

Antioxidant and antidiabetic activity of fruit extracts of *Parkia biglandulosa* (Wight and Arn.)

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Abstract

This study clarifies the investigation of antioxidant and anti diabetic activity of fruit extracts of *Parkia biglandulosa*. The antioxidant assays was done by DPPH and ABTS assay, Then the antidiabetic was done by α -Amylase inhibition assay and glucose uptake in yeast cell. The result reveals that FME as shown good inhibition and IC₅₀ value in both antioxidant and antidiabetic activity. The work reveals the *Parkia biglandulosa* fruits contain better antioxidant and diabetic agent to cure oxidative stress and diabetic disorders.

The antioxidants exploration from natural sources has acknowledged much courtesy and hard work was identify the compounds which can act as proper antioxidants to substitute artificial ones. In addition, the formulated anti-oxidants accruing naturally to give nutraceuticals. Those occurring in the body that can help to prevent oxidative damage.⁷

However, the DPPH and ABTS techniques are broadly used to determine anti-oxidant activity as well as natural plant extracts. The DPPH is a stable free radical with an absorption band at 515nm and ABTS is 734nm. The ABTS method has the extra elasticity in that it can be used at different p^H

levels. Unlike DPPH, this is sensitive to acidic p^H. The ABTS is generated by reacting a strong oxidizing agent with the ABTS salt. Reduction of blue green ABTS radical colored solution by hydrogen donating antioxidant is measured by the suppression of its characteristic long wave absorption spectrum⁴.

Still a challenge to the medical community for the management of diabetes without any side effects. The practice of the medicines is controlled by their pharmacokinetic belongings, secondary failure rates and supplementary side effects⁶. Accordingly, the searching for a innovative class of composites is necessary to overwhelmed diabetic complications. Thus, there is constant search

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for substitute medicines⁵.

With this preview in this study shows the investigation of anti oxidant activity of fruit and anti cancer activity of root extracts of *Parkia biglandulosa*.

Antioxidant activity of fruit extracts :

The antioxidant activity of fruit extracts was investigated by using DPPH and ABTS assay methods. The extracts used for this study was(PEE, CHE, EAE, ME and DWE) followed by Yogashree *et al.*,⁸.

Antidiabetic activity of fruit extracts :

α -Amylase inhibition

The fruit extracts (PE, EAE and ME) were checked for anti-diabetic activity through α -Amylase inhibition. The extracts were dissolved in DMSO to obtain concentrations of 3 μ g/mL, 2 μ g/mL and 1 μ g/mL. 500 μ l of each concentration was taken and 500 μ l of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α -amylase solution (0.5mg/ml) were incubated for 10 minutes at 25°C. After pre-incubation, 500 μ l of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each tube at 5s intervals. This reaction mixture was then incubated for 10 minutes at 25°C. 1ml of DNS reagent was added to stop the reaction. These test tubes were then incubated in boiling water bath for 5 minutes and cooled to room temperature. Finally this reaction mixture was again diluted by adding 10ml distilled water following which absorbance was measured at 540nm. Metformin was taken as positive control and DMSO as negative control.

Calculation :

$$\% \text{ of Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Glucose uptake in Yeast cells :

Commercial baker's yeast will be wash by repeated centrifugation(3,000 \times g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension will be prepare in distilled water. Plant extracts (PE, EAE and ME) extracts (1 ml) were added to 1mL of glucose solution (25 mM) and incubated together for 10 min at 37°C. Reaction will be started by adding 100 μ l of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 \times g, 5 min) and glucose was estimated in the supernatant. Metformin served as standard drug. The percentage increase in glucose uptake by yeast cells were calculated using the following formula.

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs Control}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

Anti-oxidant activity :

The DPPH and ABTS assay was done by fruit extracts (FPE, FCE, FEAE, FME and FDWE) of *Parkia biglandulosa*. FM extract shows good % inhibition both in DPPH and ABTS assay compared with the gallic acid used as a standard.in DPPH result reveals the IC₅₀ value in FME is 2.34 μ g/ml in the table 3 and in ABTS IC₅₀ value in FME is 2.938 μ g/ml as shown in table 4.

Table-1. ABTS Assay %inhibition and IC₅₀ values of fruit extracts.

FRUIT EXTRACTS		%inhibition (µg/ml)					
Sl.No.	conc.in µg/ml	FGA	FPE	FCE	FEAE	FME	FDWE
01	1	36.8	19.13	23.9	35.57	35.57	13.0
02	2	51.4	23.45	27.5	43.26	43.26	17.37
03	3	60.8	24.82	36.7	60.48	60.48	21.94
04	4	74.6	26.98	39.4	73.46	68.3	26.71
05	5	89	29.53	43.9	86.92	75.6	28.99
	IC ₅₀	2.02	13.36	6.02	2.25	2.35	9.87

FGA- Fruit gallic acid, FPE- Fruit pet ether extract, FCE- Fruit chloroform extract, FEAE-Fruit ethyl acetate extract, FME- Fruit methanol extract, FDWE- Fruit dist.water extract

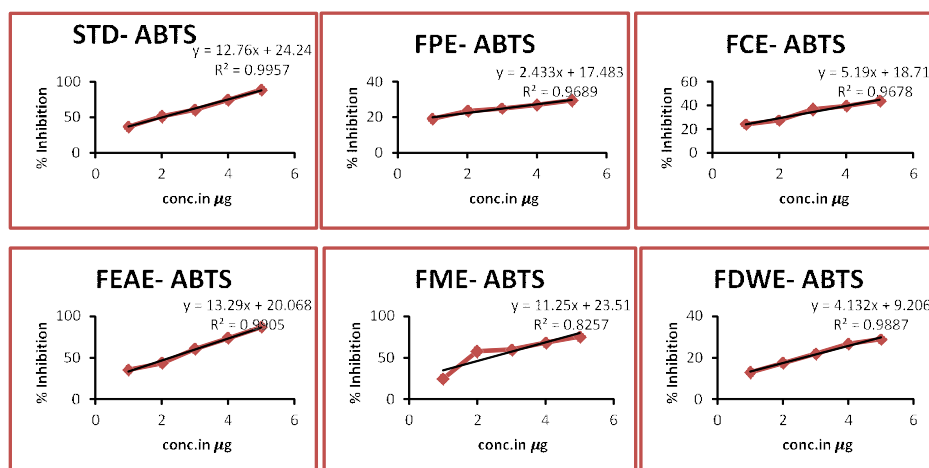


Image 1: % Inhibition ABTS Assay of fruit extracts

Table-2. DPPH Assay % inhibition and IC₅₀ values of Fruit extracts.

FRUIT EXTRACTS		%inhibition (µg/ml)					
Sl.No.	conc.in µg/ml	FGA	FPE	FCE	FEAE	FME	FDWE
01	1	33.3	5.81	4.0	16.92	34.72	3.77
02	2	60.2	10.22	8.51	21.44	37.25	4.57
03	3	67.6	12.42	10.62	28.07	57.05	5.96
04	4	75.4	17.03	14.42	33.94	60.20	8.15
05	5	82.2	19.33	19.43	36.05	63.24	11.33
	IC ₅₀	1.80	13.94	13.49	7.50	2.938	26.125

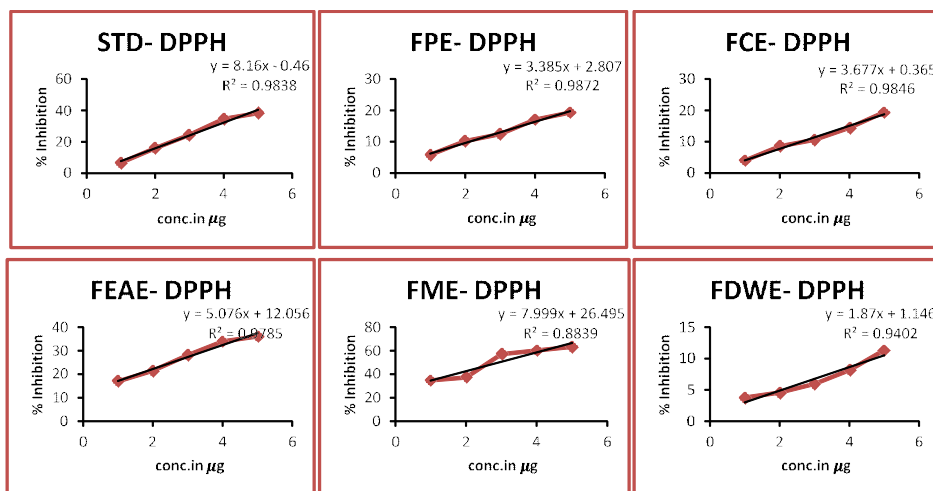


Image 2. % Inhibition DPPH Assay of fruit extracts

Anti diabetic activity :
 α -Amylase inhibition :

mL to 2µg/mL to 3µg/mL as shown in table 3.

Table-3. α -Amylase inhibition percentage of fruit extracts of *Parkia biglandulosa*

The result obtained in α -amylase inhibition reveals that ME has exhibited excellent activity followed by ME and EAE in fruit. ME and EAE has shown significant activity in the fruit extract when compared with standard drug Metformin. The extracts have shown effect in dose dependent manner and the IC 50 is rich in ME of fruit compared with the standard. Activity has increased from 1 µg/

Fruit sample	α -Amylase inhibition (%)			
	3µg/mL	2µg/mL	1µg/mL	IC ₅₀
PE	14.3	11.2	9.9	19.36
EAE	16.4	14.6	12.8	17.86
ME	21.5	19.8	17.6	17.57
Metformin	23.35	22.95	18.14	12.94

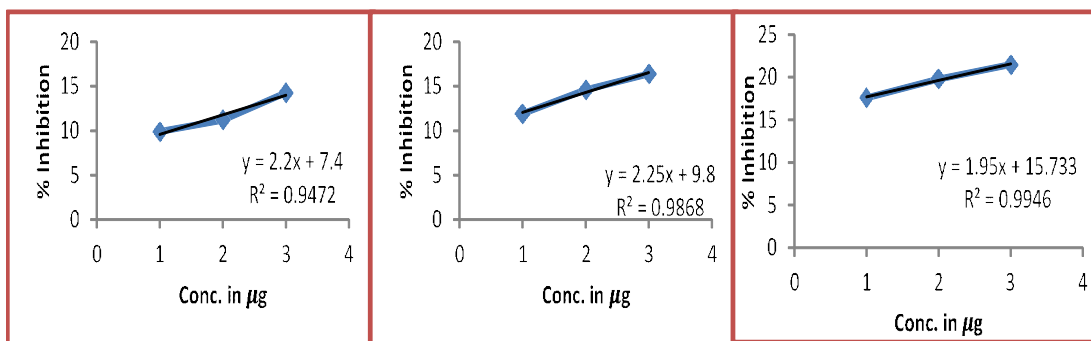


Image 3: % inhibition of α -amylase activity of fruit extracts.

Glucose uptake in Yeast cells :

In this assay reveals the EAE and ME shows good % inhibition and IC₅₀ very much compared to the standard metformin in fruit extracts of *Parkia biglandulosa*. The percentage inhibition increased from 1 µg/mL to 2 µg/mL to 3 µg/mL and the IC₅₀ value is very much near to the standard *i.e* fruit methanol extracts is 5.23 as shown in the below table-4.

Table-4. Glucose uptake in Yeast cells (%) of fruit extracts of *Parkia biglandulosa*

Fruit sample	α-Amylase inhibition (%)			
	3µg/mL	2µg/mL	1µg/mL	IC ₅₀
PE	23.47	20.13	18.46	13.701
EAE	28.37	24.36	21.30	9.16
ME	34.32	25.32	19.8	5.23
Metformin	36.05	31.5	28.45	6.73

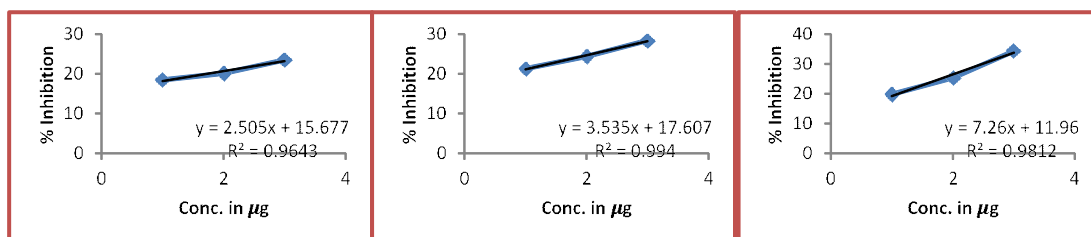


Image 4 % inhibition of increase in glucose uptake of fruit extracts.

Diabetes mellitus is a worldwide chronic metabolic disorder characterized by very high blood glucose level called hyperglycemia that affects the metabolism of protein, carbohydrates and fats¹. Controlling of diabetes no side effects is quiet a challenge to the medicinal community. The drugs usefulness is limited by their pharmacokinetic properties, secondary disaster rates and additional side effects. Consequently, examining for a new class of composites is necessary to overcome diabetic complications³.

The process of production of free radicals due to oxidative stress as well as reactive oxygen and reactive nitrogen species (ROS and RNS). These are unavoidable drawbacks of aerobic absorption by-products, which injury the structure and function of cell

organelles.

The ROS and RNS in metabolism are associated to the development of diseases due to increased the concentration, Including, cardiovascular, cancer and neurological diseases. Additionally, ROS can extensively cause cells and tissues damage, during infections and numerous degenerative illnesses, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer². In this present study we evaluated *In vitro* anti oxidant and anti diabetic activity of crude extracts of fruit of parkia biglandulosa shows significant % inhibition activity as shown in table 1,2,3, and 4.

The plant showed significant inhibition activity, so further the purified compound used

as a drug which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent and anti oxidative agent.

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