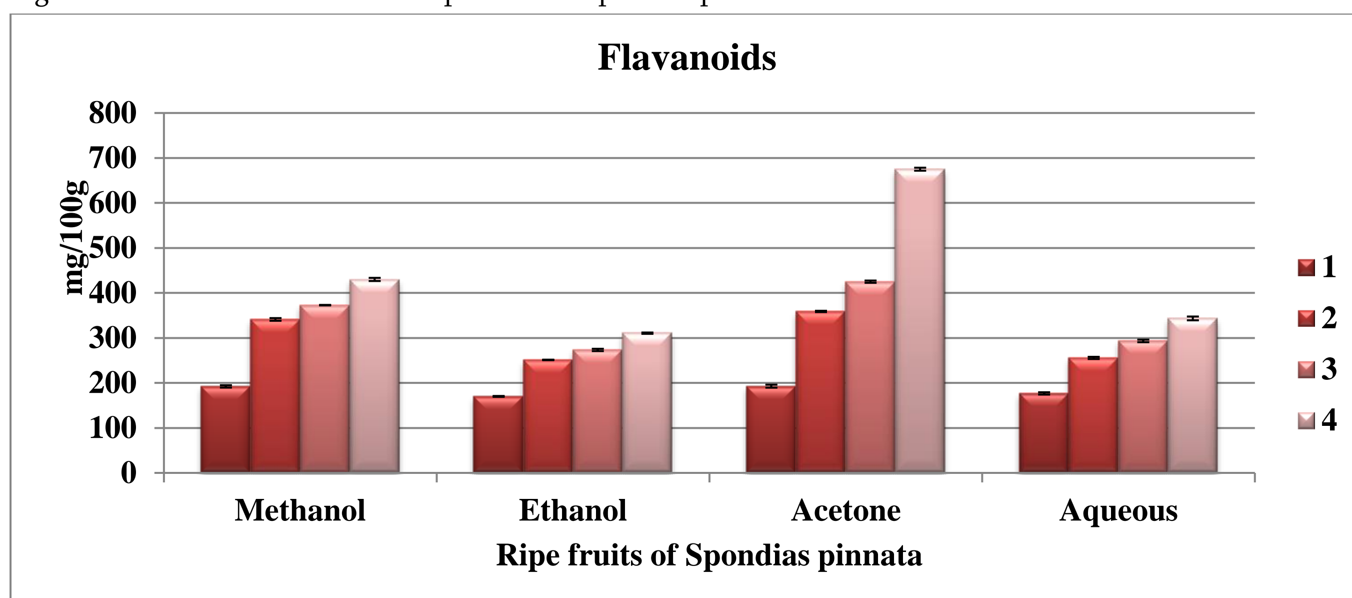


Figure No.6 Flavonoid content of Unripe fruits of Spondias pinnata

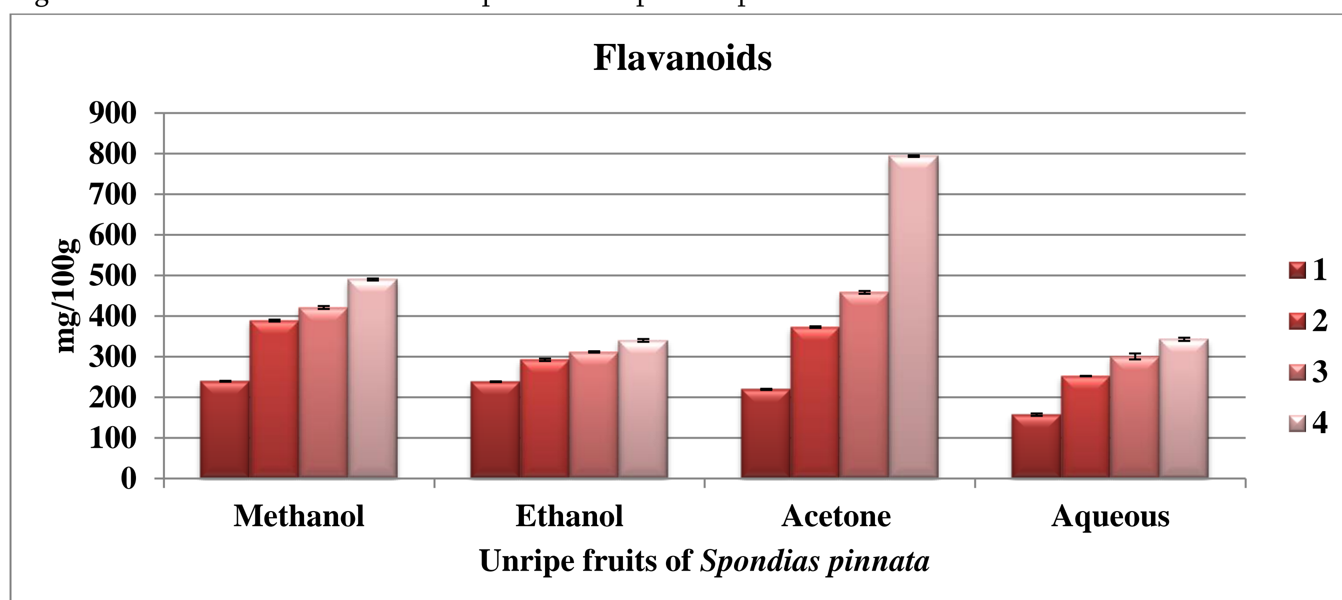


Figure No.7 Alkaloid content of ripe fruits of Spondias pinnata

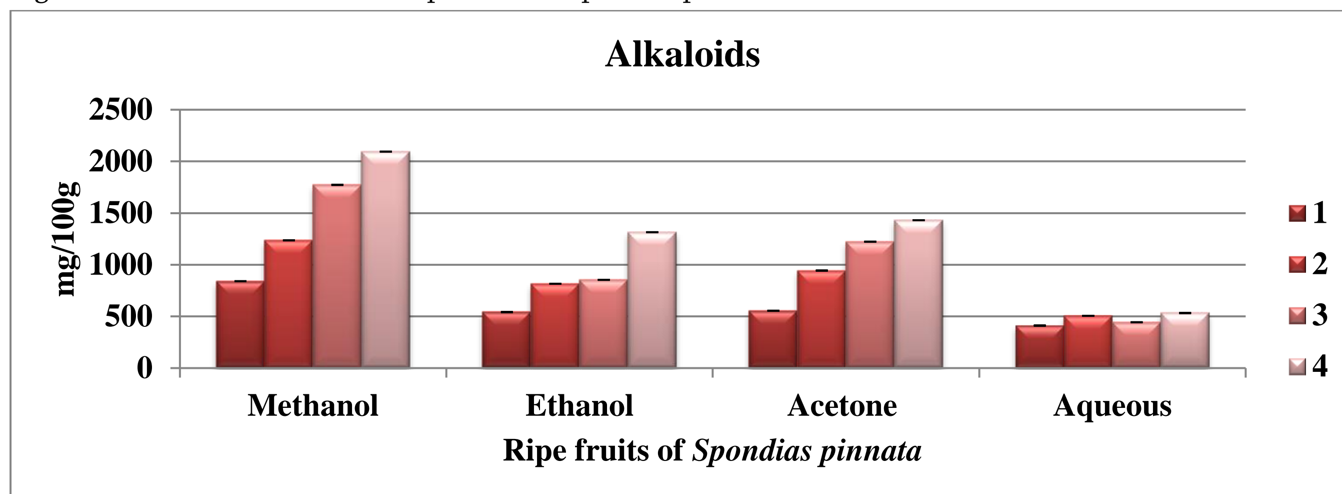
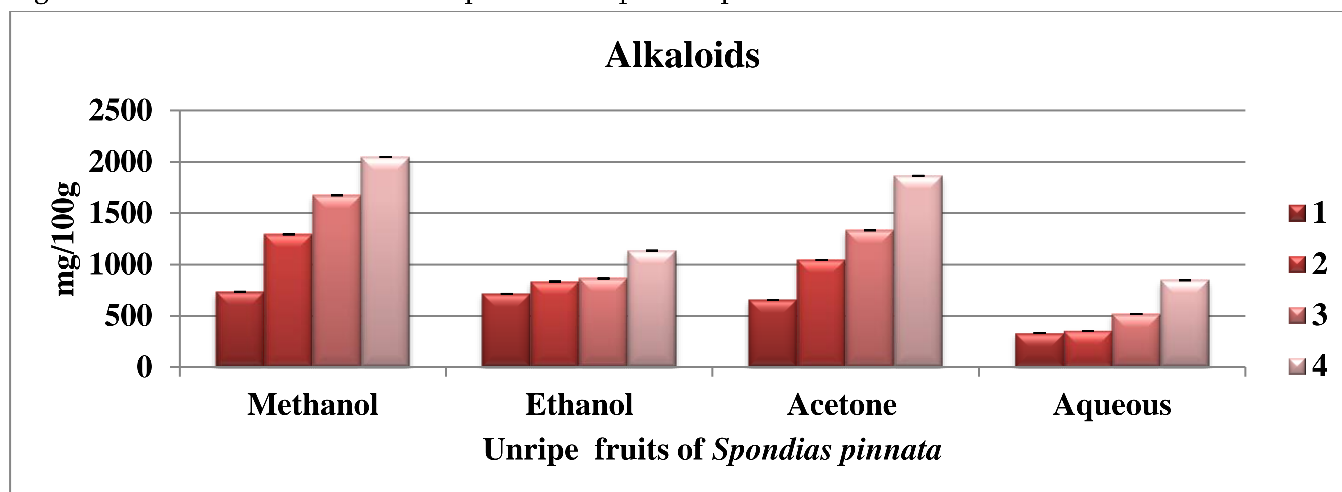


Figure No.8 Alkaloid content of Unripe fruits of Spondias pinnata



IV. CONCLUSION

The present study demonstrates that the pulp of Spondias pinnata fruit can be considered as a valuable source of an antioxidant activities and secondary metabolites. In brief, all concentration manifests good antioxidant and secondary metabolite activity. Based on the findings of present study, it was concluded that high antioxidant and free radical scavenging activities are exhibited by 70% methanol, ethanol, acetone and aqueous extract of S. pinnata pulp, which contains significant amounts of flavonoids and alkaloid compounds. It also chelates iron and has the ability to decrease it. S. pinnata fruit extracts are a major source of natural antioxidant that may be helpful in preventing various progressing form of diseases. The correlation result showed that Flavonoid is strong correlate with antioxidant activity.

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