RJC

RASĀYAN ***J. Chem.***
Vol. 12 | No. 2 |907 - 914| April - June | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

PHYTOCHEMICAL SCREENING, ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITIES OF *Kigelia africana* (LAM.) and *Sterculia foetida* L.

K. Swarnalatha[1,2], CH. Venkata Kishore Babu[3] and B. Hari Babu[1,*]
[1]Department of Chemistry, Acharya Nagarjuna University, NNagar, Guntur-522510, AP., India.
[2]Department of Chemistry, Ch.S.D.St. Theresa's College for Women, Eluru, AP., India
[3]Department of Chemistry, Avanti Institute of Engineering & Technology, Vizianagaram, AP-India
*E-mail: dr.b.haribabu@gmail.com

ABSTRACT

To explore antidiabetic and the anti-oxidant activities of crude plant extracts of *Kigeliaafricana (stem),* and *Sterculia foetida (stem),* primarily,selected plant samples collected, extracted with four solvents based on the ascending order of the polarity of n-hexane, ethyl acetate, methanol, and water. Later,the crude extracts of plant samples were analyzed for phytochemicals such as alkaloids, flavonoids, phenols etc. The quantitative estimation of phenols, flavonoids, alkaloids was performed with the help of double beam U.V spectrophotometer. The evaluation of antioxidant activity was determined by the DPPH method. To assess the antidiabetic activities of crude extracts, *in vitroα*-amylase inhibition method was performed and IC_{50} values were calculated. It is concluded that aqueous extract of *Kigeliaafricana* (stem), the methanol extract of *Sterculia foetida* (stem) are a potential source of anti-oxidant and the anti-diabetic properties.

Keywords:*Kigelia africana, Sterculia foetida,*Anti-diabetic activity, Antioxidant activity, DPPH,*in vitroα*- amylase inhibition.

INTRODUCTION

Several medicinal plants and their products have been used both in the prevention and cure of diseases[1]. The bioactive compounds extracted from the plants were used in the herbal or traditional medicine [2]. Traditional medicine is safe, clinically effective and less expensive[3]. Currently due to the potential side effects of modern synthetic drugs and increasing contraindication to their usage a popular resurgence has materialized for the use of medicinal plants. Diabetes mellitus is a chronic metabolic disorder in which the person suffers from high blood glucose due to inadequate production of insulin or problem in using insulin to process glucose from food. The chronic complications of diabetes include microvascular impairment, renal problems, cardiac problems, nerve injury and loss of sight. Natural anti-oxidants play a significant potential in the prevention and treatment of oxidative stress-induced metabolic disorders[4]. Increased levels of antioxidants reduce diabetes complications. The consumption of medicinal plants as therapeutic agents was growing worldwide because of the readily available and low cost with minimum toxic side effects than modern synthetic drugs[5]. Ethnomedicine keenly views the different medical practices of indigenous cultures in search of different curative plants. The intention of the present analysis was thephytochemical screening, quantitative estimation of phytochemicals, antioxidant and antidiabetic activity of two medicinal plants namely*Kigelia africana (Lam.)* and *Sterculia foetida (L.).*

Kigelia africana belonging to family *Bignoniaceae* popularly called the sausage tree bearing long, pendulous racemes of spotty dark flower, which appear like candelabra. Its fruits are long, appears as sausage. *Kigelia africana* plant has many amazing medicinal properties due to the presence of secondary metabolites. It is mainly found in riverine areas where distribution restricted into the wetter areas[6]. An infusion made from grounded bark and fruits used to treat stomach problems in children. Its bark is used

http://dx.doi.org/10.31788/RJC.2019.1225168

to treat pneumonia, snakebite, melanoma, renal cancer. The plant has many medicinal uses against many diseases which include ulcer, malaria, tumors etc[7]. Due to its wide range of medicinal uses,*Kigeliaafricana* could be used as an alternative to currently used medicine for both animals and human beings[8].

Sterculia foetida belongs to *Sterculiaceae* which is also called as java olive, bartered poon tree, hazel *Sterculia*, poon tree, skunk tree, samrong in Thai. In India, it is known as Jangli badam (Hindi, Bengali), adavi badam (Telugu). It is a large tree with 40m height and 3m trunk diameter. The flowers are the green or purple color with foul smell found as panicles and this species is dioecious, hermaphroditic and lack petals but have a 5 lobed calyx that appears as petals. Each fruit generally contains 10-15 black ellipsoid seeds. *Sterculia foetida* seed has starchy cotyledons and is straight with a small radical. *Sterculia foetida* seed oil may help combat belly fat and insulin resistance by resensitizing receptors[9].

EXPERIMENTAL

Chemicals

The organic solvents used in the experiments were of analytical grade and purchased from Fisher scientific, Mumbai, India.

Plant Samples

The studied plant samples *Kigelia africana* (stem) collected from Avanigadda of Krishna district and *sterculia foetida* (stem) collected from Eluru of West Godavari district respectively, the prominent places in coastal state Andhra Pradesh, India. The plant samples were authenticated at the NISCAIR (CSIR), New Delhi. Authentic samples were deposited in the raw materials herbarium and museum, Delhi (RHMD), reference numbers. NISCAIR/RHMD/consult/2016/2981-08-1 for *Kigelia africana* (Lam.), NISCAIR/RHMD/consult/ 2016/2981-08-3 for *Sterculia foetida* (L). The plant samples were cleaned and rinsed with water to remove any related debris. The cleaned fresh materials were shade air dry and chopped into a fine powder.

Plant Extractions

The dried sample powder of each selected plant parts were weighed (Table-1) and packed into a soxhelet apparatus and extracted with the solvents n-hexane, ethylacetate, methanol, and water. The extract was filtered by means of Whatman filter paper and then made it dry by means of a rotary evaporator. The final crude extracts were weighed and collected for further phytochemical assessments. The weight of the crude extract obtained in each solvent is noted in Table-1.

Table-1: The Weights of the Crude Extracts Obtained in Each Solvent

S. No.	Compound	Weight in Grams		% of Extract Value	
		Kigelia africana	*Sterculia foetida*	*Kigelia africana*	*Sterculia foetida*
1	Sample	23.38	25.36	.....	.....
2	Hexane	0.273	0.312	1.17	1.23
3	Ethyl acetate	0.493	0.657	2.11	2.59
4	Ethanol	2.420	3.951	10.36	15.58
5	Water	2.635	2.369	11.28	9.34

Preliminary Phytochemical Screening of the Plant Extractions

Phytochemical screening was conducted to the selected plants *Kigelia africana*, *Sterculia foetida*crude extracts for the presence ofalkaloids, phenols, saponins, flavonoids, carbohydrates, glycosides, by standard phytochemical procedures.[10,11]For steroids, triterpenoids, steroidal saponins Salkowski test, Liebermann Burchard's test were conducted.

Total Phenolic Content

To 0.4 mL of Folin-Ciocalteu's reagent FCR (diluted 1:10 v/v) add 1 mL of plant extract. After 5 min adds 4 mL of sodium carbonate solution. The final volume of the solution was made up to 10 mL with

distilled water and keep for 90 min at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol as standard and total phenol content of the extract was expressed in terms of catechol equivalent per gram. Total phenolic compounds present in two crude extracts of *Kigeliaafricana* (stem), *sterculia foetida*(stem) were measured from the calibration graph ($r^2 = 0.998$).

Total Flavonoid Content

1 ml plant extract was added to 3 mL distilled water followed by adding 0.3mL 5 % $NaNO_2$. After 5 min at 25 °C, $AlCl_3$ (0.3 mL, 10 %) was added. Subsequently, after 5 min, the reaction mixture was treated with 2 mL of 1 M sodium hydroxide. Lastly, the reaction mixture was made up to 10 mL with distilled water and the absorbance was measured at 510 nm. The entire flavonoid content was calculated from a calibration curve ($R^2 = 0.999$) and the results were stated as quercetin equivalent per gram.

Total Alkaloid Content

Five mL pH 4.7 phosphate Buffer was added to 1 mL of plant extract and also add 5 mL BCG (bromo cresol green) solution, the mixture was shaken with 4 mL of chloroform. The extracts were taken in a 20 mL volumetric flask and then diluted the volume to 20mL with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The total alkaloid content was calculated from a standardization curve ($R^2 = 0.998$) and the results were stated as atropine equal per gram.

Study of Biological Activities

Measurement of Antioxidant Activity (DPPH method)

The antioxidant activity of the *Kigeliaafricana* (stem), *Sterculia foetida* (stem) crude plant extracts was determined on the depending on their scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The plant extracts of 1 mL (1-500 µg/mL) of each solution of different concentrations were added to 3 mL of 0.004 % ethanolic DPPH free radical solution. The absorbance of the preparations was taken after 30 minutes at 517 nm by a UV spectrophotometer which was compared with the absorbance of standard ascorbic acid concentrations (1-500 µg/mL)[12] and absorbance of the DPPH for blank (without plant extract) also measured finally the % antioxidant activity was calculated by the following equation.

$$\%\ \text{Antioxidant Activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Measurement of Anti-diabetic Activity

A mixture of1 mL of plant extract and 1 mL of *α*-amylase, were incubated in a test tube at 37 °C for 10 min. After pre-incubation, 1 mL of 1% (v/v) starch solution was added to each tube and incubated for 15 min at 37 °C. The reaction was terminated with 2 mL DNSA reagent, placed in boiling water bath for 5 min, cool to normal room temperature and add 5mL distilled water, and the absorbance was measured[13] at 546 nm using a UV-Visible spectrophotometer. The control reaction contains 100 % enzyme activity without any plant extract. The % inhibition of ά-amylase by each plant extract can be calculated using the following formula.

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

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Preliminary Phytochemical screening of the *Kigeliaafricana* and *Sterculia foetida* reveals the presence of steroids, alkaloids, flavonoids, phenolics, carbohydrates, and saponins. The results are expressed as +ve for the presence and –ve for the absence of phytochemicals. The phytochemicals present in the Ethyl acetate, methanol, aqueous extracts of 2 plant samples were shown inTable-2.

Table-2: Phytochemical Screening of *Kigelia africana*, and *Sterculia foetida* Extracts

S. No.	Screening Tests	*Kigelia africana(stem)*			*Sterculia foetida(stem)*		
		Ethyl Acetate Extract	Methanol Extract	Aqueous Extract	Ethyl Acetate Extract	Methanol Extract	Aqueous Extract
1.	Steroids						
(a)	Salkowski	+ve	+ve	+ve	+ve	+ve	+ve
(b)	Liebermann	+ve	+ve	+ve	+ve	+ve	+ve
2.	Triterpenoids						
(a)	Salkowski	−v e	−v e	−v e	−v e	−v e	−v e
(b)	Liebermann tests	−v e	−v e	−v e	−v e	−v e	−v e
3.	Saponins						
(a)	Foam	−v e	−v e	−v e	+ve	+ve	+ve
4.	Steroidal saponin						
(a)	Salkowski	−v e	−v e	−v e	+ve	+ve	+ve
(b)	Liebermann	−v e	−v e	−v e	+ve	+ve	+ve
5.	Triterpenoid saponin	−v e	−v e	−v e	−v e	−v e	−v e
6.	Alkaloids						
(a)	Dragendorffs	−v e	−v e	+ve	+ve	+ve	+ve
(b)	Picric acid	−v e	−v e	+ve	+ve	+ve	+ve
7.	Carbohydrates						
(a)	Benedicts	+ve	+ve	+ve	+ve	+ve	+ve
(b)	Molisch	+ve	+ve	+ve	+ve	+ve	+ve
8.	Flavonoids						
(a)	Ferric chloride	−v e	+ve	+ve	+ve	+ve	+ve
9.	Glycosides	−v e	−v e	−v e	+ve	+ve	+ve
10.	Phenols						
(a)	$Fecl_3$	+ve	+ve	+ve	+ve	+ve	+ve
(b)	Chlorogenic	+ve	+ve	+ve	+ve	+ve	+ve

In the Phytochemical screening of *Kigelia africana* (stem) methanol, an aqueous extract shows positive for steroids, carbohydrates, flavonoids, phenols. However, alkaloids were distinguished only in the water extract of *Kigelia africana* (stem). In the phytochemical screening of crude extracts of *Sterculia foetida* (stem) ethyl acetate, methanol, aqueous extracts show positive results for biological compounds like steroids, saponins, steroidal saponins, alkaloids, flavonoids, phenols, carbohydrates, glycosides. The aqueous extract of *Kigeliaafricana* (stem), Methanolic extract of *Sterculiafoetida* (stem) shows the higher intensity of precipitation in the Ferric chloride test which indicates a higher number of flavonoids.

Total Phenolic, Flavonoid, Alkaloid Contents

Phenolics are compounds with an OH group attached to an aromatic carbon. Phenolic compounds acts as antioxidants due to their ability to donate hydrogen, quench singlet oxygen and acts as metal chelators. The phenolic concentration could be the foundation for antioxidant activity. Consumption of phenolic-rich compounds plays a vital role in the dealing of various diseases like diabetes.

The quantity of phenolics present in different crude extracts of the *Kigelia africana* (stem), *Sterculia foetida* (stem) was determined by means of the Folin-Ciocalteus reagent by using UV spectrophotometer and taking standard catechol and the total phenolics was expressed as mg equivalent of catechol per gram. The total amount of phenolics present in different extracts is noted down in Table-3.

Table-3: Results of Total Phenolics Present in Different Extracts

S. No.	Compound	Absorbance λ_{max}		Amount Found mg/gram of the Extract	
		Kigelia africana	*Sterculia foetida*	*Kigelia africana*	*Sterculia foetida*
1	Hexane	.....	.....	....	.....
2	Ethyl acetate	0.187	0.121	13.889	4.72
3	Methanol	0.211	0.364	17.222	38.47
4	Water	0.362	0.204	38.194	16.25

The aqueous extract of *Kigeliaafricana* shows a higher level of phenolics 38.19mg/gm while in the *Sterculia foetida* methanol extract shows maximum phenolics 38.47mg/gm.

Flavonoids are a category of secondary plant metabolites with potential antioxidant potential. The antioxidant activity influenced by the flavon nucleus, number, position, and types of hydroxyl substitutions of flavonoids[14]. The more the hydroxyl substitutions, the stronger the antioxidant activity. Flavonoids were showing their efficiency in regulating the digestion of carbohydrate, insulin activity and release of glucose in insulin-sensitive tissues through various intracellular signaling paths. Flavonoids influence β-cell mass and maintain insulin sensitivity in peripheral tissues. The intake of flavonoids has protective effects against cancers, heart and skin diseases, osteoporosis and obesity, menopause[15].

The quantity of flavonoid content in different solvent extracts of the *Kigelia africana* (stem), *Sterculia foetida* (stem), was estimated by the usage of aluminum chloride and standard quercetin. The total flavonoid amount was expressed as mg equivalent of quercetin per gram. The total flavonoids present in the various crude extracts are depicted in Table-4.

Table-4: Results of Total Flavonoids Content Present in Different Extracts

S. No.	Compound	Absorbance λ_{max}		Amount found mg/gram of the extract	
		Kigelia africana	*Sterculia foetida*	*Kigelia africana*	*Sterculia foetida*
1	Hexane	....	....	....	....
2	Ethyl acetate	....	0.121	....	3.36
3	Methanol	0.454	0.889	34.486	75.140
4	Water	0.796	0.281	66.449	18.32

The aqueous extract of *Kigelia africana* shows a higher quantity of flavonoids 66.44 mg/gm. In the *Sterculia foetida* methanol extract shows maximum flavonoids 75.14 mg/gm.

Alkaloids are nitrogenous compounds; the availability of nitrogen is causes accumulation of alkaloids in plants. Alkaloids contain one or more nitrogen atoms in a heterocyclic ring. In the plant, they exist in Free State or as salts or as N-oxides. Alkaloids contain oxygen in adding up to carbon, hydrogen, and nitrogen. Alkaloids have a wide range of pharmacological activities include antidiabetic and antioxidant activity. The sum of alkaloids presents in different solvent extracts of the *Kigelia africana* (stem), *Sterculia foetida* (stem), was determined by using phosphate buffer and BCG (bromo cresol green) and atropine standard. The total alkaloid quantity was expressed as mg equivalent of atropine per gram. The aqueous extracts of *Kigelia africana* and *Sterculia foetida* show the highest quantity of alkaloids 20.69mg/gm,43.89mg/gm respectively. The total amounts of alkaloids present in different extracts are noted down in Table-5.

Anti-oxidant Activity

Antioxidants are the substances which remove free radical or delay oxidation. The antioxidants protect the human body from free radicles and reactive oxygen. Reactiveoxygen species damages lipids, proteins,

DNA in the cells and cell structures16 which result in cell death. DPPH assay estimates the ability to scavenge free radicals which causes damage to natural macromolecules. The percentage of DPPH activity of these two plant crude extracts with that of the standard ascorbic acid was given in Table-6. The graphs (a and b) showing the antioxidant activity of *Kigeliaafricana, and Sterculia foetida* were shown in Fig.-1.

Table-5: Results of Total Amounts of Alkaloids Present in Different Extracts

S. No.	Compound	Absorbance λ_{max}		Amount found mg/gram of the extract	
		Kigelia africana	*Sterculia foetida*	*Kigelia africana*	*Sterculia foetida*
1	Hexane	....	....	....	....
2	Ethyl acetate	....	0.131	....	6.11
3	Methanol	.....	0.212	.....	17.36
4	Water	0.236	0.403	20.694	43.89

Table-6: Results of the Percentage of DPPH Activity of the Two Plant Crude Extracts

S. No.	Concentration in µg/mL	Ascorbic acid	*Kigelia africana* *% of DPPH activity*			*Sterculia foetida* *% of DPPH activity*		
			EtOAc	MeOH	Water	EtOAc	MeOH	Water
1	5	12.941	0.962	2.567	6.310	....	10.588	...
2	10	22.353	2.567	9.518	20.107	....	20.000	7.058
3	15	37.112	7.700	17.754	35.294	3.957	36.257	13.155
4	20	56.257	18.930	37.540	54.545	8.663	53.690	31.979
5	25	75.936	29.305	54.548	76.364	13.262	77.433	41.497
6	30	77.326	52.192	57.433	80.000	18.930	84.385	47.914
7	50	89.839	65.241	68.556	89.198	26.310	89.839	75.508
8	100	91.979	66.310	79.251	91.337	41.711	92.406	78.823

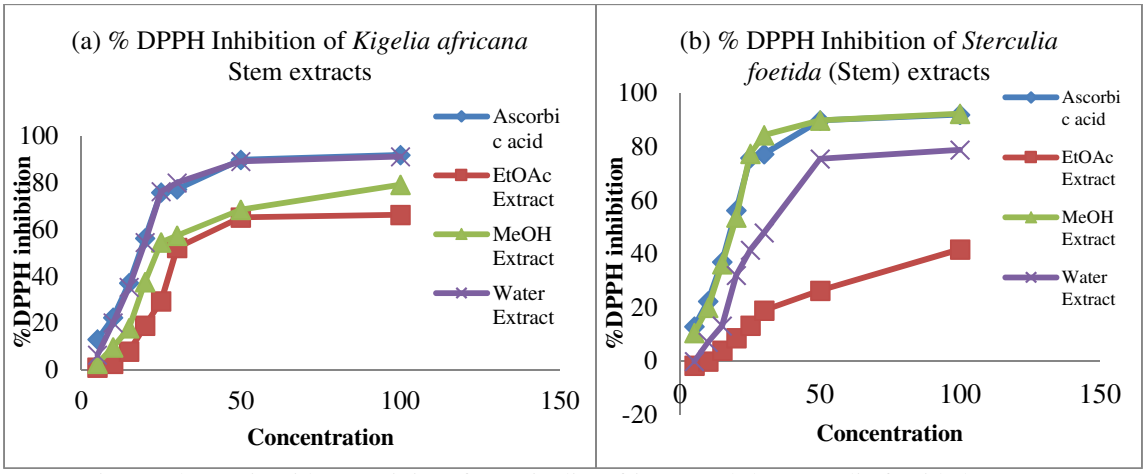

Fig.-1: The Anti-oxidant Activity of (a) Kigelia Africana and (b) Sterculia foetida by DPPH

The present study shows the extracts have a significant scavenging effect which was increasing with the concentration. The DPPH inhibition was tested for ethyl acetate, methanol, aqueous extracts. In the *Kigelia africana* (stem), the aqueous extract was found to possess an IC_{50} at 20 µg/mL in the DPPH method. The standard Ascorbic acid shows DPPH IC_{50} at the 20 µg/mL. In the *Sterculiafoetida* (stem) methanol extract was found to possess IC_{50} at the lower concentration of 20µg/mL in the DPPH method. Whereas standard ascorbic acid shows DPPH IC_{50} at 20 µg/mL. The aqueous extract of*Kigelia africana* (stem), the methanol extract of *Sterculiafoetida* (stem) shows remarkable antioxidant activity it might be due to the presence of a higher quantity of flavonoids.

Anti-diabetic Activity

The antioxidants which are available naturally can be utilized to decrease oxidative damage and dwindle the occurrence of diabetic difficulties[17]. The comparison of α-amylase inhibition of two plant crude extracts with the standard acarbose was given in Table-7. The graphs showing (a & b) antidiabetic activity of *Kigelia africana, Sterculia foetida* were shown in Fig.-2.

Table-7: Results of α-amylase Inhibition Activity of the Two Plant Crude Extracts

S. No.	Concentration in µg/mL	Standard Drug Acarbose	*Kigelia africana* % of Inhibition			*Sterculia foetida* % of Inhibition		
			EtOAc	MeOH	Water	EtOAc	MeOH	Water
1	5	14.413	0.112	9.944	20.112	...	10.503	0.670
2	10	26.927	9.385	17.765	29.832	0.335	20.223	10.391
3	15	39.553	15.419	25.363	52.514	9.050	32.737	20.112
4	20	53.631	20.782	42.570	63.575	14.078	47.039	26.480
5	25	70.391	22.011	45.922	75.978	23.464	55.531	42.570
6	50	78.883	37.653	53.519	77.765	31.397	70.503	50.279
7	100	83.799	44.357	56.760	84.134	34.413	80.112	56.760
8	200	90.056	59.665	69.944	89.162	59.330	88.492	72.179

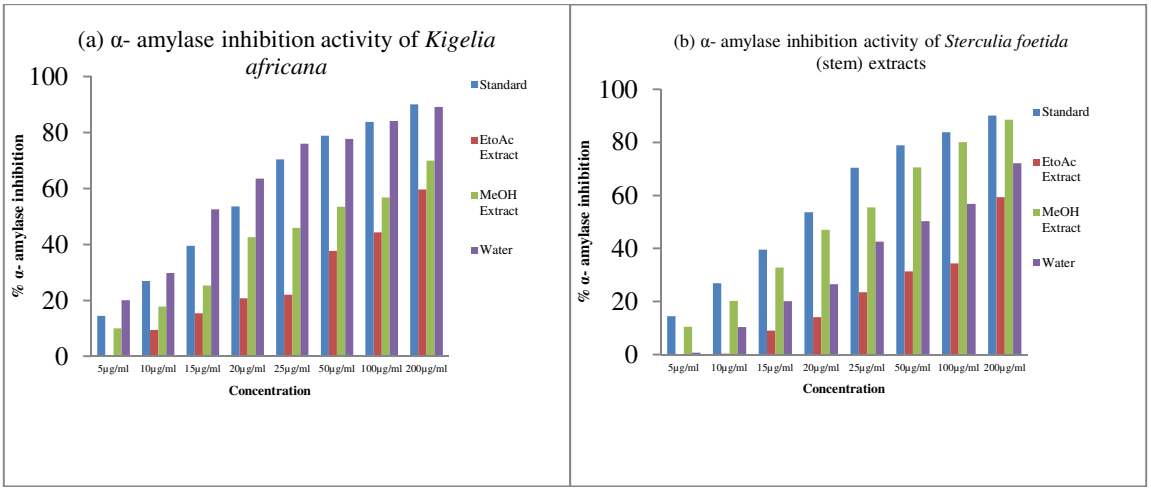

Fig.-2: The *α*-amylase Inhibition Activity of (a) *Kigelia africana*(b) *Sterculia foetida*

The antidiabetic activity *Kigelia africana* (stem), *Sterculia foetida* (stem) was performed by using *in vitroα-amylase* inhibition method by using acarbose as a standard. In the *Kigeliaafricana*(stem) aqueous extract shows, *α- amylase* inhibition IC_{50} at 15 µg/mL whereas standard drug acarbose shows *α-amylase* inhibition IC_{50} at 20 µg/mL. In the *Sterculiafoetida* (stem) methanol extract showed *α-amylase* inhibition at 25 µg/mL while standard drug acarbose shows α-amylase inhibition IC_{50} at 20 µg/mL. The aqueous extract of *Kigeliaafricana*(stem) methanolic extract of *Sterculiafoetida* (stem) shows the maximum number of flavonoids which might result in maximum *α- amylase* inhibition.

CONCLUSION

The hexane, ethyl acetate, methanol, and water extracts of plant samples *Kigelia africana (stem),* and *Sterculia foetida (stem),* were analyzed for the presence of phytochemicals such as alkaloids, flavonoids, phenols etc and were further estimated quantitatively with the help of double beam U.V spectrophotometer. Moreover, the extracts of *Kigelia africana (stem),* and *Sterculia foetida (stem),* were also explored for antidiabetic and anti-oxidant activities using *in vitroα*-amylase inhibition method and DPPH method respectively. It was found that the aqueous extract of *Kigelia africana*, the methanol

extract of *Sterculia foetida* are a potential source of anti-oxidant and the anti-diabetic properties compared to other extracts.

ACKNOWLEDGMENT

The authors thank Acharya Nagarjuna University for constant support and encouragement. The first author also acknowledges the University Grants Commission, New Delhi of India for giving an opportunity to do the work under faculty development programme [File no: ANU/UCS/CHE/ FDP/2015(48)].

REFERENCES

1. Y.G. Prakash, V. Gopal and L. Kaviarasan, *InternationalJournal of Pharmaceutical Reviewand Research*, **2(2)**, 93 (2012).
2. M.A. Hossain and M.R. Nagooru, *Pharmacognosy Journal*, **3(24)**, 25 (2011).
3. G. Ateufack, B.R.D. Feudjio, W.N. Yousseu, A.D. Atsamo and A. Kamanyi, *Journal of Biology and Life Science*, **6(2)**, 61(2015).
4. E. A. Oshaghi, H. Tavilani, I. Khodadadi and M.T. Goodarzi, *Asian Pacific Journal ofTropical Biomedicine*, **5(9)**, 720 (2015), **DOI:** 10.1016/j.apjtb.2015.06.012
5. B.H. Priya, A.M. Menkudale, M.A. Gahlot, P.U. Joshi, and M.O.Agarwal, *International Journal of Pharmacy and Pharmaceutical Science*, **5**, 163 (2013).
6. A. K. Saini, P.K. Chauhan, V. Singh and P. Sharma, *Indian Journal of Pharmaceutical and Biological Research*, **1(2)**, 46 (2013).
7. K. Shalini, K. Kavya, M. Vijusha, A. Rajani and K. Hema Malini, *International Journal of Pharmacology and Toxicol*ogy,**4(1)**, 48 (2014).
8. M. M. Dos Santos, M. T. Olaleye, R. P. Ineu, A.P. Boligon, M. L. Athayde, N. B. Barbosa and J.B.T. Rocha, *EXCLI Journal*, **13**, 323 (2014).
9. M. Kavitha, R. Vadivu and R. Radha, *Journal of Pharmacognosy and Phytochemistry*, **7(4)**, 239 (2015).
10. A. J. Harborne, Phytochemical Methods a Guide to Modern Techniques of Plant Analysis, Chapmanand Hall Ltd, London, pp 49-188(1973).
11. W. C. Evans, G. E. Trease, Pharmacognosy 11th edition, Bailliere Tindall, pp 45-50(1989).
12. T. Hatano, H. Kagawa, T. Yasuhara, and T. Okuda, *Chemical, and Pharmaceutical Bulletin*, **36(6)**, 2090 (1988).
13. V. K. Prabhakar, A. Jaidka and R. Singh, *International Journal of Scientific Research and Publications*, **3(8)**, 1(2013).
14. K. E. Heim, A. R. Tagliaferro and D.J. Bobilya, *Journal of Nutritional Biochemistry*, **13(10)**, 572 (2002).
15. C. Nguyen, J.F. Savouret, M. Widerak, M.T. Corvol and F. Rannou, *Nutrients*, **9(1)**, 45(2017), **DOI**: 10.3390/nu9010045
16. J. A. Olugbuyiro, A.S. Banwo, A. O. Adeyemi, O.S. Taiwo, and O. A. Akintokun , *Rasayan Journal of Chemistry*, **11(2),** 798 (2018), **DOI**:10.31788/RJC.2018.1121823
17. C. Rice-Evans, *Current Medicinal Chemistry*, **8(7)**,797 (2001), **DOI**: 10.2174/0929867013370

[RJC-5168/2019]