# *In vitro*antioxidant & antidiabetic activity of *Abutilon crispum & Ficus dalhousiae*

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**ABSTRACT**

Free radicals are associated with several types of diseases including arthritis,diabetes mellitus, ageing as well as cancer *etc*. In the treatment of diseases, treatment by antioxidant has grown utmost prominence. Diabetes Mellitus is ancomplicated metabolic problemthrough chronic hyperglycemia that intrudes the metabolism of carbohydrates lipids&proteins. Thus, contemporary examination attempted to explore the antioxidant &antidiabetic activities of *Abutilon crispum*&*Ficus dalhousiae*by various standard *in vitro*models.In this attempt the methanol extracts of *Abutilon crispum*&*Ficus dalhousiae*were evaluated for their activities like radical inhibitoryactionthroughsuperoxide radical action,DPPH, lipid per oxidation assay,radical inhibition assay by nitric oxide method. The*in vitro* antidiabetic activity was also carried out by diverse parameters such as glucose diffusion,alpha amylase& alpha glucosidase inhibitory activities and glucose uptake capacity through yeast cells. Revealed results ofboth plantextractshad shown better radical scavenging capacity for their antioxidant activity while compared to standard antioxidants. Likewise, *Abutilon crispum*&*Ficus dalhousiae*extractshad showneffective*in vitro* antidiabetic activity by diverse parameters.It can be concluded that *Abutilon crispum*possess good antioxidant and antidiabetic properties when contrasted to *Ficus dalhousiae*by *in vitro* assay.

**Keywords:** *Abutilon crispum*, *Ficus dalhousiae*, methanol extract, *in vitro* antioxidant& antidiabetic activity.

## INTRODUCTION

Diabetes Mellitus is an intricate problemof metabolism through chronic hyperglycemia which interrupts functions of body. The etiology of Diabetes Mellitus can contrastimmensely yet steadily for any insulin release or reverberation of tissues (Baynest, 2015). Oxidative stress displays a noteworthyportion indiabetes. Hyperglycemia developauto oxidation of glucose results in free radical formation. The outcome of the free radicals endorses the advancement of diabetes & theaccompanyingdifficulties. Antioxidantsmight act onimmeasurablepoints, impeding the organization of ROS or eliminate free radicals, or increasein cell reinforcements protection catalyst capabilities.N-acetylcysteine &vitamin C antioxidantsare compelling in declining diabetic entanglements, demonstrating that thismay perhaps be helpful either by dietary supplementor byingestion of common cell reinforcements.Typically, antioxidants are engaged to reduce the glitches of diabetes (Saritha and Afreen, 2012). The leading point of the contemporary work was to estimateits antioxidant as well asantidiabetic activities by *in vitro* models.Theintention of the contemporary investigation was to assess the antioxidant activity for*Abutilon crispum*&*Ficus dalhousiae*through i*nvitro*model by evaluating various parameters.

## MATERIALS & METHODS

### Authentication & Collection of plants

*Abutilon crispum*&*Ficus dalhousiae*whole plantswere collected in the month of July from Chittoor, Tirupati,Andhra Pradesh. The material of plantswas recognized taxonomically by Prof. K. Madhava Chetty, Plant Systematics Laboratory,BotanyDepartment, Sri Venkateshwara University, Chittoor, Andhra Pradesh, India & voucher samples were placed in herbarium against accession numbers(0477) and (0977) for future reference.

### Extraction

Powder of *Abutilon crispum*and *Ficus dalhousiae* were individually extracted through methanol by means of uninterrupted Soxhlet extraction apparatus. The solvents of extracts were separated by rotary vacuum evaporator, the residual mass of extracts wasconcerted and dried out. The extracts were deposited in desiccator for additional studies.

### *In vitro* antioxidant activity

### DPPH assay

It wasevaluatedthrough 1, 1 diphenyl 2 picryl hydrazyl strategy (Tailor and Goyal, 2014). Numerousconcentrationslike 5, 10, 20, 40 and 80 µg/ml compositionswere organized by addingextractsof methanol to refined water. DPPH 0.1 milli molar composition was made by pouring in ethanol. To 2 ml of preparedstandard,4 ml plant extracts of diversecompositions were mixedindividually &laterfinal combination was vortexed to attainnormal temperature. Absorbance was assessedby spectrophotometer at 517 nm. Quercetintaken as standard for reference

### Nitric oxide assay

Dissimilar compositions of *Abutilon crispum*and *Ficus dalhousiae*(5 to 160 µg/ml) extractsremainedorganized individually. Sodium nitroprusside 3 ml to 0.5 ml of buffer of saline

phosphate was combinedthroughdissimilarcompositions of extract of methanol of plants& for 3 hours it was incubated at $30^0$C. Ethylenediamine dihydrochloride, 1 ml of Griess reagent and buffer made of phosphate (pH-8.3) are added on completionto incubation period. The composition was incubated to40-60 minutes and the absorbance wasassessed at 440 nmat room temperature. Standard referencewasRutin(Parul et al, 2013).

**Lipid peroxidation assay**

Methanol concentrates of *Abutilon crispum*&*Ficus dalhousiae*and rat liver microsomal part of diverse compositions (10-160 µg/ml) aretaken individually(Bouchet et al., 1998)to resolve the thiobarbituric acid receptive substances in this study. Working arrangement of 200 µl of plant extracts, liver microsomal portion400 µl & $FeCl_3$ (1mM) 100 µl were merged independently, finally vitamin C 100 µl was poured in it. For an hour, compositionsare incubated at 39 °C and applying the outcome with thiobarbituric acid, lipid per oxidation was evaluated. At 532 nm the response was assessed. All responses arerepeated. StandardVitamin Ewas employed.

**Scavenging activity of Superoxide anion radicals**

This activitywasaccomplished by model ofNishimikiet al., 1972. Consecutivecompositions(5-160 µg/ml)areorganizedseparately. 1ml of nitroblue tetrazolium & nicotinamide adenine dinucleotide were added to each dilution.100µl of phenazine methosulphate solution was added&the response was started. At 560 nmabsorbance was recorded. Curcumin employed as standard compound.

**Scavenging activity of radicals of hydroxyl group**

Diverse compositions (10 to 160 µg/ml) were organized by extract of methanolof *Abutilon crispum*and *Ficus dalhousiae*separately(Gayathri et al., 2014). Methanol extracts 400 µl at diverse compositions were added to 200 micro liter of 2-deoxy 2-ribose & 200 micro literof 1.04 mM EDTA. Later,equal quantity of ferric chloride & 100 micro liter of 1.0 mM $H_2O_2$ were poured to it. Finally, 100 micro liters ofnutrient C mixed to it. Subsequent one hour completion of incubation,trichloroacetic acid&thiobarbituric acideach 1ml mixed to the response composition&again incubated to31 minutes. It was assessed at 498 nm. Vitamin E utilized as standard.

**Antidiabetic activitythrough*in vitro*model**

**Inhibitory activity of alpha amylase**

0.5MTris-HCl(0.4 ml)comprising $CaCl_2$was added with 4 mgstarch azure. Cylinderscomprisingcomposition mixtureare warmed for few min &subsequentlyat 38°C preincubated for few min. Extracts of methanol of*Abutilon crispum*and *Ficus dalhousiae*wereplaceddistinctly in DMSO to get 5-100 µg/mL compositions. Later, 0.2 mL *Abutilon crispum*and *Ficus dalhousiae*extracts of precisecompositionshavemixeddistinctly to the cylindercomprising the compositionmixture. Likewise, Tris-HCl with porcine pancreatic amylase 0.2 mL was mixed to the particulartube comprising the extractsof *Abutilon crispum*&*Ficus dalhousiae*and compositionmixture. It was centrifuged for 7 min at 6°C, at an rpm of 3000. At 595 nm the absorbance was assessed employing spectrophotometer. Acarbose was employed as

standard. The experiments were repetitive thrice. The inhibitory action of α-amylase was evaluated by applyingthemethod:

Composition of acarbose &extractof plant required to suppressportion of α-amylase actionbelow the circumstancesremainedconsideredto $IC_{50}$regard. The repressiveaction of α-amylase of plant distillates&even acarbose areevaluated in addition its $IC_{50}$regards were resolved (Iniyan et al., 2010).

**Alpha glucosidase inhibitory activity**

For this activity, α-glucosidase gotfragmentedto 0.2 U/ml concentration in buffer (100 mM) of phosphateof pH 6.9& alsosodium azide 0.2 g/liter,bovine serum albumin 2 g/liter was taken as source of enzyme andas a substrate paranitrophenyl-α-d-glucopyranoside was employed. Methyl alcohol concentrates of *Abutilon crispum and Ficus dalhousiae*and successive dilutions of 5-100 µg/ml were completedindividually byequal volumes of distilled water&DMSO.10 microliters of dilutions of extract were incubated with enzyme sourceof 50 µl for 5 min. Substrate (50 µl) was mixed after incubation & for 5 minincubatedfurtherat room temperature.Absorbances wereassessed on a microplate at 406 nm. Each examination was repetitive& the average value was utilized to estimated. Acarbose employed as astandard(Kavitha et al., 2013).

**Glucose diffusion inhibitory study**

In this test, 4 cm parts of the dialysis layer were detached& loaded by2 ml of 0.16 M NaCl comprising glucose 19 mM &2 ml of *Abutilon crispum* &*Ficus dalhousiae*methanol extract distinctly.Utilizing a nylon string, they were tied at the two closures and placed in tumblercomprising39 ml of 0.16 M NaCl &9 ml purified water to alter the strength of media. Theapparatus then placedin orbital shaker &maintained at normalenvironment. Control sample consists NaCl encompassing glucose & distil water1 ml. Test samples were collected from each measuring cup& glucose concentration was evaluated by employing reagent. For 180 mins the study was repeated thrice(Rastogi et al., 2013).

**Glucose uptake capacity evaluation by yeast cells**

Investigation was accomplishedthroughCirillo model (Rehman et al., 2018). For preparing 1% baker's yeast suspension, it was liquefied in purified water andmaintained at 25◦C.

Later,at 4200 rpmfor 5 minutes, the yeast cell suspension was exposed to centrifugation.

Sequenceisreworkedthrough adding distilled water to it tillthe upper layer was developed. Smallportions of topmostclearfluids are mergedthrough distilled water to obtain yeast cellsuspension. About6 mg extract of plant was combined with DMSO. Mixture was later improvedthroughdiverse compositions of 1mL of preparationof glucose & incubated for few min. Forinducing a response, yeast suspension 100 $\mu$Lwas dispensedto blend,mixed well and incubated at $37^0$Cfor another59 min. The cylinders were centrifuged at 3800 rpmfor 6 minutes after incubation and glucose was evaluatedon 521 nmby spectrophotometer. Absorbance was noted on a comparablestandard value. In this, control is comprising entirely reagents but lack of test component. Standard drug taken was metronidazole. It wasexecuted for *Abutilon crispum*&*Ficus dalhousiae*methanol extract independently to determine capacity of yeast cells for glucose uptake.

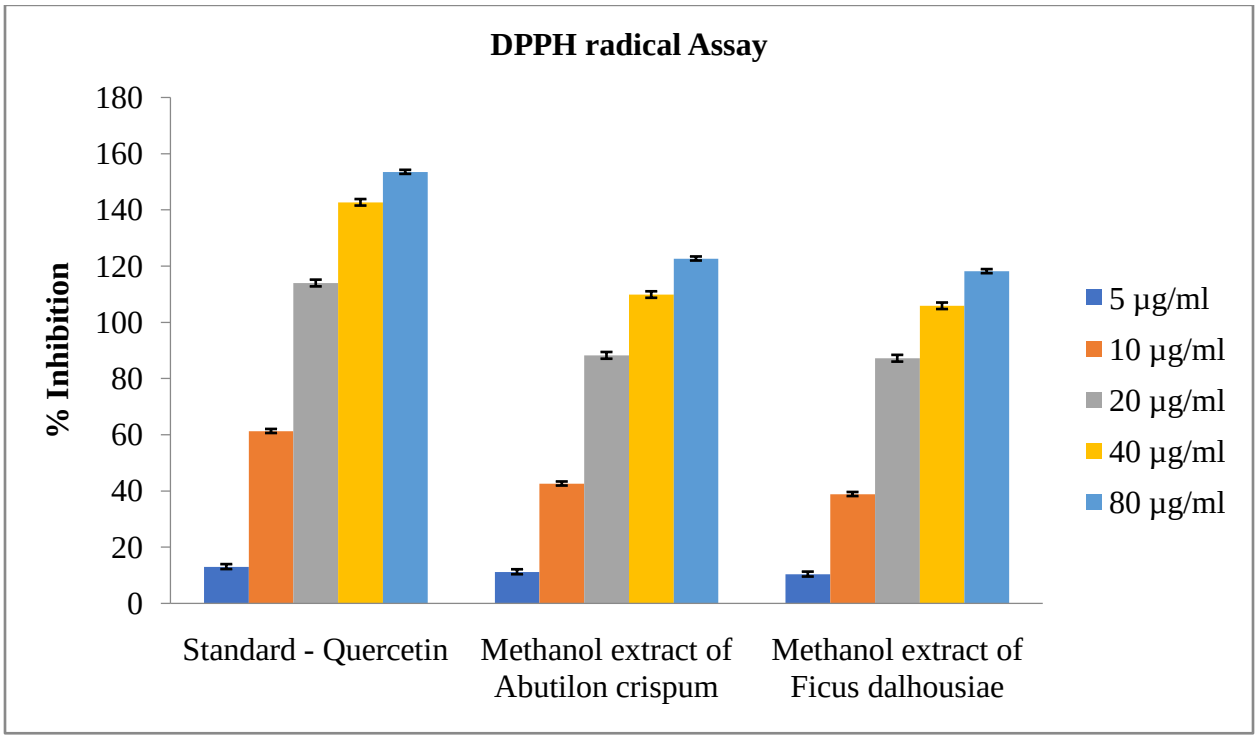
## RESULTS

### *In vitro* antioxidant activity

### DPPH assay

DPPH assayof *Abutilon crispum*&*Ficus dalhousiae*were checked and appeared in Figure1.The $IC_{50}$ estimation ofextract ofmethanol of *Abutilon crispum*was discovered as12.64 µg/ml and extract of methanol of *Ficus dalhousiae* was 12.41 µg/ml and itwas 8.53 µg/ml for quercetin standard

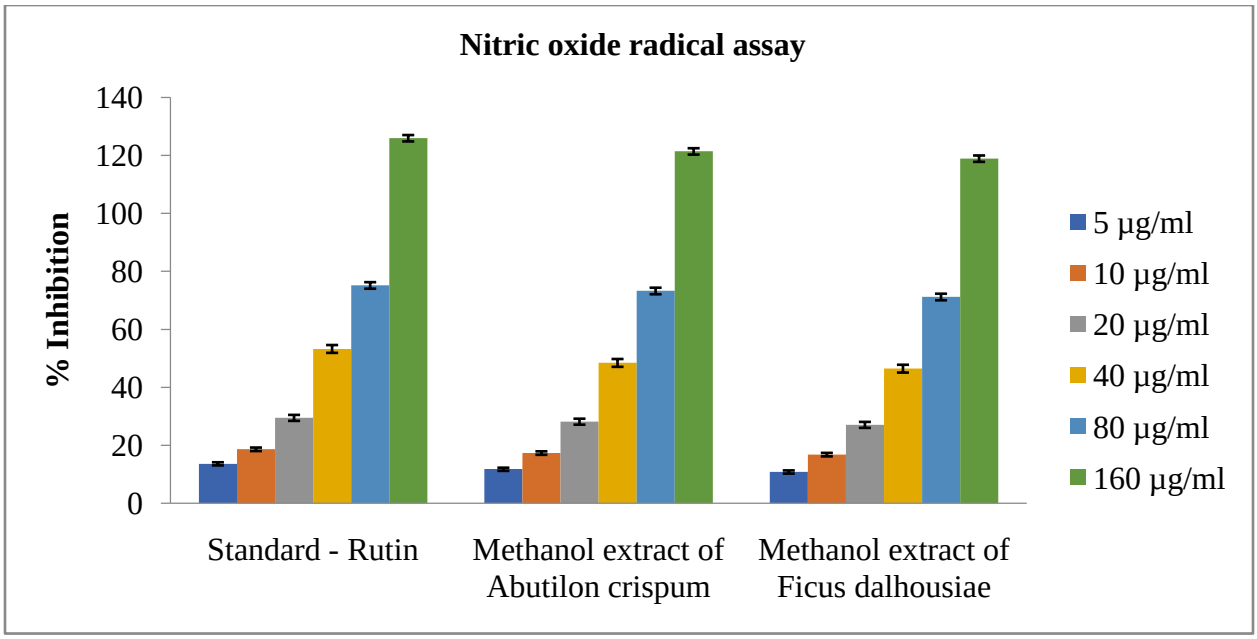
**Figure 1: DPPH radical Assay**




### Nitric oxide (NO) radical inhibition assay

It was recognized from $IC_{50}$ estimations for methanol extracts of *Abutilon crispum*as 43.69 µg/ml, for methanol extract of *Ficus dalhousiae* as 44.91 µg/ml& for rutin standard wasidentified as 36.34 µg/ml. The conclusions were displayed in Figure 2

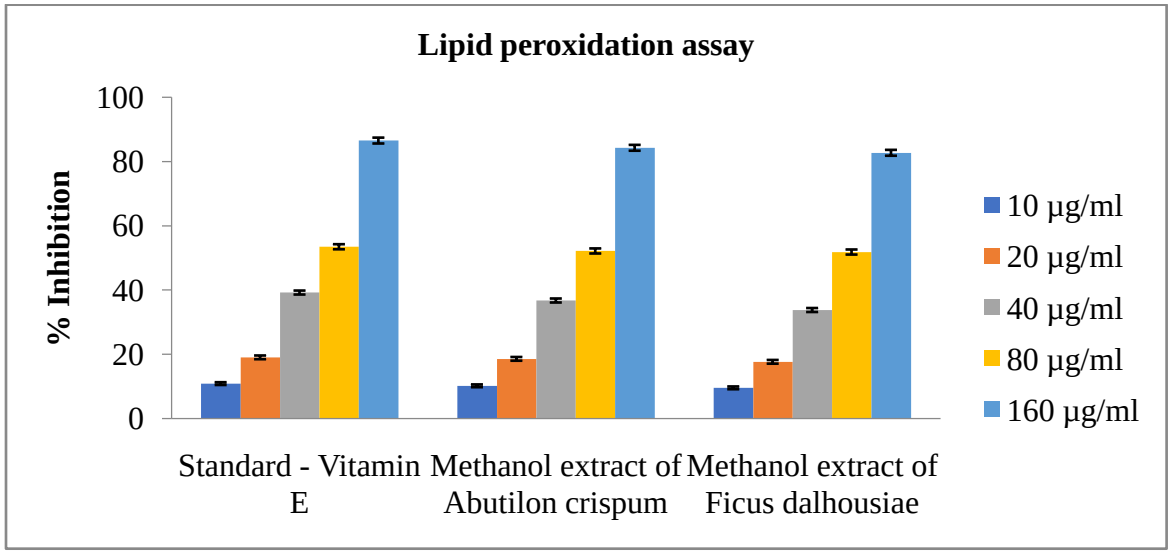
**Figure 2: Nitric oxide radical assay**




**Lipid peroxidation assay**

The methanol extracts of *Abutilon crispum*, *Ficus dalhousiae*& vitamin E standard presentedstable scavenging result of hydroxyl group at diverse compositions, presented in Figure3. The $IC_{50}$assessmentextracts of methanol of *Abutilon crispum* was distinguished as 75.52 µg/ml, for methanol extract of *Ficus dalhousiae*it was 71.38 µg/ml&vitamin E standardit was denoted as 71.48 µg/ml.

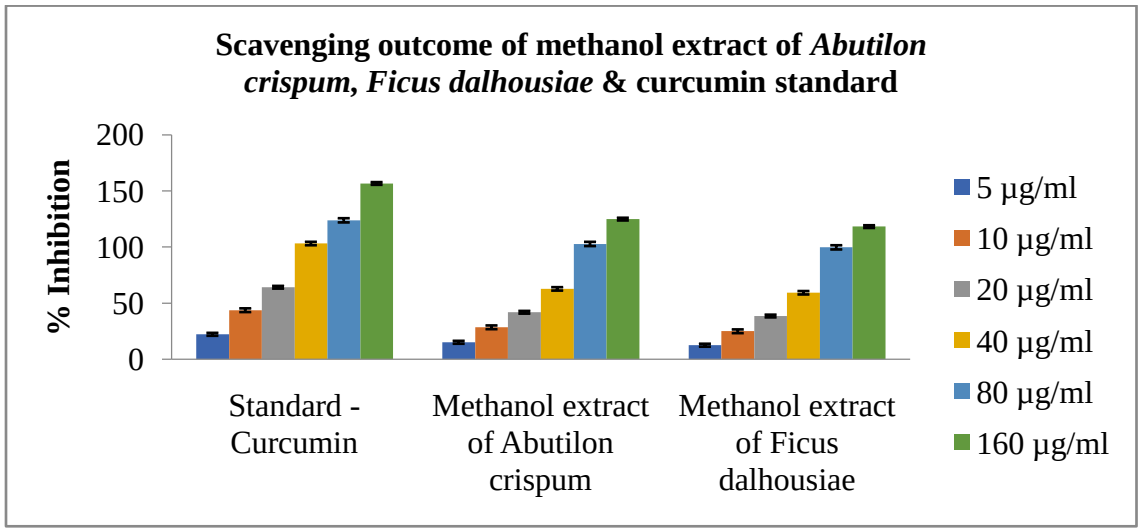
**Figure 3: Lipid peroxidation assay**



**Superoxide anion radical scavenging activity**

Outcomes of assay reveals that methanol extract of *Abutilon crispum*with $IC_{50}$approximations of 28.95 µg/ml, for methanol extract of *Ficus dalhousiae*the $IC_{50}$ estimations of 32.11 µg/ml&curcumin standardwith $IC_{50}$assessment as 13.15 µg/ml exhibited in Figure 7.4.

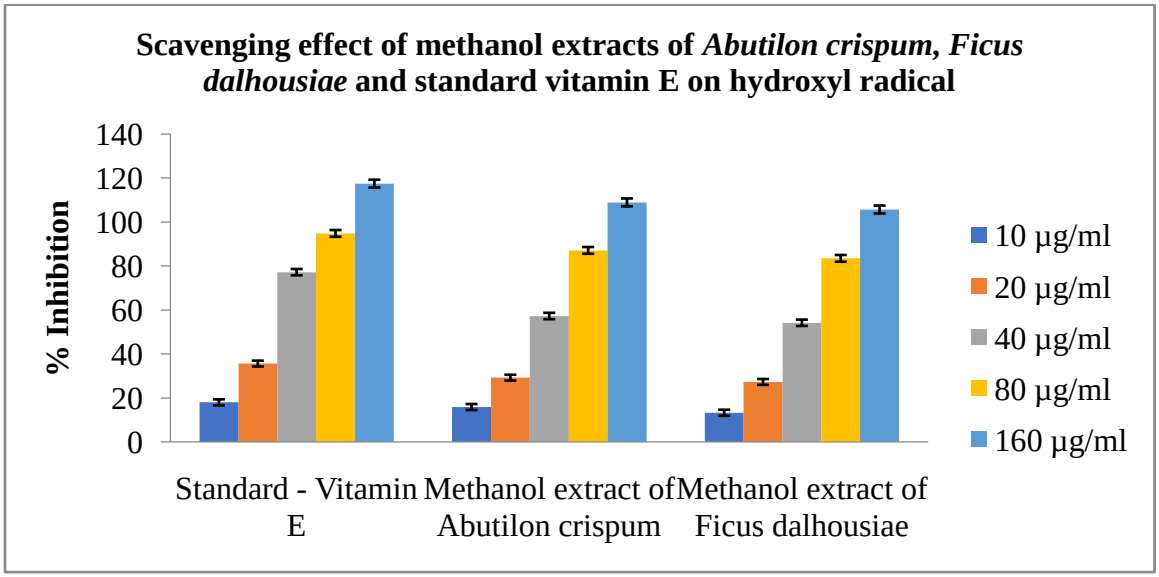
**Figure 4: Scavenging outcome of methanol extract of *Abutilon crispum, Ficus dalhousiae*&curcuminstandard.**




**Scavenging activity of hydroxyl radical**

It was assessedthroughresponse of fenton &consequencesexhibited below. The concentrations of $IC_{50}$observed for methanol extract of *Abutilon crispum*as 37.47 µg/ml, *Ficus dalhousiae*as 33.25 & for vitamin Estandardit was observed as 27.62 µg/ml.

**Figure 5: Scavenging impact on hydroxyl radical**

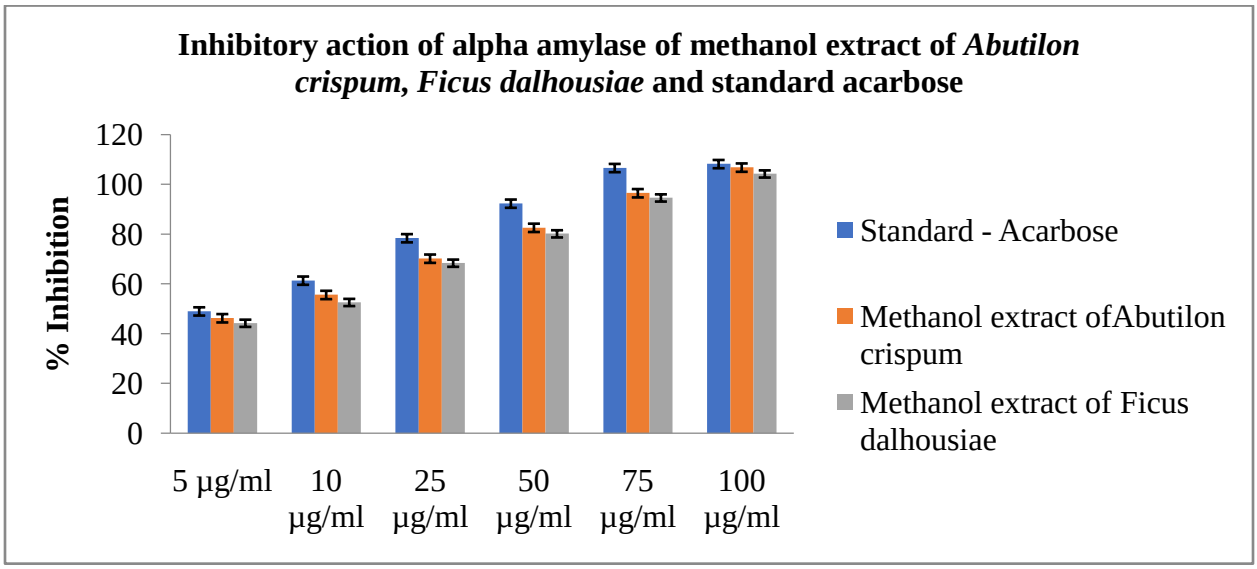


***In vitro*antidiabetic activity**

**Inhibitory activity of Alpha amylase**

The investigation reveals the outcomes of inhibitory activity of *Abutilon crispum*, *Ficus dalhousiae* &alpha amylase inhibitory acarbose in Figure 7.6. The $IC_{50}$ estimations of methanol extracts of *Abutilon crispum*was7.83 µg/ml and for methanol extract of *Ficus dalhousiae* was found to be 8.35 µg/ml,are better on comparisonwith acarbose standard5.42 µg/ml.

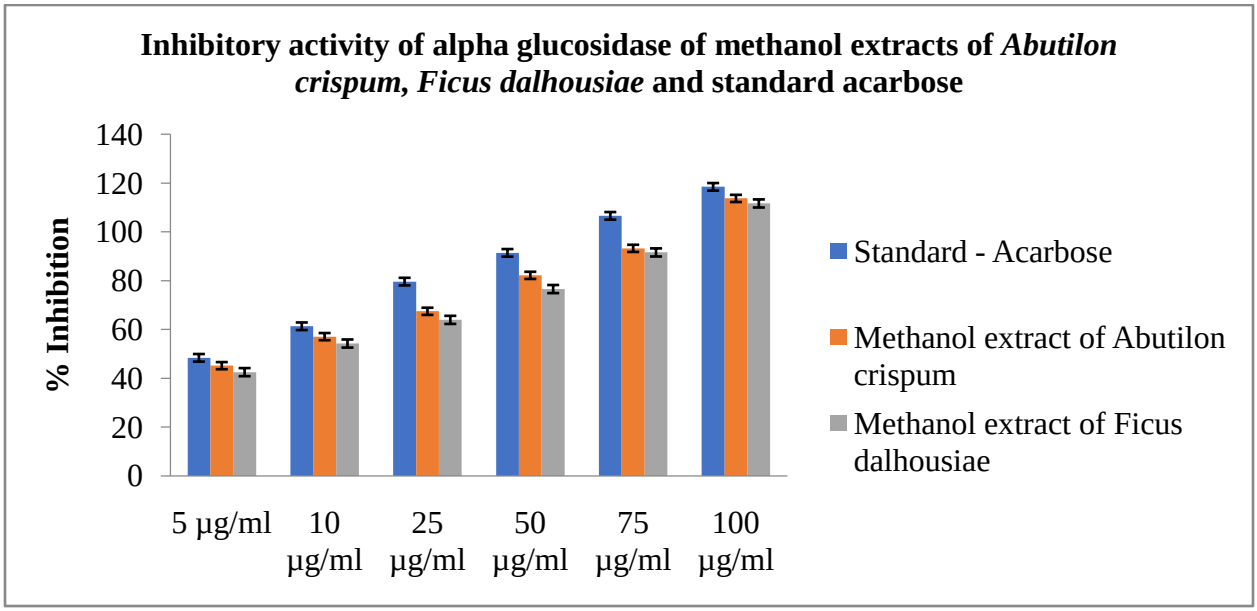
**Figure 6: Inhibitory action of alpha amylase of methanol extract of *Abutilon crispum, Ficus dalhousiae*and standard acarbose**




**Inhibitory activity of Alpha glucosidase**

The repressive activity of alpha glucosidase of extracts of methanolof*Abutilon crispum*and *Ficus dalhousiae* were contrasted & basic standard alpha glucosidase inhibitor acarbose areexhibited inFigure 7. The $IC_{50}$ estimations of methanol extract of *Abutilon crispum*was7.12 µg/ml and for methanol extract of *Ficus dalhousiae*was 8.3 µg/ml separatelyare better on comparison with acarbose standard5.58 µg/ml.

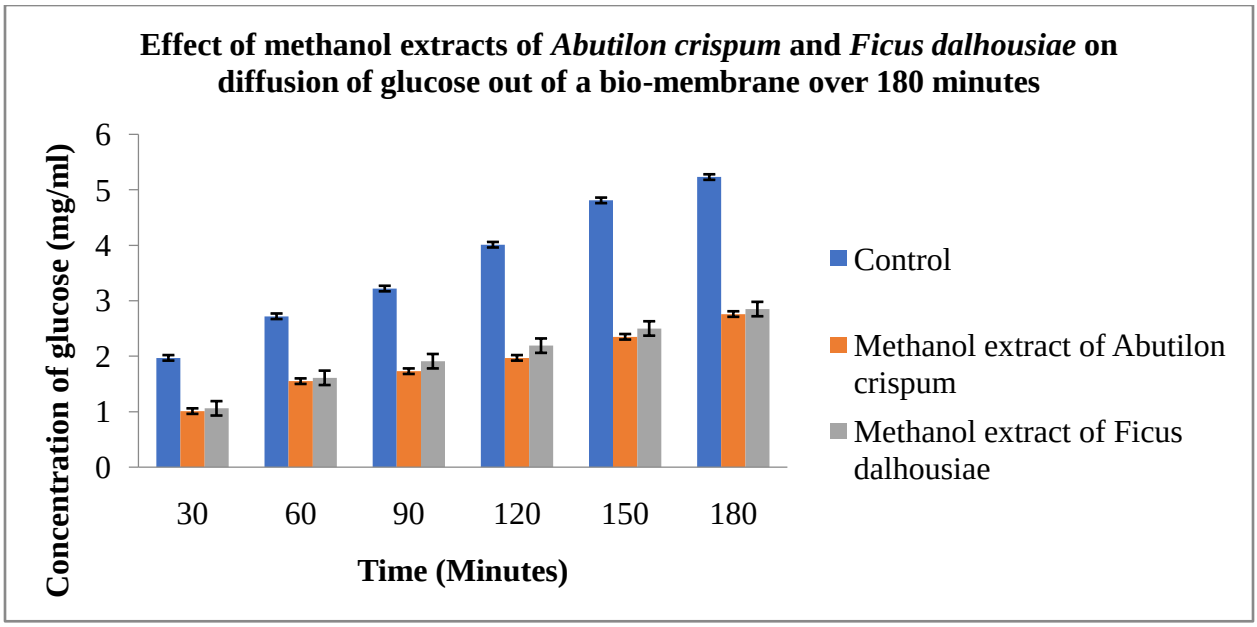
**Figure 7: Inhibitory activity of alpha glucosidase of extract of methanol of *Abutilon crispum, Ficus dalhousiae*and standard acarbose**




**Glucose diffusion inhibitory investigation**

Outcomes of study of*Abutilon crispum*and*Ficus dalhousiae*wereexhibited inFigure 8. The methanol extract of both plants had showninterruption of glucose diffusion through the dialysis layer, at 180 minutes the comparative movement regarding control was 54.12 ± 2.69&55.78 ± 2.69independently.

**Figure 8: Glucose diffusion inhibitory investigation**



**Determination of glucose uptake capacity through yeast cells**

Uptake of glucose by yeast cellsof *Abutilon crispum*&*Ficus dalhousiae*methanol extract were evaluated and consequencesareexhibited in Figures 9, 10 and 11.

**Figure 9: Effect of methanol extract of *Abutilon crispum*&*Ficus dalhousiae*on glucose uptake through yeast cells at 5 mM glucose concentration**

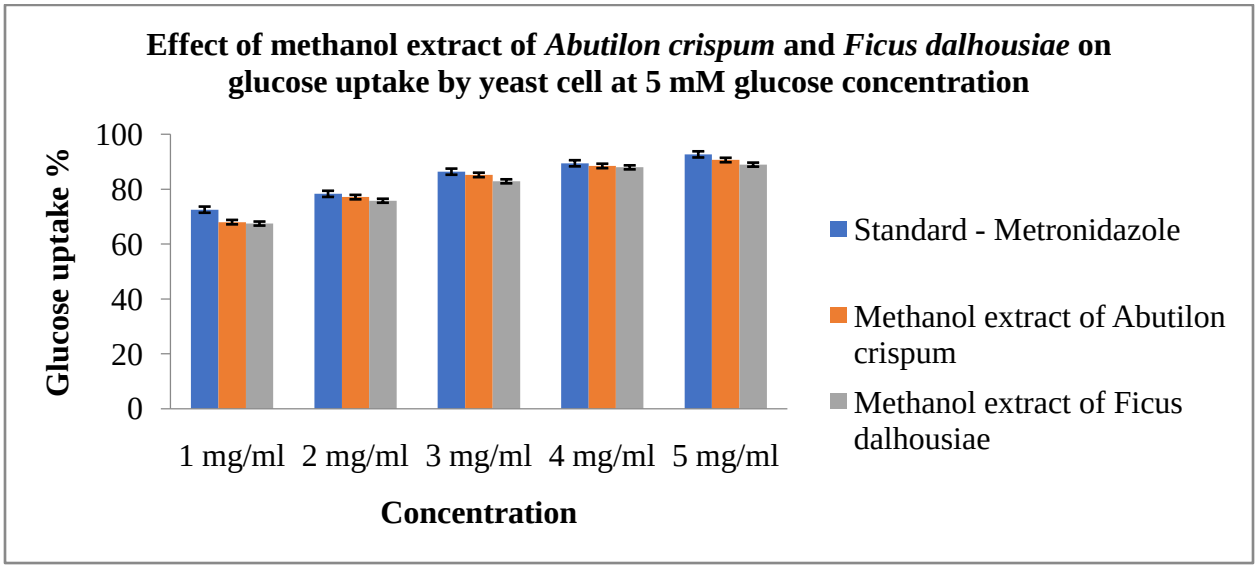



**Figure 10: Effect of methanol extract of *Abutilon crispum* and *Ficus dalhousiae*on uptakeof glucose through yeast cell at 10 mM glucose concentration**

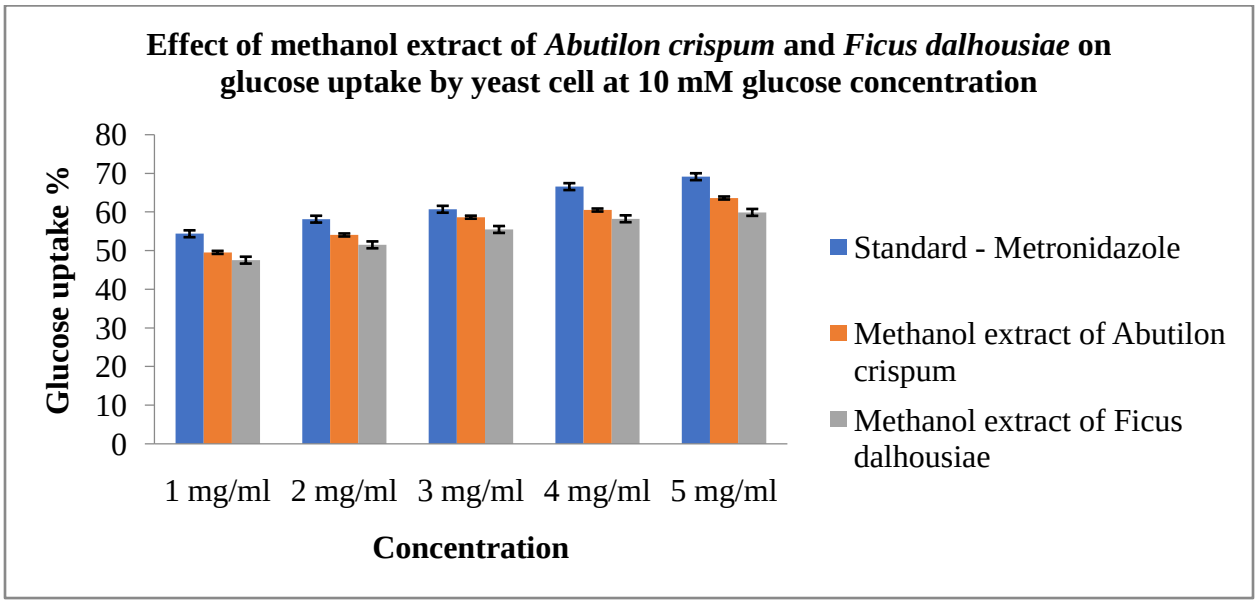
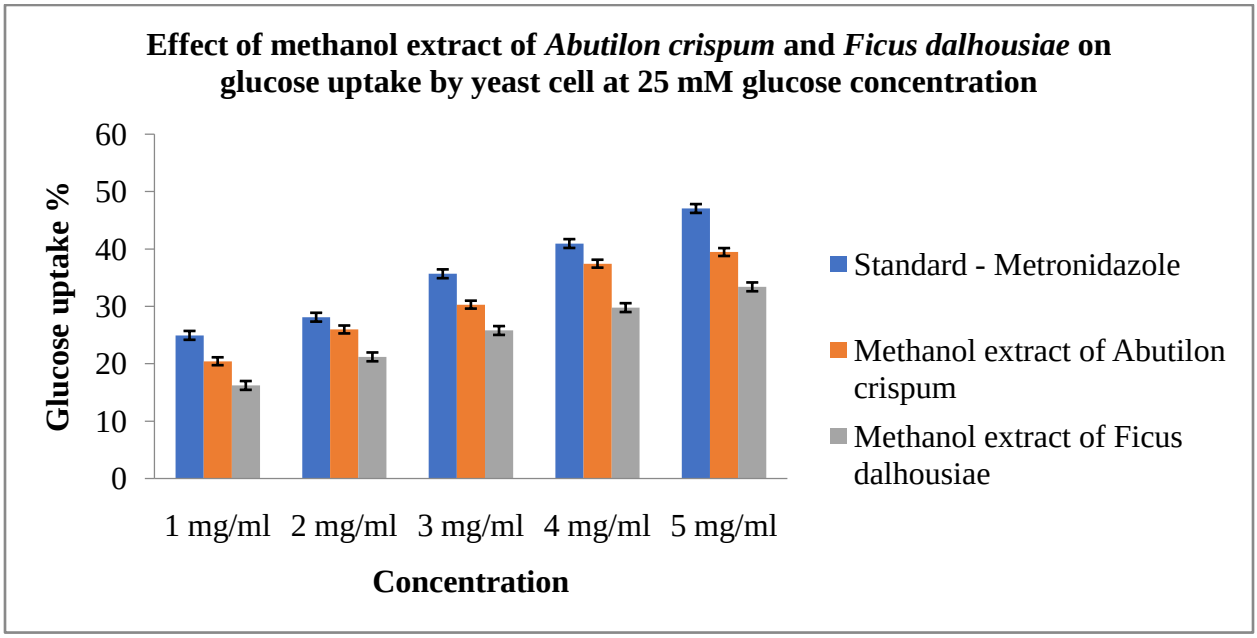


**Figure 11: Effect of methanol extract of *Abutilon crispum* and *Ficus dalhousiae*on uptake of glucose through yeast cell at 25 mM glucose concentration**




## DISCUSSION

### *In vitro* antioxidant activity

Methanol extracts of bothplants discoveredeffective radical scavenging activity against DPPH assay.The scavenging activity of *Abutilon crispum* and *Ficus dalhousiae*on DPPH radical might be due to presence of flavonoids, conferring to Bors model.The hydroxyl portionmerged B ring of flavonoid elementmightmovearound as diminishing specialist thatmightdesignate hydrogen molecule for deactivation of free radical of (Bors et al., 1990).A progressive decline in absorbance was recordedaccordingly with nitritesconcentration. Declineof absorbance could be due to the extract of plant,thatrival's oxygen to respond by nitric oxide instigatingdeclineby nitric oxide availability. Itcould be due toexistence of flavonoids in extract of plant, thatconfines the nitric oxide releaselike nitric oxide scavenger (Vanacker et al., 1990).The $IC_{50}$assessments of methanol extract of *Abutilon crispum* and *Ficus dalhousiae*revealed potent radical scavenging action on comparison with standard. The sharpdeclinein lipid peroxidation through the extract of *Abutilon crispum* and *Ficus dalhousiae*is due to the occurrence of steroids&phenols(Raja and Ramya, 2017).Both plants inhibited lipid peroxidation in dose reliant way on comparison to standard.Formazan staining bydecline in absorbance in existence of extracts of plant displayssuperoxide anion consumptionthroughterpenes of plant in responseblend. The outcomesdiscovered that extracts of methanol of *Abutilon crispum* and *Ficus dalhousiae*ownaddedeffective activity on comparison to standard curcumin.Hydroxyl radical is effective reactive oxygen species in living system thatalso reacts through polyunsaturated fatty acid moieties of cell membrane phospholipids & origins damage to cell. $IC_{50}$values for extracts of methanolare more potent for methanol extract of *Abutilon crispum* and *Ficus dalhousiae*to vitamin E standard.

**Antidiabetic activity by *In vitro*model**

**Alpha amylase inhibitory activity**

It isresponsible for carbohydrates digestion &to producevaried products of glucose thatmight be liableto hyperglycemia besidesprogress of diabetes mellitus. The extract of methanolof *Abutilon crispum* and *Ficus dalhousiae*repressed the alpha amylase action and declines the elevated glucose levels of blood. The extractsof methanol werehighlyeffective on comparisonto acarbose standard. The α-amylase inhibitoryimpact regulated by plantis asupportivepractice for diabetesmanagement.

**Inhibitory activity of alpha glucosidase**

The alpha glucosidase enzyme illustratescomparable activityto alpha amylase. The repressive activity of alpha glucosidase of methanol extract of *Abutilon crispum* and *Ficus dalhousiae*wascomparedto standard inhibitor acarbose,amongst them the extract of methanol of plant displayseffectiveaction.One strategy that has been developed to treat type-2 diabetes is inhibition of the activity of alpha-glucosidases using synthetic drugs

**Glucose diffusion inhibitory study**

We can concludefrom the consequences that,extract of methanol of *Abutilon crispum*&*Ficus dalhousiae*obstructs the diffusion of blood glucose across dialysis membrane. There are diversetransportersin the body that work in organizationthroughdissimilar molecules to transport glucose. The glucose atoms require a transporter element to passacrosscells, in the contemporary investigation glucose was set up in NaCl&this was executed by sodium particles. Consequencesdemonstrates that glucose diffusion is a probableconstituent of antihyperglycemic action of plants.

**Determination of glucose uptake capabilitythrough yeast cells**

The glucose uptake of *Abutilon crispum* and *Ficus dalhousiae*wasalmostalikeby that of known standard metronidazole. The influence of metronidazole on uptake of glucose via yeast cells are negligibly higher on comparedtoextracts of methanol of *Abutilon crispum*&*Ficus dalhousiae*. The uptake limit of glucose by the yeast cells were prolongedconcerningincrease in concentration of extracts of *Abutilon crispum*&*Ficus dalhousiae*. However, raise in the glucosemolar concentrationdisplays the converseassociation on uptake of glucose through yeast cells areobservedamongstcomparablequantity forextracts of methanol of *Abutilon crispum*&*Ficus dalhousiae*. Frequently, by facilitated diffusion the yeast cells will uptake glucose as opposite to enzyme system of phosphotransferase & other method.

## CONCLUSION

The contemporaryexaminationhad revealedthat extracts of methanolof*Abutilon crispum*&*Ficus dalhousiae*mightpossess*in*vitro antioxidant in addition antidiabetic activity. The plant material extracteddisplayequivalentoutcomes to that of particular standard employed. The extracts of

methanol of *Abutilon crispum*hadshown maximum *invitro* antioxidant and antidiabetic activity when compared to *Ficus dalhousiae*. Thus,both plantshave auspicious natural sources of antioxidants andcan be used in nutritional or pharmaceutical fieldsfor the elimination of diseases mediated by free radicals.

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