International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 16-11-2021, Accepted: 01-12-2021, Published: 17-12-2021
Volume 6, Issue 6, 2021, Page No. 1130-1138

Bioprospecting of *Rhynchosia rufescens* (Willd.) DC. an underutilized wild vegetable

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Abstract
In the current study immature and mature seeds of *Rhynchosia rufescens* were used for the analysis of antioxidant, secondary metabolites and antibacterial activities. For enzymatic and non-enzymatic antioxidant analysis there are four different solvents like methanol, ethanol, acetone and aqueous with four different concentrations 1mg, 2mg, 3mg and 4mg was used. A unique standard approach was used to assess the antioxidant potential and secondary metabolites of all levels in terms of total alkaloid, total flavonoid, total phenolic, DPPH free radical scavenging potential, ferric ion reducing antioxidant power, reducing potential, total antioxidant capacity, ABTS free radical scavenging potential, ferrous ion chelating ability. In contrast to the other four solvents, acetone plant extract showed the highest antioxidant and secondary metabolite activities. The activities increase as the sample concentration increases from 1 mg to 4 mg. Two Gram positive bacteria, such as *Staphylococcus aurous* and *Bacillus cereus*, and two Gram negative bacteria, such as *Proteus vulgaris* and *Escherichia coli*, were utilized in antibacterial research. Using the agar well diffusion method, the inhibition zones were calculated. Plant crude extract was compared to standard antibiotic discs and evaluated using bacterial strains. *Bacillus cereus* was the most active antibacterial strain among the four.

Keywords: antioxidant, secondary metabolites, antibacterial activities, *Rhynchosia rufescens*, Immature and mature seeds

Introduction
Rhynchosia is a genus of the legume family Fabaceae, subfamily Faboideae, tribe Phaseoleae, and subtribe Cajaninae (Lackey 1981) [21]. In India the genus is represented by 25 species, as well as one variety and one subspecies, of which 7 species are endemic to India. In India, a great diversity of the *Rhynchosia* species, around 60%, are found in the Eastern Ghats (Prasad & Narayana Swamy, 2014) [27]. The genus *Rhynchosia* L. consists of approximately 200 species and distributed in both the eastern and western hemisphere in warm temperate and tropical regions (Grear, 1978) [13].
Rhynchosia rufescens is an erect shrub with trailing branches common in dry deciduous and open forest. It is a tribal pulse that belongs to the Fabaceae family and is commonly utilized by tribal people in India, Bangladesh, Sri Lanka, Cambodia, Indonesia, and Malaysia for a variety of diseases (Panneerselvam & Shanthi K 2017) [26]. Total seed protein in *Rhynchosia rufescens* has a higher amino acid composition and essential amino acid score than the FAD/WHO (1991) recommended pattern (Kalidass & Mohan2012). Medicinal plants are a worthy source of biologically active compounds for the production over the past centuries of new therapeutic drug candidates (Harvey 2008; Koehn and Carter 2005; Newman and Cragg 2012) [14, 20, 24]. Also today, plant species are commonly used by people in many countries as a cure for the treatment of different forms of diseases such as infectious, cardiovascular, diabetic, intestinal, renal, mental-nervous, dietary, respiratory, reproductive, neurological, skin infections and many wounds (González- Tejero *et al*., 2008) [12]. Therefore, the plants provide a valuable source of natural compounds and played a major role with scientifically enhanced effectiveness in the new drug development as well as less side effects (Butler 2004) [5]. Antioxidants are chemical substances that minimize oxidative stress, which is caused by free radicals, which are constantly produced in the body and are responsible for cell damage, diabetes, and cancer. Many medicinal plants include natural antioxidants such as flavonoids, vitamin C, and other phenolic chemicals that are effective against a range of infections (Dehshari *et al*., 2012) [8]. The goal of this study was to determine the antioxidant, secondary metabolite, and antibacterial characteristics of *R. rufescens* immature and mature seeds in four different solvents at four different concentrations.

Material and methodology
1. Collection and Preparation of Plant Material
Plant material of *Rhynchosia rufescens* were collected from Nandi hills Karnataka, between the period of November to March from 2018 to 2021. The immature and mature seeds are collected and brings to lab air dried at room temperature. A mechanical grinder is used to make tiny powders of both immature and mature seeds. Then this powder was used to study different antioxidant, secondary metabolites and antibacterial assay. The plant extract was prepared in four different solvents: methanol, ethanol, acetone, and aqueous, with concentrations of 1mg, 2mg, 3mg, and 4mg for antioxidant and secondary metabolites. For antibacterial study the plant extract was prepared in two different solvents methanol and acetone each with three different concentrations like 50%, 75%, 100%.

2. Antibacterial activity analysis
The antibacterial activity of a plant extract was tested by the agar well diffusion method of Akbar, A. and K. A. Anal (2014) [1]. Lawn cultures of target bacteria were prepared by

spreading 100 micro liter of fresh broth culture of Gram positive (*B. cereus*, *Staphylococcus aureus*), Gram negative (*E. coli*, *Proteus vulgaris*) .Wells of 5mm diameter were bored in agar plates and different concentration of plant extract was placed in each well. Plates were incubated at 37c for overnight and inhibitory zone was measured in mm accordingly.

3. Antioxidant analysis

3.1- Enzymatic antioxidants

a. Catalase

The activity of catalase was determined using a slightly modified Sadasivam and Manickam (1992) technique. In 10ml 0.1 M phosphate buffer, 500mg fresh plant material was homogenized (ph-7.0). The extract was then filtered using four layers of muslin fabric (moistened with phosphate buffer). After that, the filtrate was centrifuged for 10 minutes at 0 to 4^0 C at 10,000 rpm. The supernatant was then employed as a source of enzymes. 3 mL H_2O_2 phosphate buffer in the assay mixture. Then, using a UV-VIS double beam spectrophotometer, 0.2 ml enzyme was combined immediately and the change in optical density was measured every minute at 240 nm.

b. Peroxidase

The Maehly method was used to determine the activity of peroxidase (1954). By dissolving 0.5g of fresh plant material in 10ml of 0.1 M phosphate buffer, the enzyme was recovered (pH- &.7.0). The filtrate was then centrifuged at 10,000 rpm for 10 minutes at 0 to 4^0 C after being filtered through four layers of muslin cloth soaked with phosphate buffer. The supernatant was then utilized as a source of enzymes. 2 ml phosphate buffer (pH-7.0), 1 ml 20 mm guaiacol, and 0.5 ml enzyme were used in the enzyme assay. After that, 0.1 ml of 20mm H_2O_2 was added to start the reaction. The change in optical density due to guaiacol oxidation was measured per minute at 470 nm using a dual beam UV-VIS spectrophotometer with regular stirring of the reaction liquid with a glass rod. The enzyme's activity is then measured in O.D min-1, mg-1 protein.

c. Superoxide dismutase

The approach of (Beaucham and Fridovich technique-1971) with modest modifications was used to calculate superoxide dismutase (SOD). To protect the enzyme against polyphenol action, o.5 g fresh plant material was homogenised in 10 ml of 150 mM cold potassium phosphate buffer (pH-7.8) containing 1 percent PVP. The extract was then filtered through four layers of muslin fabric, and the filtrate was centrifuged for 10 minutes at 10,000 rpm. The supernatant is then utilized as an enzyme source. The supernatant was then suspended in phosphate buffer pH 7.8 (1ml) and used to calculate SOD activity. The reaction is started by adding 0.4ml riboflavin to test tubes at room temperature. After 15 minutes, the response took on a light blue color. To stop the reaction, the tubes were coated in carbon black paper. Then, using a spectrophotometer, the absorbance was measured at 560 nm.

3.2- Non- Enzymatic antioxidants

a. Carotenoids

The carotenoids were calculated using Krik and Allen's technique (1965). In a cold mortar pestle in the dark, the fresh 0.5 g plant material was homogenised in 80 percent chilled acetone with a pinch of magnesium carbonate. The resulting extract was then filtered under suction using Buchner's funnel with whatman no-1 filter paper. The residue from the filter was thoroughly cleaned 2 to 3 times with 80 percent acetone. The filtrate was then reduced to a final volume of 100 ml using 80 percent acetone. The filtrate was then transferred to a conical flask that was wrapped in black paper to prevent the pigments from photo oxidizing. Finally, using a UV-VIS double beam spectrophotometer and 80 percent acetone as a blank, absorbance was measured at 480 nm.

b. Polyphenols

The polyphenols were determined using the Folin and Denis method (1915) [11]. With the addition of a pinch of magnesium carbonate, 0.5 g of fresh plant material was crushed in a mechanical mixer and extracted in 30ml of 80 percent acetone at 0 to 40^0 C in the dark. The residual was then properly cleaned 2-3 times with 80 percent acetone. Using 80 percent acetone, the final volume of the produced filtrate was increased to 100 ml. Then 2 ml of plant extract was combined with 10 ml of 20% Na_2Co_3 and distilled water to make a volume of 35 ml. Then 2 ml of Folin and Denis reagent (100 g sodium tungstate and 20g phosphomolybdenic acid were dissolved in roughly 800 mL distilled water, 50 ml of 85 percent phosphoric acid was added, and the mixture was refluxed for 2-5 hours) was added to the mixture Finally, using distilled water, dilute the mixture to 50 ml. The absorbance was measured at 660 nm using a UV-VIS double beam spectrophotometer after the color was created. The standard polyphenol curve was created using a standard tannic acid solution.

c. Ascorbic acid

The ascorbic acid concentration was determined using a trimetric approach proposed by Sadasivam and Manikam (1992) [32]. 5 ml of working standard solution was added to a conical flask, followed by 10 ml of 4 percent oxalic acid and titration against dye. (To make the dye solution, combine 42 mg sodium carbonate with 52 mg 2, 6- dichlorophenol indophenol in a small amount of distilled water and dilute to 200 ml with distilled water.) After that, a 0.5 g extract of plant material was created in 4 percent oxalic acid, and the volume was increased to 100 ml using 4 percent oxalic acid. After that, the extract was centrifuged for 10 minutes at 10,000 rpm. Then, 5 ml of the supernatant was pipetted into a conical flask, and 10 ml of 4% oxalic acid was added. The extract was then titrated against the dye. After a few minutes, a pink color developed, indicating that the reaction had reached its conclusion.

Free radical scavenging antioxidants analysis

DPPH radical scavenging activity

With minimal modifications, Wang *et al*., (1998) described the DPPH technique. For each extract, 100 µl of plant extract was mixed with 2.9 ml of DPPH methanol solution (20 g/ml) at different concentrations (1, 2, 3, 4, mg/ml). A UV-visible spectrophotometer was used to evaluate the absorbance against blank methanol after a 30-minute incubation period. The radical scavenging activity (percent) was estimated using the following formula: DPPH scavenging activity (percent) = [(AB-AT) / AB] X 100, where AB and AT are the absorbance of blank and plant content, respectively. The percentage of scavenging activity

of each extract was compared to L-Ascorbic acid, a positive control.

Ferric Ion Reducing Antioxidant Power (FRAP Assay)
The Benzie and Strain method was used to calculate FRAP activity (1996). In a 10: 1: 1 ratio, 300 mm acetate buffer, 10 mm TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mm HCL and 40 mm HCL $FeCl_3$ to $6H_2O$ (20 mm) are combined. Obtain the FRAP reagent. The absorbance of the plant extract (100 l) was measured at 593 nm after mixing it with 2.9 ml of FRAP reagent.

iii. Reducing Power
The Oyaizu (1986) [25] method was used to calculate the reducing power of all *R. rufescens* extracts. 1 mL of each extract (1, 2, 3, 4, mg/mL), 2.5 mL of phosphate buffer, and 2.5 mL of 1 percent potassium ferricyanide were mixed with double-distilled water. After incubation at 50°C for 20 minutes, 2.5 mL of 10% trichloroacetic acid (TCA) was prepared and centrifuged for 10 minutes at 3000 rpm. 2.5 mL of the supernatant top layer was mixed with 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%). Instead of using additives, water was utilized as a blank, and L-Ascorbic acid was employed as a positive control. The absorbance was measured on UV spectrophotometer at 700nm.

iv. Total Antioxidant Capacity (Phosphomolybdenum)
The total antioxidant capacity (TAC) of the crude methanolic extract was determined through phosphomolybdenum method by Prietro *et al*., (1999). 0.2 ml each extract of methanol, ethanol, acetone and aqueous with a concentration of 0.2 mg/ml was coupled 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 95^0C with 90 min. A UV-visible spectrophotometer set to 695 nm was used to calculate the absorbance against a blank containing 3 ml of reagent solution. The crude extract's total antioxidant activity was measured in milligrams per 100 grams of dry weight as an L-ascorbic acid equivalent.

v. Chelation Power on Ferrous (Fe2+) Ions
The ability of the plant extract to chelate ferrous ions was determined using the method proposed by Decker and Welch (1990) [7]. 2 ml of methanolic plant extract (100 g / ml) was mixed with 0.1 ml of 2 Mm FeCl2, and 0.2 ml of 5 mm ferrozine solutions was able to react at room temperature for 10 minutes. A spectrophotometer was used to measure the absorbance at 562 nm. Instead of a ferrozine solution, D. W. was utilized as a blank. By comparing the test findings to those of L-Ascorbic acid (100 g / ml), the percentage of ferrous ion inhibition was calculated.

vi. ABTS radical scavenging assay
The ABTS radical cation decolorization test was used to investigate the free radical scavenging activity of *R. rufescens* plant material (Pellegrini 1996). The reaction between 7mM ABTS in water and 2.45mM potassium persulfate (1:1) produced ABTS + cation, which was kept in the dark at room temperature for 12-16 hours before use. After diluting the ABTS solution with methanol, an absorbance of 0.700 at 734nm was obtained. The absorbance was measured 30 minutes after the addition of 20 µl of plant extract to 180 µl of diluted ABTS solution. In each test, a suitable solvent blank was used. The ABTS scavenging effect (+) = ((AB-AA)/AB) 100 (2) formula was used to obtain the percent inhibition of absorbance at 734nm. The standard for this experiment was ascorbic acid.

4. Secondary metabolites analysis
a. Total alkaloids
Singh *et al*., (2004) [33] defined the total alkaloid content using the 1, 10 Phenanthroline technique, with slight modifications. The extracts were made by homogenizing 0.5 g of plant material in 10 ml of 70 percent methanol, ethanol, acetone, and aqueous. This was filtered through a Buchner funnel and centrifuged at 5000 rpm for 10 minutes. Supernatant was employed for the extra computation. The reaction mixture contained 100 l of plant extract. 1 ml of 0.05 M phenanthroline solution in different solvents and 1 ml of 0.025 M $FeCl_3$ solution in 0.5 M HCL solution in a water bath set to 70°C, the reaction mixture was incubated for 30 minutes. At 510 nm, the red-colored substance's absorption was measured against the blank reagent. The typical colchicine curve was used to assess and determine the concentration of alkaloids.

b. Total flavonoids
The Luximon-Ramma *et al*., (2002) [22] method was used to calculate the total flavonoid content. A solution of quiercitine (100, 200, 400, 600, 800, and 1000 mg / ml) was applied to an aliquot of extracts (0.1 ml) or standard in a 10 ml volumetric flask containing 4 ml Distilled water. The flask was filled with 0.30 ml of 10% $AlCl_3$. After 5 minutes, 2 ml of 1 M NaOH was added, bringing the total to 10 ml distilled water. The solution was mixed, and absorbance was measured at 510 nm against a blank.

Results and Discussion

Table 1: Non enzymatic and enzymatic antioxidant of immature and mature seeds of *Rhynchosia rufescens.*

	Non Enzymatic			Enzymatic		
Plant Name	**Carotenoid (mg/100g)**	**Total polyphenol (mg/100g)**	**Ascorbic acid (mg/100g)**	**Catalase (mg/100g)**	**Peroxidase (mg/100g)**	**Superoxide dismutase (mg/100g)**
RRI	46.56±0.69	3.127±0.102	105.2± 2.9	0.010±0.001	4.670±0.189	0.58±0.003
RRM	45.73±2.75	3.40±0.0434	89.6± 2.7	0.038±0.001	1.826±0.031	0.37±0.03

RRI = *Rhynchosia rufescens* immature seeds, RRM = *Rhynchosia rufescens* Mature seeds.

In a non-enzymatic antioxidant assay: The carotenoids, Polyphenols and ascorbic acid were studied. Overall carotenoid (46.56±0.69mg/100g) and Ascorbic acid (105.2±2.9 mg/100g) was rich in immature seeds; whereas total polyphenols rich in mature seeds (3.40±0.0434mg/100g) than the immature seeds (3.127±0.102mg/100g) of *R. rufescens*, (Table 1).
In an enzymatic antioxidant analysis: The catalase, peroxidase and superoxide dismutase were analyzed. The highest catalase activity was noticed in mature seeds

0.038±0.001mg/100g, while lower amount was observed in immature seeds 0.010±0.001 mg/100g. Immature seeds of *R. rufescens* showed maximum amount of peroxidase 4.670±0.189 mg/100g and Superoxide dismutase 0.58±0.003 mg/100g, than the mature seeds (Table 1).

Free radical scavenging analysis

In the estimation of DPPH free radical scavenging assay, in immature seeds of *R. rufescens* high percent of inhibition was observed in ethanolic seed extract at 4mg concentration (80.54±1.28), whereas low percent of inhibition were observed in methanolic seed extract at 1mg concentration (24.41±0.82) (Fig-1.).While mature seeds showed high amount of inhibition in acetonic seed extract at 4mg concentration (79.7±0.66) and low percent of inhibition in aqueous seed extract at 1mg concentration (19.5±0.81) (Fig-2). In FRAP (Ferric Ion Reducing Antioxidant) analysis, in immature seeds highest amount of Ferric ion reducing antioxidant potential was observed in acetonic extract at 4mg concentration (262.11±1.59 mg/100g)(Fig-3).Whereas mature seeds showed high amount of ferric ion reducing antioxidant potential in 4mg concentration of acetonic extract (282.43±1.59mg/100g) (Fig -4).

In the assessment of Ferrous (Fe2+) Ions Chelation Power ability, the highest percent of inhibition was observed in immature seeds at 4 mg concentration of aqueous extract (87.11±0.24), while the lowest percent of inhibition was observed at 1 mg concentration of methanolic extract (83.29±0.16) (Fig-7). In comparison to immature seeds, mature seeds showed the highest level of inhibition at 4 mg methanolic extract concentration (86.27±0.32) and the lowest level of inhibition at 1 mg aqueous extract concentration (81.05±0.48) (Fig-8) In terms of total antioxidant potential or phosphomolybenum analysis, the highest activity was observed in immature seeds at 4 mg concentration of acetonic extract (234.12±0.58mg/100g), while the lowest activity was observed at 1mg concentration of methanolic extract (14.09±0.40mg/100g) (Fig-5).In comparison to immature seeds, mature seeds demonstrated the highest level of activity at 4mg concentration of acetonic extract (365.85±0.28mg/100g) and the lowest level at 1mg concentration of methanol extract(14.89±1.02mg/100g)(Fig-6). In the ABTS free radical scavenging analysis, the maximum percent of inhibition was observed in immature seeds at 4 mg concentration of aqueous extract (92.52±0.21), while the minimum percent of inhibition was observed at 1mg concentration of same aqueous extract (71.27±0.21) (Fig-11). In comparison to immature seeds, mature seeds reported a high level of inhibition at 4mg concentration of acetonic extract (93.7±0.16) and the lowest level of inhibition at 1 mg concentration of aqueous extract (64.17±0.12) (Fig-12.). In reducing power activity analysis, the maximum amount of activity has been noticed in immature seeds at 4 mg concentration of acetonic extract (412.91±0.043mg/100g) and the least amount of activity was noticed at 1mg concentration of ethanolic extract (26.25±0.19mg/100g) (Fig-9). In contrast to immature seeds, mature seeds exhibited the highest amount of activity at 4mg concentration of acetonic extract (902.35±0.17mg/100g) as well as the lowest level of activity at 1 mg concentration of ethanolic extract (31.71±0.12mg/100g) (Fig-10).

Table 2: Secondary metabolites analysis in immature and mature seeds of *Rhynchosia rufescens.*

Plant names		Alkaloids (mg/100g)				Flavonoids(mg/100g)				TPC (mg/100g)			
		1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml
RRI	M	464.99±3.63	660.83±2.88	814.99±3.63	854.16±3	55.58±2.93	91.69±2.88	107.8±2.42	126.69±2.54	10.38±1.17	12.88±0.44	14.35±1.7	17.01±0.93
	E	389.16±2.2	577.49±3	762.49±3	815.83±3	75.58±4.54	90.58±2	103.08±2.18	123.91±3.37	14.18±1.09	19.28±2.51	21.28±2.69	28.31±1.34
	A	577.49±2.2	714.99±2.2	783.33±2.88	909.16±4.4	88.58±0.98	108.14±2.69	127.25±1.11	131.14±4	15.11±2.69	21.05±1.2	25.35±1.04	34.31±0.76
	Aq	454.99±5.06	611.66±2.2	792.49±3.63	852.49±3	57.14±2.88	73.36±3.46	90.58±2.42	11.14±1.46	9.31±1.61	10.01±0.44	11.48±2.69	16.05±0.33
RRM	M	385.83±2.88	661.66±2.2	768.33±3.81	980.83±4.33	73.97±2.22	88.91±3.88	107.25±3.37	123.91±0.55	10.38±2.19	12.75±2.53	22.11±1.04	34.15±2.32
	E	429.99±4.63	708.33±1.44	828.33±2.88	978.33±2.88	59.3±3.55	73.36±3.33	88.97±3.96	108.08±3.24	13.15±0.6	20.31±0.44	27.28±0.44	31.31±0.33
	A	559.99±2.2	684.99±6.5	788.33±4.33	1016.66±2.2	76.14±2	111.14±2.42	127.25±0.55	140.58±2.93	15.15±2.17	21.28±2.53	27.21±0.44	35.11±1.09
	Aq	459.99±6.82	559.16±3	686.66±4.4	783.33±1.44	55.03±1.92	72.25±2.42	91.14±0.55	107.8±2	10.18±1.77	12.35±0.87	22.05±1.45	28.41±1.7

RRI = *Rhynchosia rufescens* immature seeds, RRM = *Rhynchosia rufescens* mature seeds, M= Methanol, E= Ethanol, A= Acetone, Aq= Aqueous.

Secondary metabolites analysis

In the estimation of total alkaloids content, the immature seeds showed their highest total alkaloid content in 4mg concentration of acetonic extract (909.16±3.63mg/100g) and lowest content were showed in 1mg concentration of ethanolic extract (389.16±2.88mg/100g). In contrast to immature seeds, mature seeds exhibited the highest level of total alkaloids content at 4mg concentration of acetonic extract (1016.66±3mg/100g) and the lowest content were noted at 1mg concentration of methanolic extract (385.83±2.88mg/100g). In the determination of total flavonoid content, the maximum amount of flavonoid content was noticed in mature seeds at 4mg concentration of acetonic extract (140.58± 0.55mg/100g), while minimum amount of flavonoid was observed in 1mg concentration of aqueous extract (55.03± 0.51mg/100g).While in immature seeds the highest values was observed at 4mg concentration of acetonic extract (131.14±1.11mg/100g) and lower amount was observed at 1mg concentration of methanolic extract (55.58±2.93mg/100g). In the analysis of (TPC) total phenolic content, the highest amount of total phenolic content was noted in mature seeds at 4mg concentration of acetonic extract (35.11±0.44mg/100g) whereas lowest amount of phenolic content were noted at 1mg concentration of aqueous extract (9.31±1.61mg/100g) in immature seed (Table-2). Plants are abundant in phenolic chemicals, which have the capacity to neutralize free radicals (Alam *et al*., 2015). The redox characteristics of phenolic compounds in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and degrading peroxides are primarily responsible for their antioxidant action. These vital plant components give off hydrogen atoms from hydroxyl groups and combine with free radicals to produce stable phenoxyl radicals, which aid in antioxidant activity (Lin *et al*., 2016). Because flavonoids contain many hydroxyl groups, they have stronger antioxidant activity against peroxyl radicals than phenolic acids. The quantity of phenolic compounds in plant extracts

must be determined in order to determine their antioxidant capability. Through their physiological activity and oxidative inhibition, antioxidants have the potential to enhance human health (Yashin *et al*., 2017) [36]. Phenolic acids, flavonoids, tannins, lignans, and quinones are all total phenolic substances that can be identified (Huang *et al*., 2018) [15]. Carotenoids, which contain solely carbon and hydrogen atoms, and oxocarotenoids (xanthophylls), which contain at least one hydrogen atom, are the two types of carotenoids. Singlet molecular oxygen and peroxyl radicals are scavenged by carotenoids (Edge and Truscott, 2018) [9]. Carotenoid compounds react more efficiently with peroxyl radicals generated under oxidative circumstances, protecting the cellular membrane and lipoprotein from oxidative damage (Kiokias *et al*., 2018) [18]. In the presence of antioxidants, the ferric complex is converted to the ferrous form (Alam *et al*., 2014) [2].

Vinoth Kumar *et al*., (2020) [34] investigated the antioxidant properties *of Rhynchosia minima* and discovered that the total phenol content of the ethanol and aqueous extracts were 84.12±2.41 and 76.38±1.28mg of Gallic acid equivalents per gramme of dry extract, respectively and the total flavonoids content of ethanol extract was found to be 158.14± 3.64mg, whereas in aqueous extract it was 148.32±2.34mg of rutine equivalent per gramme of dry extract. The phenolic compounds found in medicinal plants are renowned for their antioxidant properties and capacity to prevent oxidative damage. They can directly trap free radicals and/or scavenge them via a series of enzymatic reactions with antioxidant enzymes.

Rammohan *et al*., (2015) [30] studied the flavonoids isovitexin, isoorientin, mangiferin, and 2-hydroxy-3, 4-dimethoxybenzophenone were isolated from *Rhynchosia suaveloens* flowers and tested for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Mangiferin and 2-hydroxy-3, 4-dimethoxybenzophenone demonstrated significant antioxidant activity, with IC50 values of 51.7 and 57.7 g/mL, respectively, comparable to the positive control ascorbic acid (IC50 34.2 g/mL). In another work (Praveena *et al*., 2013), the aerial sections of *Rhynchosia capitata* were investigated using DPPH and nitric oxide antioxidant principles, yielding five C-glycosylflavanoids: vitexin, isovitexin, vicenin-2, orientin, and isoorientin. The chemical characteristics, components, and mechanism of radical scavenging ability of C-glycosyl flavonoids were investigated using density functional theory (DFT). The antioxidant activity of C-glycosyl flavonoids was revealed in this work by donating electrons rather than seizing free radicals.

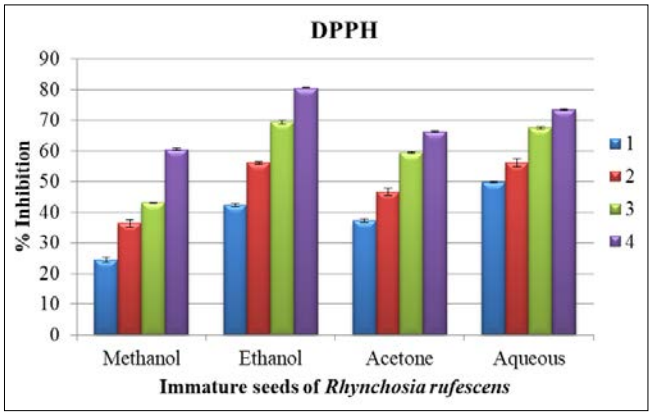

Fig 1: DPPH scavenging activity of immature seed of *R. rufescens.*

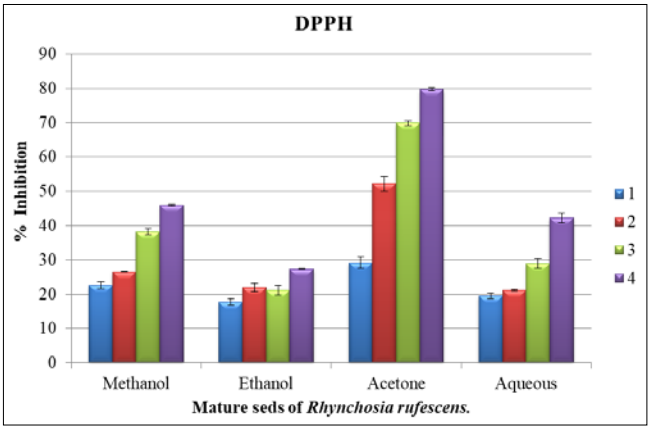

Fig 2: DPPH scavenging activity of mature seeds of *R. rufescens.*

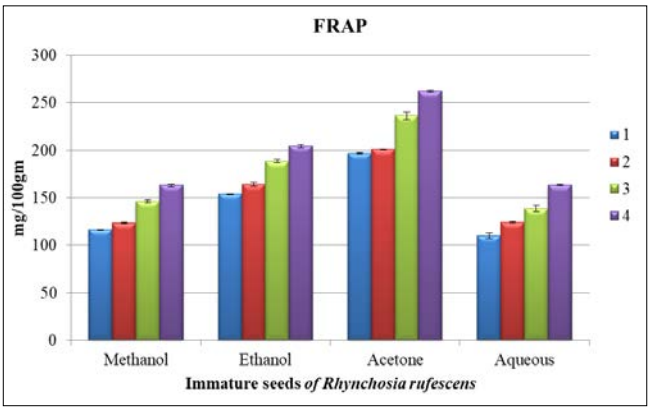

Fig 3: FRAP activity of immature seeds of *R. rufescens.*

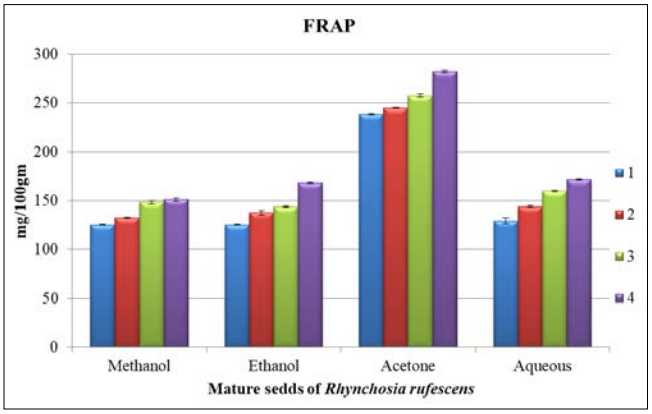

Fig 4: FRAP activity of mature seeds of *R. rufescens*

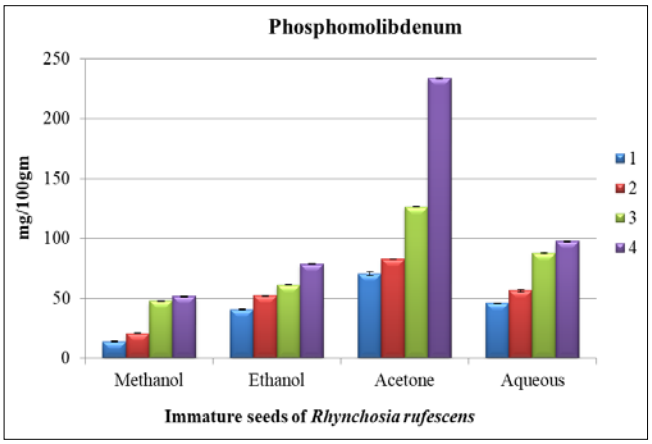

Fig 5: Total antioxidant activity of immature seeds of *R. rufescens.*

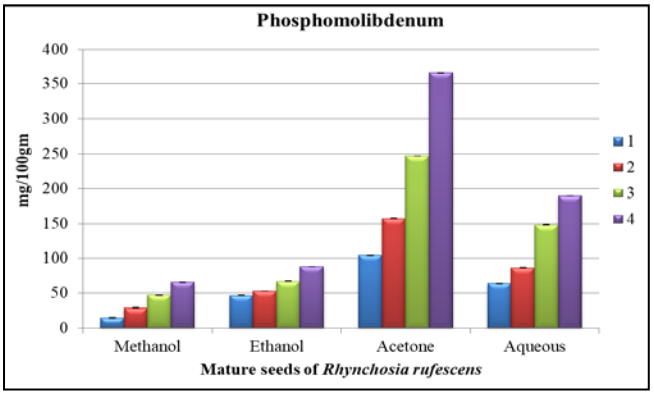

Fig 6: Total antioxidant activity of mature seeds of *R. rufescens.*

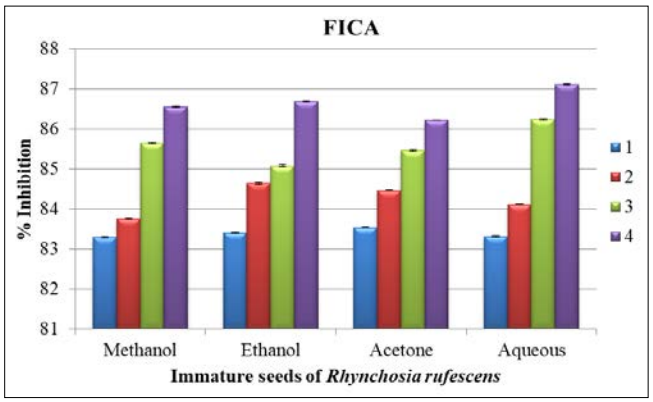

Fig 7: Ferrous ion chelating ability of immature seeds of *R. rufescens*

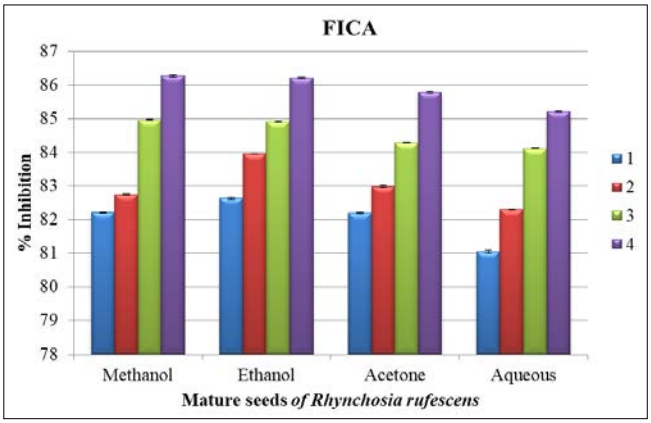

Fig 8: Ferrous ion chelating ability of mature seeds of *R. rufescens*

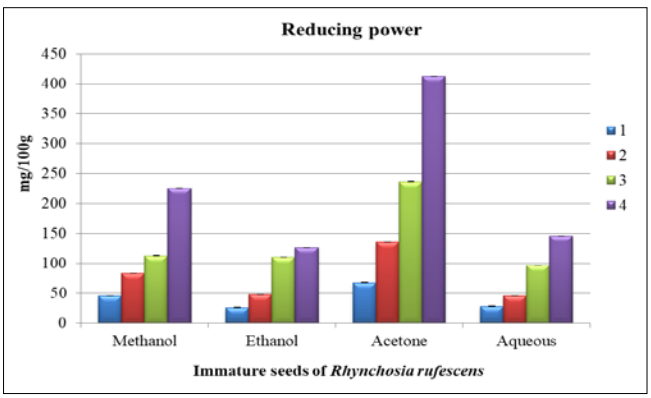

Fig 9: Reducing power activity of immature seeds of *R .rufescens.*

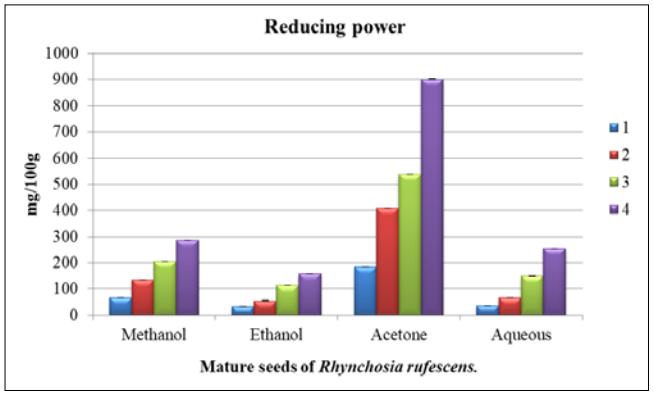

Fig 10: Reducing power activity of mature seeds of *R. rufescens.*

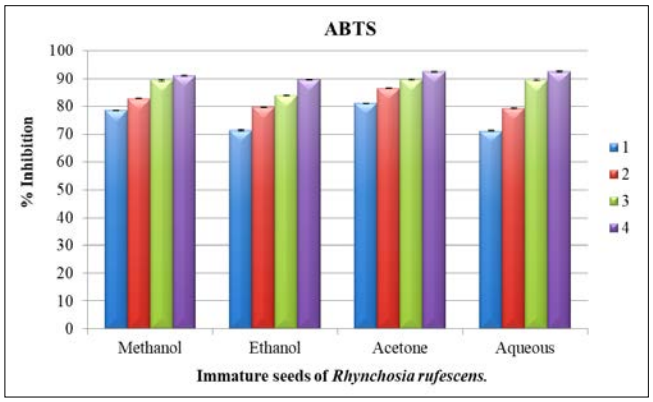

Fig 11: ABTS radical scavenging activity of immature seeds *of R. rufescens.*

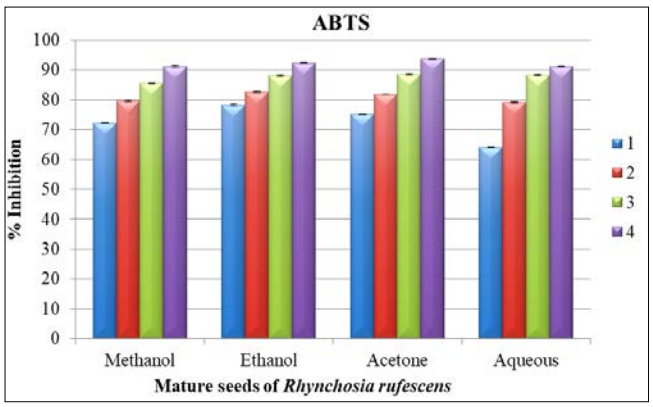

Fig 12: ABTS radical scavenging activity of mature seeds *of R. rufescence*

Table 3: Zone of inhibition showed by acetone and methanol seeds extract of *R. rufescens* against bacterial strains.

Plants name	Solvents	Plants conc. %	Zone of inhibition in mm			
			Staphylococcus aureus	***Bacillus cereus***	***Proteus vulgaris***	***Escherichia Coli***
Rhynchosia rufescens.	Methanol	50%	8	10	8	8
		75%	9	11	10	11
		100%	10	14	11	15
	Acetone	50%	8	8	9	9
		75%	9	9	10	11
		100%	14	16	11	12

Antibacterial activity of plant extract was observed against four different bacterial strains using agar well diffusion method. The activities of plant sample were compared with antibiotic streptomycin. The highest zone of inhibition of acetonic extract (100%) of healthy seeds of *R. rufescens* was demonstrated against the *Bacillus cereus* 16mm. Next to *Bacillus cereus*, *Staphylococcus aurous*, *E.coli* and *Proteus vulgaris* showed an inhibition of 14mm, 12mm, and 11mm respectively against acetonic plant extract of healthy seeds of *R. rufescence*. The methanolic plant extract of *R. rufescens* showed inhibition zones of 15mm, 14mm, 11mm and 10 mm against the *E.coli*, *Bacillus cereus*, *Proteus vulgaris* and *Staphylococcus aurous* (Table- 3).

Four flavonoids, isovitexin, isoorientin, quercetin-7-O-methylether, and biochanin A, were recently identified from *Rhynchosia beddomei* flowers (Rammohan *et al*., 2019), Using the disc diffusion method, all of the compounds were evaluated for antibacterial activity against gram-positive and gram-negative bacteria and fungi. Isoorientin and quercetin-7-O-methylether inhibited *Pseudomonas aeruginosa* and *Candida albicans* at doses of 20.1 and 15.8 nm; and 20.4 and 15.7 nm, respectively. With the redox indicator Alamar Blue, the roots of *Rhynchosia precatoria* DC were examined for in vitro inhibitory and bactericidal activity against *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* (Coronado-Aceves et al. 2017) [6]. Six flavonoids were found in *Rhynchosia precatoria*: lupinifolin, lupinifolinol, cajanone, precatorin C, precatorin A, and precatorin B.

Except for compound lupinifolinol, all of the isolated compounds were tested for antimycobacterial activity. Khan and Shoeb (1984) [17] investigated the antibacterial activity of an ethanol extract of the whole plant of *Rhynchosia suaveloens* against *Bacillus subtilis* and *Staphylococcus aureus* bacteria. Purification of the active portion yields two biphenyls: 4-(3-methyl-but-2-enyl)-5-methoxy-(1, 1′-biphenyl)-3-ol and 2-carboxy-4-(3-methyl-but-2-enyl)-5-methoxy-(1, 1′- biphenyl)-3-ol. Both biphenyls had action against *Bacillus subtilis and Staphylococcus aureus*, with MICs of 15.63 and 31.25 g/mL, respectively.

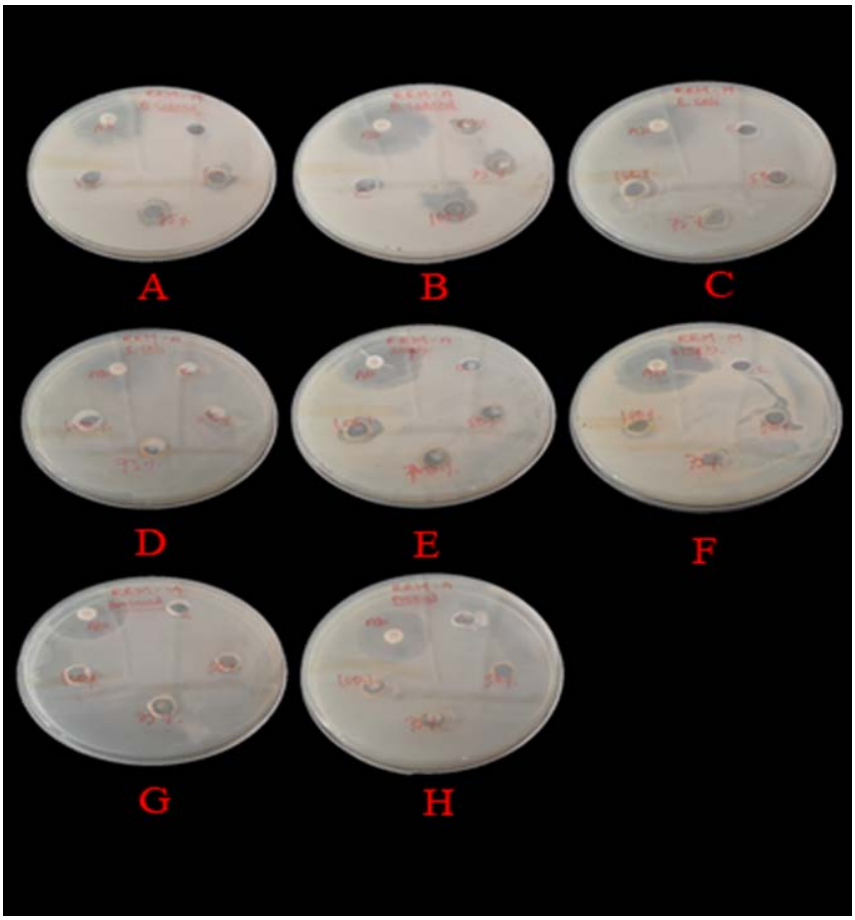

Plate 1: Antibacterial analysis of mature seed of *Rhynchosia rufescens* A. *Bacillus cereus* (Methanol), B. *Bacillus cereus* (Acetone), C. *E.coli* (Methanol), D. *E.coli* (Acetone), E. *Staphylococcus aureus* (Methanol), F. *Staphylococcus aureus* (Acetone),G.*Proteus vulgaris* (Methanol), H. *Proteus vulgaris* (Acetone).

Conclusion

According to the above findings, seeds of *R. rufescens* has a significant amount of antioxidant power to naturally scavenge free radicals, as well as antibacterial power against various gram (+) and gram (-) bacterial strains. In Non-enzymatic and enzymatic antioxidant analysis, Immature seeds of *R. rufescens* showed a high level of carotenoid, ascorbic acid, peroxidase and superoxide dismutase than the mature seeds, while mature seeds showed high amount of total polyphenols and catalase. In comparison to the other three solvents like, ethanol, methanol, and aqueous, the acetonic plant extract of both mature and immature seeds demonstrated a substantial amount of inhibition in free radical scavenging analysis and secondary metabolites estimation. In antibacterial studies *Bacillus cereus* was the most active antibacterial strain among the four. Wild legume *Rhynchosia* is a rich source of phytochemicals and nutrition and provides possibilities for advancement as a stand-by for cultivated species as a vegetable. The several species of *Rhynchosia* exhibit antioxidant, antimicrobial, anti-nutritional, antidiabetic, anti-inflammatory and anticancer activities that suggest they have a range of medicinal properties as well as exceptional nutritional potential.

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