WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 909-920. Research Article ISSN 2277– 7105

EVALUATION OF POTENT PHYTOCHEMICALS AND ANTIDIABETIC ACTIVITY OF *FICUS RACEMOSA LINN.*

Jayashree Sethuraman[1,3], Harini Nehru[1,3], Kumaran Shanmugam[1] and Purushothaman Balakrishnan[1,2]*

[1]Department of Biotechnology, Periyar Maniammai University, Vallam, Thanjavur, Tamilnadu, India.

[2]TanBio R and D Solution, No.213, 1st floor, Periyar Technology Business Incubator, Periyar Maniammai University, Vallam, Thanjavur, Tamil Nadu, India.

[3]Evenly Contributed.

Article Received on
26 Sept. 2017,

Revised on 16 Oct. 2017,
Accepted on 06 Nov. 2017

DOI: 10.20959/wjpr201715-10140

***Corresponding Author**
Purushothaman Balakrishnan
Department of Biotechnology, Periyar Maniammai University, Vallam, Thanjavur, Tamilnadu, India.

ABSTRACT

Ficus racemosa is an annual perennial tree which belongs to the family Moraceae widely spread all over the. It is used in Indian traditional medicine to treat various diseases. In order to evaluate its antidiabetic activity, methanolic extract of *Ficus racemosa* leaves were performed with addition to phytochemical analysis, antioxidant. Phytochemical screening confirms the presence of terpenoids, flavonoids, tannins, steroids, glycosides and coumarins in it. These phytochemicals were placed in Thin layer chromatography (TLC) to get the Rf values of each extract. Scavenging free radicals (DPPH, reducing power) total antioxidant, glucose diffusion, glucose uptake, α-amylase assays has been performed. In the present study, antidiabetic activity of *Ficus racemosa* was studied.

KEYWORDS: *Ficus racemosa*, antioxidant, glucose diffusion, glucose uptake and α-Amylase.

INTRODUCTION

Ficus genus includes more than 750 species spread all over the world especially in the tropical and subtropical forests. *Ficus racemosa* is a moderate-sized avenue tree belongs to family Moraceae. It is popular in Indian traditional Indigenous System of Medicine like Ayurveda, Siddha, Unani and Homoeopathy (Ayyanar M and Ignacimuthu S, 2009). It is

used to cure various ailments like dysentery, diarrhoea, diabetes, bilious affections, stomach ache, menorrhage, haemoptysis, and piles and as carminative and astringent. It is also used to cure diabetics and also has wound healing properties.

Ficus racemosa possess some pharmacological activities such as antimicrobial (Saranya *et al.,* 2017), anti-inflammatory, hepatoprotective, anthelmenitic, antioxidant and antidiabetic activities due to the phytochemical present in it like alkaloids, tannins, sterols and flavonoids (Ahmed F & Urooj A., 2010; Joseph B& RAJ SJ., 2010). In the present study, antidiabetic activity of methanolic extracts of *F.racemosa* was studied. In addition to that, phytochemical screening, glucose diffusion, glucose uptake and α- Amylase inhibition assays were performed.

MATERIALS AND METHODS

Plant collection

Ficus racemosa plants have been collected from nearby Tiruchirapalli, Tamilnadu, India. All the chemicals used were purchased from Thermo fisher scientific with analytical grade and the glassware used were fully sterilized & the suitable environment was maintained throughout the experiment.

Preparation of extract

The powder of *Ficus racemosa* (50 g) was extracted with 90% methanol for 24 h in soxhlet's apparatus. The extract was boiled in a heating mantle until a viscous liquid is obtained.

Qualitative determination of the chemical constituents

It was mainly performed to determine the presence of phytochemicals such as alkaloids, terpenoids, flavanoids, tannins, steroids, glycosides, coumarins in the methanolic extract of *Ficus racemosa* leaves and various fractions was confirmed individually by the following procedures (Trivedi CP *et al.,* 1969).

Table. 1: Qualitative determination of the phytochemicals.

S.no	Phytochemical	Reagent mixture	Amount of Methanol extract (ml)	Confirmation
1	Terpenoids	2 ml of chloroform + conc.H_2SO_4 (after the addition of *F.racemosa*)	2	Appearance of reddish brown color
2	Flavanoids	3 drops of 10% lead acetate	1	Appearance of yellow color.
3	Saponins	5 ml of distilled water + 3 drops of olive oil (shaked vigorously)	5	Formation of oil emulsion.
4	Tannins	2 ml distilled water + 3 drops of 0.1% ferric chloride (after the addition of *F.racemosa*)	2	Appearance of green color.
5	Alkaloids	3 drops of Hager's reagent	2	Formation of yellow precipitate.
6	Steroids	2 ml of chloroform + 5 drops of conc.H_2SO_4	2	Reddish brown ring was formed.
7	Glycosides	2 ml of chloroform + 2 ml of acetic acid	2	Color change from Violet to blue to green.
8	Phlobatannins	2 ml of 1% HCl	2	Formation of red precipitate.
9	Proteins	1 ml of conc.H_2SO_4	1	Formation of white precipitate.
10	Coumarins	3 ml of 10% NaOH	2	Appearance of yellow color.

Quantification of Phytochemical compounds

It was performed to determine the presence of Phytochemical such as tannins, flavonoids, alkaloids and total phenolics with Quantification measures.

Determination of tannins

For quantification of tannins, 0.2g of sample was added to the 20ml of methanol and heated in a water bath for 1 hour. Then it was filtered with the No.1 whatmann filter paper in the volumetric flask. Then 20 ml of distilled water, 2.5 ml of folin's reagent and 10 ml of sodium carbonate were added to the filtrate and made to 100 ml. Absorbance was taken at 725 nm.

Determination of flavonoids

To a 0.5 ml of extract 0.5 ml of aluminium chloride-methanol mixture was added and left for 1 hour. After 1 hour absorbance was taken at 430 nm.

Determination of alkaloids

To a 5 g of extract 50 ml of acetic acid-ethanol solution was added and left for 4 hour. Then filtered and kept for some time to evaporate ethanol in the filtrate.0.1 N NaOH was added and precipitate was formed. The precipitate was dried and reweight for the determination of amount of alkaloids and the absorbance was taken at 435 nm.

Determination of total phenolics

To a volume of 0.5 ml of extract, 2.5 ml of folin's reagent and 2 ml of sodium carbonate was added and incubated for 30 minutes. Appearance of bluish green was regarded as positive for total phenolics. Then absorbance was taken at 650nm.

Pharmacological activities

Invitro antioxidant assays

A stock solution (20µg/ml) of the methanol extract was prepared for various antioxidant and reducing assay. Antioxidant power of each assay was compared with efficacy of standard chemicals (Sirisha N *et al.*, 2010).

DPPH radical scavenging activity

The antioxidant potential of the extract was assessed by using 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) assay. Stock solution of the extract was prepared at various concentrations. 1 ml of DPPH was added to each concentration of the extract and incubated in dark for 30 minutes. Absorbance was taken at 520 nm (Ramalingam PS *et al*., 2017). Ascorbic acid was used as standard for the *F.racemosa*. It is calculated by the following formula,

$$\text{Percentage inhibition} = \frac{(\text{control absorbance- leaf sample absorbance}) \times 100}{(\text{Control absorbance})}$$

Total antioxidant assay

0.1 ml of extract at various concentrations was taken and mixed with 1 ml of reagent (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) and incubated at 95˚c in a water bath for 90 minutes. Absorbance was recorded at 680 nm. When it attains room temperature. Ascorbic acid was used as standard. The total antioxidant capacity was calculated by using the following formula,

$$TAC = \frac{C \times v}{M}$$

Where, V= volume of plant extract. M=concentration of plant extract. C=x.

Reducing power assay

For this assay, 2.5ml phosphate buffer and 2.1 ml of potassium ferricyanide were added to the test tubes containing various concentration of the extract. Test tubes were kept at 50˚C in a water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. Then to the upper layer (2.5 ml), 2.5 ml of distilled water and 0.5 ml of ferric chloride were added. Absorbance was recorded at 700 nm.

Glucose diffusion method

To perform glucose diffusion method, 2ml of 0.15M Nacl containing 22mM D-glucose was loaded into a dialysis tube and sealed. Then the sealed tube was placed in a centrifuge tube containing 45ml of 0.15M Nacl and incubated in an orbital shaker at room temperature. The diffusion of glucose into the external solution was measured by the amount of glucose concentration in the external solution for every 60 minutes (Ahmed F & Urooj A., 2009).

Glucose uptake assay

For glucose uptake assay, 1g of yeast was diluted in a 10ml of distilled water and centrifuged at 3000rpm for 5 minutes. After centrifugation 1ml of supernatant was diluted in 10ml of distilled water. To a 500µl of different concentrations of plant extract 1ml of glucose solution (5mM, 10mM, 25mM) was added at different concentrations and kept incubated for 37ºC for 10 minutes. After incubation, 100µl of yeast suspension was added and vortexed. Incubation was repeated for 60 minutes at 36°C. Then centrifuged at 2500rpm for 5 minutes and glucose was estimated in the supernatant (Veerapur VP et al., 2012).

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

α- Amylase inhibition assay

To a 500µl of various concentrations of *F.racemosa* leaves extract 500µl of 0.02M sodium phosphate buffer (pH 6.9) containing alpha amylase solution was added and pre-incubated for 10 minutes at 25ºC. After that 500µl of 1% starch solution in 0.02M sodium phosphate buffer was added at timed intervals and then further incubated at 25ºC for about 10 minutes. This reaction was terminated by adding 500µl of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in water bath for 5 minutes and cooled to room temperature. The reaction mixture was diluted with 100ml distilled water and the absorbance was measured at

540nm (Ponnusamy S *et al.*, 2011). While the control solution was prepared using the same procedure by replacing the extract with distilled water.

$$\%\text{inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs of control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical analysis of methanol extract of *Ficus racemosa* leaves indicated the existence of terpenoids, flavonoids, tannins, steroids, glycosides, proteins and coumarins.

Table. 2: Phytochemical analysis of methanol extract of *F.racemosa* leaves.

S.no.	Phytochemical screening	Presence	Absence
1.	Terpenoids	+	
2.	Flavonoids	+	
3.	Saponins		-
4.	Tannins	+	
5.	Alkaloids		-
6.	Steroids	+	
7.	Glycosides	+	
8.	Phlobatannins		-
9.	Proteins	+	
10.	Coumarins	+	

+ represent Present, - represent Absent.

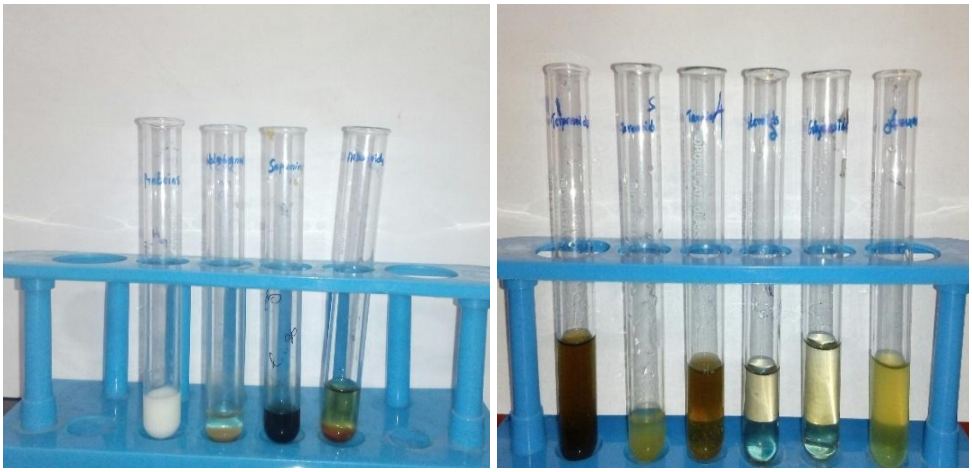

Fig 1: phytochemical analysis of methanol extract of *Ficus racemosa* leaves

DPPH radical scavenging activity

The results revealed that the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *Ficus racemosa* leaves was high at larger concentrations of the extracts. The absorbance were measured at 570nm with Ascorbic acid as standard.

Table 3: DPPH radical scavenging activity of methanol extract of *Ficus racemosa* leaves.

S.no	Concentration (µg/ml)	Absorbance at 570nm	
		Ascorbic acid	*Ficus racemosa*
1.	Blank	13.4	12.2
2.	0.2	13.5	13.6
3.	0.4	15.4	15.2
4.	0.6	17.2	17.8
5.	0.8	20.3	19.0
6.	1.0	21.8	19.8

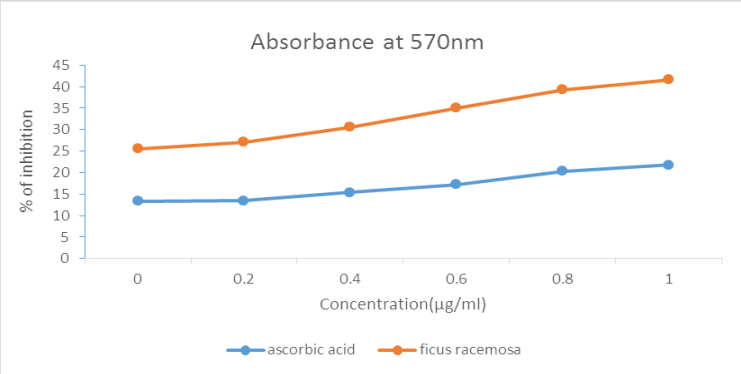

Fig 2: DPPH radical scavenging activity of methanol extract of *Ficus racemosa* leaves.

Total anti-oxidant assay

The Anti-oxidant assay was performed and absorbance was taken at 570nm using Ascorbic Acid as standard and graph was plotted.

Table 4: Determination of Total anti-oxidant activity of the *Ficus racemosa* leaves.

S.no.	Concentration(µg/ml)	Absorbance at 570nm	
		Ascorbic acid	*Ficus racemosa*
1.	Blank	0.0	0.04
2.	0.2	0.05	0.09
3.	0.4	0.10	0.15
4.	0.6	0.18	0.28
5.	0.8	0.26	0.3
6.	1.0	0.38	0.42

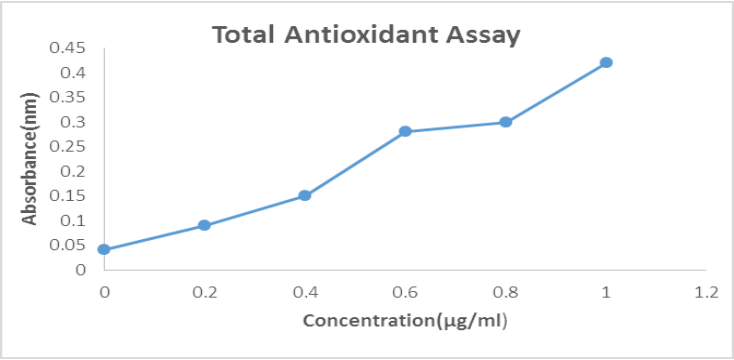

Fig 3: Determination of Total anti-oxidant activity of the *Ficus racemosa* leaves.

Reducing power assay

The reduction of ferric cyanide complex to ferrous form by donating an electron indicates the presence of reductants in plant extract.

Table 5: Determination of reductants presents in the *Ficus racemosa* leaves.

S.no.	Concentration(µg/ml)	Absorbance at 570nm	
		Ascorbic acid	*Ficus racemosa*
1.	Blank	0.14	0.16
2.	0.2	0.3	0.25
3.	0.4	0.32	0.3
4.	0.6	0.38	0.32
5.	0.8	0.4	0.34
6.	1.0	0.41	0.37

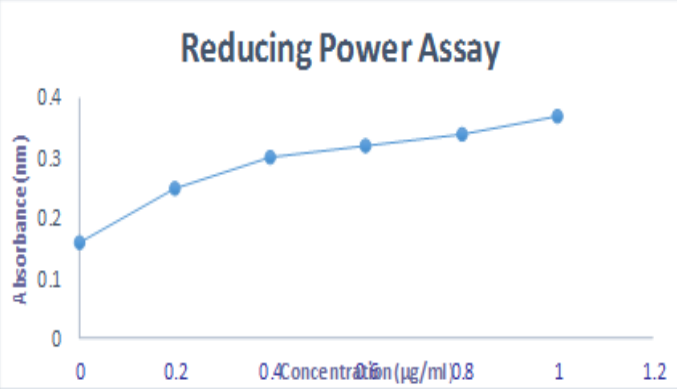

Fig 4: Determination of reductants presents in the *Ficus racemosa* leaves.

Glucose diffusion assay

The level of inhibition of glucose movement by the plant extract at various intervals of time was analyzed and compared with the control in the absence of plant extract. Methanol extract of *F.racemosa* leaves significantly decreased the glucose movement across the membrane when compared to the control.

Table 6: Glucose diffusion rate of methanol extracts of *Ficus racemosa leaves.*

S.no.	Control	Sample
1.	0.27	0.38
2.	0.3	0.44
3.	0.29	0.46
4.	0.29	0.52
5.	0.36	0.56
6.	0.27	0.61

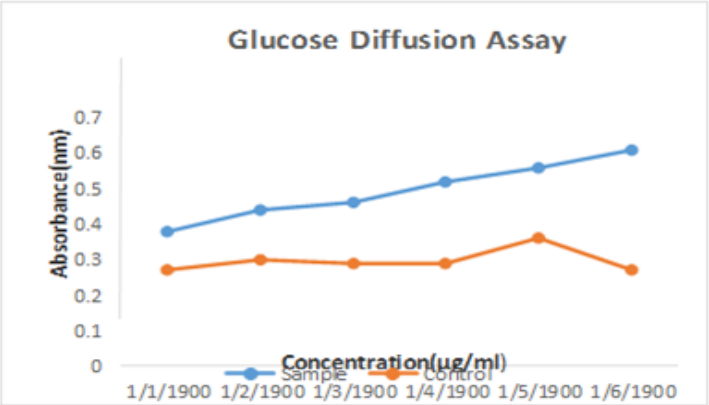

Fig 5: Effect of methanol extract of *F. racemosa leaves* on glucose diffusion.

Glucose Uptake assay

The results reveal that the glucose uptake rate was increased with the increasing concentration of the *F. racemosa* leaves extract and decreased with the increasing extracellular glucose concentration (Fig. 5). It is stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced. The obtained results clearly suggests that the methanol extract of *F. racemosa* has the capability to enhance the glucose uptake and effective glucose utilization, thereby it is controlling blood glucose level. *F. racemosa leaves* have better anti-diabetic property which can be used for the control of diabetics.

Table 7: Effect of methanol extract of *F. racemosa* on glucose uptake by yeast cells.

Sl.no.	5mM	10mM	25mM
1.	8	4.16	4.84
2.	8	4.16	4.84
3.	12	4.16	5.5
4.	12	4.16	6.4
5.	12	6.5	9.6

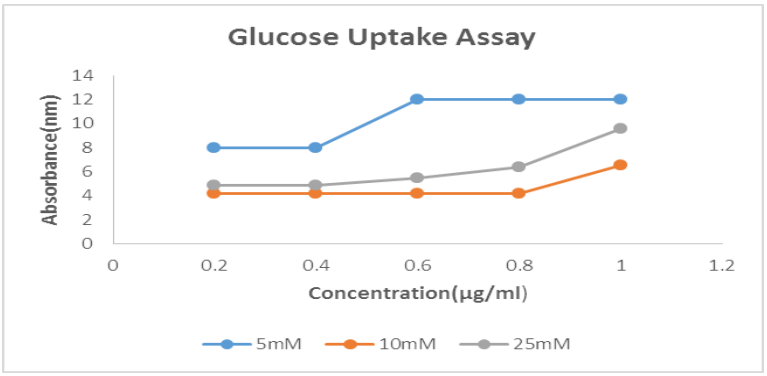

Fig 6: Effect of methanol extract of *F. racemosa* on glucose uptake by yeast cells.

α – amylase Inhibition assay

In vitro inhibitory assay of α-amylase was performed for extracts of the *F.racemosa* leaves. It was found that methanol extract of *F. racemosa* showed significant inhibitory activity. The inhibition varied from 8 to 25% in the concentration range of 500 to 1000µg/ml. The results obtained clearly suggest that the methanol extract of *F. racemosa* is capable of effectively inhibiting α- amylase activity.

Table 8: Inhibitory effects of extracts of the *F. racemosa* on α – amylase Inhibition.

Sl.no.	Absorbance	Inhibition by different concentrations (µg/ml)			
		250	500	750	1000
1.	0.16	0	0	0	0
2.	0.13	5.6	8.22	10.4	12.2
3.	0.11	11.8	16.66	18.88	22.5
4.	0.1	12.0	16.66	18.88	25.8
5.	0.9	22.2	25	27.5	25.8
6.	0.8	22.2	25	27.5	30.0

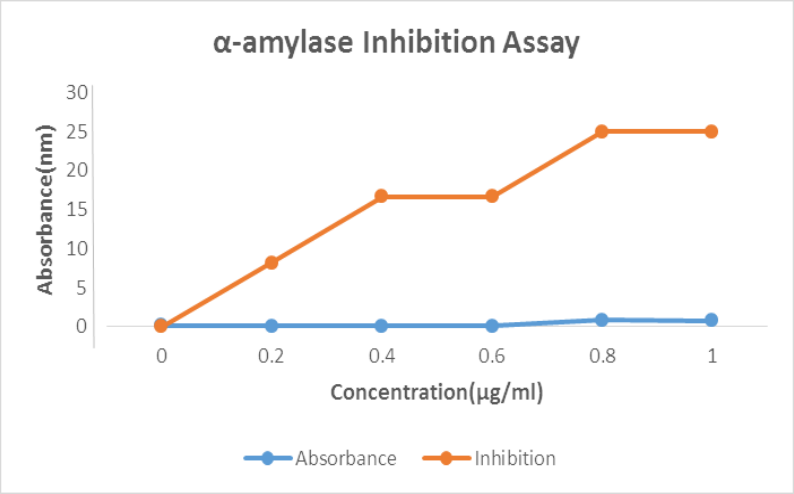

Fig 7: Inhibitory effects of extracts of the *F. racemosa* on α – amylase Inhibition.

CONCLUSION

The present study reveals that the qualitative and quantitative determination of phytochemicals present in the methanolic extract of *Ficus racemosa leaves*. It also deals with the antioxidant, glucose diffusion, glucose uptake and α- Amylase inhibition assay of the leaves sample. Our results revealed that *F. racemosa* has potential antidiabetic activity. Due to its effective pharmacological activities, in future it may be used for the treatment of many diseases like diabetics. As we are keenly interested in the study of traditional medicinal plants and our team have also have published our articles in the plants such as Allium sativum (Rathnasamy S *et al.*, 2014), Solanum trilobatum (Balakrishnan P et al., 2015), *Leucas*

aspera (Nagarasan S, Boominathan M 2016), *Adhatoda vasica* (Nagarasan S, Boominathan M 2016).

REFERENCES

1. Kobmoo N, Hossaert-mckey M, Rasplus JY and kjellberg F. *Ficus racemosa* is pollinated by a single population of a single agaonid wasp species in continental South-East Asia. Molecular Ecology., 2010; 19(13): 2700–2712.
2. Ahmed F and Urooj A. Traditional uses, medicinal properties and phytopharmacology of *Ficus racemosa*: A review. Pharmaceutical biology., 2010; 48(6): 672-681.
3. Ahmed F and Urooj A. In vitro studies on the hypoglycemic potential of *Ficus racemosa* stem bark. Journal of the Science of Food and Agriculture., 2009; 90(3): 397–401.
4. Ayyanar M and Ignacimuthu S. Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. International Journal of Applied Research in Natural Products., 2009; 2(3): 29-42.
5. Joseph B and Raj SJ. Phyto-pharmacological and phytochemical properties of three Ficus species - an overview. International Journal of Pharma and Bio Sciences., 2010; 1(4): 246-253.
6. Trivedi CP, Shinde S and Sharma RC. Preliminary phytochemical and pharmacological studies on Ficus racemosa (Gular). Indian J Med Res., 1969; 57(6): 1070-1074.
7. Sirisha N, Sreenivasulu M, Sangeeta K and Madhusudhana C. Antioxidant Properties of Ficus Species – A Review. International Journal of Pharm Tech Research., 2010; 2(4): 2174-2182.
8. Veerapur VP, Prabhakar KR, Thippeswamy BS, Bansal P, Srinivasan KK and Unnikrishnan MK. Antidiabetic effect of Ficus racemosa Linn. stem bark in high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats: A mechanistic study. Food Chemistry., 2012; 132: 186–193.
9. Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S and Kumar AR. Evaluation of Traditional Indian Antidiabetic Medicinal Plants for Human Pancreatic Amylase Inhibitory Effect In Vitro. Evid Based Complement Alternat Med., 2010. doi:10.1155/2011/515647.
10. Prasanna srinivasan Ramalingam, Monisha Antony Sagaya Raj, Priyalakshmi Ravichandran, Kumaran Shanmugam, Suganthi Nagarasan and Purushothaman Balakrishnan. Lipid peroxidation and anti-obesity activity of *nigella sativa* seeds. World Journal of Pharmaceutical Research, 2017; 6(10): 882-892.

11. Rathnasamy S, Auxilia LR, Purusothaman. Comparative Studies on Isolation and Characterization of Allinase from Garlic and Onion using PEGylation- a Novel Method. Asian J. Chem., 2014; 26(12): 3733-3735.
12. Balakrishnan P, Gani TAM, Subramanium S, Shanmugam K. A perspective on bioactive compounds from Solanum trilobatum. J. chem. Pharm. Res., 2015; 78: 507- 512.
13. Nagarasan S, Boominathan M. Perspective pharmacological activities of *leucas aspera*: an indigenous plant species. Indo Ame. J. Phar Res., 2016; 6(09): 6567-6572.
14. Nagarasan S, Boominathan M. Invitro studies on the primitive pharmacological activities of *Adhatoda vasica.* Int. J. of Life Sci., 2016; 4(3): 379-385.
15. Saranya, Kalimuthu J, Balakrishnan P, Ramalingam PS, Parthasarathi S, Ganesan B, Kaliyaperumal R, Ranganathan B and Shanmugam S. Isolation and characterisation of cellulolytic activity of bacteria and fungi from the soil of paper recycling unit at periyar maniammai university. Indo Ame J. Pharma Res., 2017; 7(6): 8253-8264.
16. Aruna AP, Balakrishnan P, Shanmugam K and Rajendran K. Biological finger printing of microbial diversity in vermicompost of different raw materials. Indo Ame J. Pharma Res., 2017; 7(04): 8497-8501.