

In vitro α -Glucosidase Inhibitory potential and Free Radical Quenching Activity of Some Selected Medicinal Plants

Total poly phenolic content and its pharmacological activity of medicinal plants

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Abstract : The present study aimed to evaluate Total poly phenolic content and its pharmacological properties of selected five medicinal plants (*A. catechu*, *C.procera*, *S. asoca*, *T. indicus*, *D. letifolia*). plant sample were extracted with methanol, Standard Biochemical procedures were used to carry out their phyto-chemical analysis and screened for their pharmacological properties like free radical quenching potential (DPPH Scavenging activity), antidiabetic activity (α -Glucosidase inhibition potential) and evaluate their TPC by using FC reagent. Among the selected plants, Kattha (*A. catechu*) showed maximum polyphenolic content (.0412mg/ml), maximum α -Glucosidase inhibition activity (0.351mg/ml) and also maximum DPPH scavenging activity (0.0453mg/ml). The Pearson correlation between polyphenolic and biological activity curve statistically analyzed for understanding the role of polyphenols in biological activity. It showed positive correlation between the polyphenolic content and IC₅₀ values of α -Glucosidase inhibition activity and poor regression line having R²=0.554 were found. Further the polyphenols were correlated with IC₅₀ values of free radical scavenging activity which was highly fitted in linear regression line R² =0.985 value. The overall result reveals that the polyphenols are responsible for biological activity.

Key words- α -Glucosidase inhibition, DPPH, Free radical quenching, Polyphenols, IC₅₀ value, antidiabetic properties, correlation.

I. INTRODUCTION

Hyperglycemia is diabetes related risk factor, a cause of generation of free radicals which results increased oxidative stress in various tissues¹. Free Radicals are responsible for many diseases due to damage of important molecules like DNA and per-oxidation to biomembranes, the imbalance between ROS (Reactive Oxygen species) and antioxidant defense system may increase the oxidative burden which leads to tissue damage thus cause the number of disease². ROS (Reactive Oxygen Radicals) generated by a number of biotic and abiotic factor such as irradiation, environmental factors, pollutants, stress or byproducts of metabolic processes^{3,4}. About 5% or more of the inhaled oxygen (O₂) is converted to reactive oxygen species (ROS) such as O₂^{•-}, H₂O₂ and OH[•] by univalent reduction of O₂⁵. This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process⁶. Oxidative stress induces diabetes complications, both microvascular and macrovascular which causes mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, and also in the myocardium⁷. Therefore, Glucose homeostasis is the solution for the treatment of Diabetes⁸. Enzyme α -Glucosidase located at the brush border of the small intestine regulates glucose metabolism by degrading complex carbohydrates to absorbable monosaccharide⁹. Enzyme α -Amylase degrades complex carbohydrates to oligosaccharides and disaccharides, which are ultimately converted into monosaccharides by α -Glucosidase, liberated glucose is then absorbed by the gut and results in post prandial hyperglycemia. Therefore, inhibition of intestinal α -Glucosidase limits postprandial glucose level in the management of type 2 diabetes¹⁰.

α -Glucosidase inhibitors such as acarbose, miglitol, voglibose are known to reduce post prandial hyperglycemia by interfering with carbohydrate digestive enzymes and retarding glucose absorption¹¹. Even though these oral hypoglycaemic agents or drugs are effective but they are having side effects such as liver disorders, flatulence, abdominal pain, renal tumours and hepatic injury¹². Inhibitors of these enzymes have been recently developed from natural sources¹³. Some plants contain α -Amylase and α -Glucosidase inhibitors and now a day's researchers are focusing on these natural sources for potential anti-diabetic drugs^{14, 15}.

Some plants containing polyphenols have both the properties of enzyme inhibition as well as free radical scavenging activity¹⁶. Antioxidants play a pivotal role in the prevention of human diseases¹⁷. These compounds may function as free radical scavengers, complexing agents for pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation¹⁸. Antioxidants prevent the autoxidation of the oils and fatty foods; therefore, the importance of search for natural antioxidants has greatly increased in the recent years¹⁹.

Medicinal plants are of great economic value from beginning Indian medicinal system (Ayurveda) include the detailed information about hundred of medicinal plants²⁰. The search for suitable plant extracts which possess strong bioactive compounds that are important in treating infections and managing various human diseases have been increased since last decade²¹⁻²³. Therapeutic value of these plants is due to their efficiency, low operating costs, availability in rural areas, also utilized as traditional medicines and comparatively cheaper than modern medicines²⁴⁻²⁷.

Plant derived phytochemicals like Alkaloids, Glycosides, Flavonoids, Phenolics, Tannins etc. are of consequence because of their potential antioxidant, antimicrobial and antidiabetic properties²⁸. A considerable free-radical scavenging (antioxidant) activity has been exhibited by phenolic compounds, which is determined by their reactivity as hydrogen- or electron- donating agents^{29,30}. Major types of phenolic compounds from most of the herbs were preliminarily screened (identified and analyzed), are phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids³¹. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark³². Utilization of antioxidants is beneficial for diabetic patients, not only to maintain antioxidant level in the body but also to treat the long term complications³³. An effective antidiabetic compound should possess both antihyperglycemic and free radicals scavenging properties, with minimal or no side effects³⁴.

In the present scenario many plants are used to treat various diseases. As we have mentioned above that there is a great variety of phytochemicals and many are unknown for their chemical characters as well as their biological activities. The main purpose of this work is to evaluate the biological activities through the spectroscopic technique and analysis of phytochemicals found in some selected medicinal plant. In this study we have chosen 5 plants of named Ashoka (*S.asoca*), Emali (*T.indica*), Shisham (*D.latifolia*), Madar (*C. procera*) and Kattha (*A.catechu*). Some of the selected plants have been reviewed by Pandeya et al.³⁶ (2013) as antidiabetic medicinal plants.

Ashoka (*S. asoca*) is considered to contain medicinal properties, generally known as a “ashok briksh” and also reported to contain several phytoconstituents like carbohydrates, proteins, tannins and saponins and have multiple uses in Ayurveda, Unani and Homeopathy³⁷. These phytoconstituents may be responsible for various pharmacological activities like antibacterial³⁸, anti cancer³⁹, anti menorrhagic⁴⁰ and anti oxytoxic⁴¹ activities.

Emali (*T. indica*) is an extensively used traditional medicine of which almost every part gives⁴², either nutritional or medicinal value. It is used for treatment of dysentery, jaundice, erysipelas, hemorrhoids and various other ailments gingivitis, asthma and eye inflammations⁴³. Phenolic compounds of this plant may have many biologic effects in terms of health promotion⁴⁴. Tamarind seed was also investigated for their antioxidative activity⁴⁵. This plant shows an important protective effect is reduction of oxidative damage, mediated by lipid peroxidation, which in living systems is strongly associated with mutagenesis, carcinogenesis, ageing, and atherosclerosis⁴⁶.

Shisham (*D. latifolia*) is the main remedy ingredient for the treatment of diarrhoea, worms, indigestion, dyspepsia, diarrhoea, obesity, cutaneous infections and leprosy⁴⁷⁻⁴⁹. It is used as anthelmintic, antipyretic, analgesic, anti-allergic, anti-androgen, anti-arthritis, anticancer, antidiabetic, antifertility, anti-inflammatory, antimicrobial agent⁵⁰⁻⁵³.

Madar (*C. procera*) flowers have shown strong cytotoxic activity in the patients of colorectal cancer. It was used in cases of cutaneous diseases, intestinal worms, cough, ascites, asthma, bronchitis, dyspepsia, paralysis, swellings, intermittent fevers, anorexia, inflammations and tumors⁵⁴.

Kattha (*A. catechu*) is a multipurpose and moderate size deciduous tree with crooked and forked trunk⁵⁵. This medicinal plant is commonly known as kattha or karangali, shows various pharmacological effects like an astringent, anti-inflammatory, antibacterial, antifungal, hypoglycaemic and hepato-protective agent has been reported⁵⁶⁻⁵⁸. It is used to cure high blood pressure, leucorrhoea, diarrhea, dysentery, leprosy, colitis, gastritis, bronchitis and cough, and also gargled for gingivitis, toothache, sore throat and mouth infections has also been reported⁵⁹. The medicinal properties of plants have been sufficiently harnessed. The difficulty encountered with alternative medicine is the lack of reliable documentation of traditional plants for curing chronic diseases.

The main objectives of this study were to determine the total polyphenolic contents of five selected traditional medicinal plant leaves of Chitrakoot region for their pharmacologic activities and understanding the role of polyphenols in biological systems.

II. RESEARCH METHODOLOGY

2.1 Collection of Plant Materials:

The plant leaves of Ashoka (*S. asoca*), Emali (*T. indica*), Seesham (*D. latifolia*), Kattha (*A. catechu*) and Madar (*C. procera*) were collected in March 2013 from Chitrakoot region and identified. All plant leaves were collected, washed with fresh water and dried under shade at room temperature separately. The leaves were powdered and stored separately in sterile and air tight container for further use.

2.2 Chemicals:

Methanol, water, DMSO, tris-HCl, Folin & Ciocalteu's Phenol Reagent (FCP), p-nitrophenyl- α -D-glucopyranoside, Sodium carbonate and ascorbic acid were obtained from SRL, India. While 2,2- Diphenyl-1-picryl hydrazyl (DPPH), Catechol were purchased from Alfa acer, Great Britain. Acarbose, α - glucosidase rat intestinal acetone powder procured from Sigma Chemicals, USA. All solvents were HPLC grade while chemicals were AR grade and used without further purification.

2.3 Preparation of Plant extracts:

100 mg powdered sample of plant leaves were extracted with 10 ml HPLC grade methanol through open air reflux process at 40°C for 6 hours till dried than make the volume again 10 ml with methanol and reflux, this process was repeated several times. The extracts were filtered through filter paper (Watman no.1) to remove free un-extractable substances. The filtrates of plant extract were evaporated at room temperature at dryness, finally dissolved with 10 ml with DMSO and preserved at 4-5°C for further process.

2.4 Determination of Total Polyphenolic Content:

Total polyphenolic content of extracts of plant leaves were measured using Folin-Ciocalteu reagent method, adopted as it is described by Tripathi et al.⁶⁰ (2013). The 25 µl of plant extract diluted with 125 µl water followed by addition of 150 µl of Folin-Ciocalteu reagent (1N) & 25 µl of Na₂CO₃ (20% w/v) incubated at 45°C for 60 min absorbance was measured spectrophotometrically at 765nm (Synergy H₄ multimode micro plate reader, biotek instrument, inc Winoosci, VT, USA). Quantification was performed with respect to the standard curve of Catechol. ($y = 0.003X + 0.023$, $R^2 = 0.964$) results were expressed as milligram of catechol equivalent per ml of extract.

2.5 DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay:

The assay for free radical DPPH was done by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method adopted as it is described by Tripathi et al.⁶⁰ (2013). In brief, a 96-well microplate, 25µl of various dilutions of methanolic extract 125 µl of tris -HCl buffer (0.1M, pH 7.4) and 125 µl of DPPH solution (0.004 in methanols) were added. The reaction mixture was shaken well. The DPPH decolorisation was recorded at 517 nm on a Biotek Synergy H₄ hybrid multimode micro plate reader after 30 min incubation in dark. The percentage of DPPH scavenging by plant extracts obtained in terms of ascorbic acid equivalent concentration. Quantification was performed with respect to the standard curve of ascorbic acid ($Y = 0.733X + 14.6$, $R^2 = 0.948$). Results were expressed as milligrams of ascorbic acid equivalent per ml of the extract.

2.6 Determination of α -Glucosidase Inhibition activity:

Method for determination of α -Glucosidase were adopted from Misra S. et al.⁶¹ 2011. Rat intestinal acetone powder (Sigma chemicals, USA) was sonicated properly in normal saline (100:1 w/v) and after centrifugation at 3000 rpm \times 30 mins the supernatant was treated as crude intestinal α -Glucosidase. 50 µl various dilutions in DMSO (0.1mg /ml solution) were mixed and incubated with 50 µl of enzyme in a 96-well microplate for 5 mins. Reaction mixture was further incubated for another 10 mins with 50 µl substrate (5 mM, p-nitrophenyl- α -D-glucopyranoside) prepared in 100 mM phosphate buffer (pH~6.8) and release of nitrophenol was read at, 405 nm spectrophotometrically (Synergy H₄ multimode micro plate reader, biotek instrument, inc. Winoosci, VT, USA). All the samples were run in triplicate and Acarbose was taken as standard reference compound. Several dilutions of primary solution (5mg/ml DMSO) were made and assayed accordingly to obtain concentration of the test sample required to inhibit 50 % activity (IC₅₀) of the enzyme. Quantification was performed with respect to the standard curve of acarbose ($Y = 26.63X + 46.26$, $R^2 = 0.958$) results were expressed as milligram of Acarbose equivalent per ml of extract. Percent α -Glucosidase inhibition was calculated using following equation:

$$\alpha\text{-Glucosidase inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A₀ is the absorbance of the control and A₁ is the absorbance in the presence of the sample.

2.7 Statistical Analysis:

Data reproduced during the experimental work, were analyzed using Origin Pro8.5 software. All parameters were triplicately recorded for accurate result. Tables (1-4) show mean value and standard deviation (\pm) of reproduced data. Graphs (1-7) were also plotted using OriginPro 8.5 software.

III. RESULT AND DISCUSSION

It is of particular interest to investigate antioxidants, particularly in those intended to prevent the presumed deleterious effects of diabetes. So there is a preference for antioxidants and α -Glucosidase inhibitors from natural rather than from synthetic sources because it is cheaper than synthetic one. Therefore, this experiment was designed in such a way that the maximum extractability of the poly-phenolic compounds occur, we chose to extract raw materials with methanol. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on bioactive components of plant materials for their potential medicinal value. Present studies indicates the % 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, α -Glucosidase inhibition activity and their correlation with total polyphenolic constituents present in the leaves of Ashoka (*S. asoca*), Emali (*T. indica*), Seesham (*D. latifolia*), Kattha (*A. catechu*) and Madar (*C. procera*) methanolic extracts at different concentrations with reference standard.

3.1 Total Polyphenolic Contents:

Polyphenolic compounds are commonly found in both edible and inedible plants, and reported for multiple biological effects, including antioxidant activity⁶². The antioxidant effect of plant phenolics has been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders⁶³. It was reported that phenolic

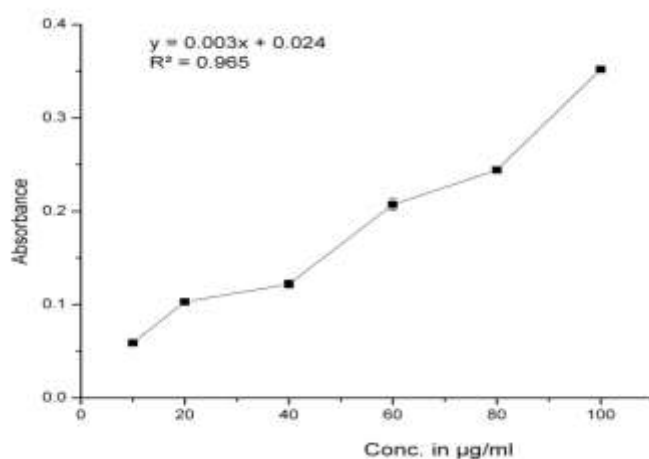
compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation^{64, 65}. As demonstrated in Table 1, the total phenolic content of methanolic extracts of plant leaves. Graph 1 was plotted between absorbance Vs concentration of Catechol, which is used as the standard curve for the quantification of total polyphenolic content equivalent to Catechol $\mu\text{g/ml}$ in methanolic extracts of selected plant leaves.

Table 1:- tpc of selected medicinal plant leaves.

S.N	Plants Name (Botanical name)	Total Polyphenolic Content equivalent to Catechol $\mu\text{g/ml} \pm \text{SD}$
1	Kattha (<i>Acacia catechu</i>)	41.22 ± 0.0006
2	Ashoka (<i>Saraca ashoca</i>)	34.89 ± 0.0006
3	Seesham (<i>Dalbergia latifolia</i>)	29.11 ± 0.001
4	Emali (<i>Tamarindus indica</i>)	10.67 ± 0.002
5	Madar (<i>Calotropis procera</i>)	5.89 ± 0.003

Ramesh et al.⁶⁶ (2009), estimated total phenolic contents of Madar (*C. procera*) leaves i.e. 3.6 mg.g-1 dry weight. Escalona-Arranz et al.⁶⁷ (2011), evaluated 25.972 mg/ml for TP (total phenolic content) in Emali (*T. indica*) leaves. Khalid et al.⁶⁸ (2011), assessed the total polyphenolic contents $210 \pm 1.16 \mu\text{g/ml}$ in bark extract of Seesham (*D. latifolia*). A. K. Pandey et al.⁶⁹ (2011), evaluated total polyphenolic content of Ashoka (*S. asoca*) bark samples, the maximum concentration of total phenols ($7.25 \pm 0.94\%$) was found in girth class 61-90 cm and minimum concentration of total phenols ($6.54 \pm 0.71\%$) was found in girth class 15-30 cm.

In the present study we have estimated Madar (*C. procera*), Emali (*T. indica*), seesham (*D. latifolia*), Kattha (*A. catechu*) and Asoka (*S. asoca*) leaves it contain the $5.89 \mu\text{g/ml}$, $10.67 \mu\text{g/ml}$, $29.11 \mu\text{g/ml}$, $41.22 \mu\text{g/ml}$ and $34.89 \mu\text{g/ml}$ polyphenolic contents respectively.



Graph 1- Standard Curve Of Catechol For Estimation Of Total Polyphenolic Content

3.2 Free Radical Scavenging Activity:

Natural free radical scavengers are closely related to their bio-functionalities. Free radicals are highly reactive molecules or chemical species capable of independent existence. Generation of highly Reactive Oxygen Species (ROS) is an integral feature of normal cellular functions like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation, and fertilization but their higher production however, multiplies several folds during pathological conditions. Oxygen, because of its bi-radical nature, readily accepts unpaired electrons to give rise to a series of partially reduced species collectively known as ROS including, superoxide ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl (HO^{\cdot}), peroxy (ROO^{\cdot}), alkoxy (RO^{\cdot}) and nitric oxide (NO^{\cdot})⁷⁰.

Assay based upon the use of DPPH is the most popular spectrophotometric methods for determination of the free radical scavenging capacity of food, beverages and vegetable extracts⁷¹. This chromogen radical compound can directly react with antioxidants. Additionally, DPPH scavenging method has been used to evaluate the antioxidant activity of compounds

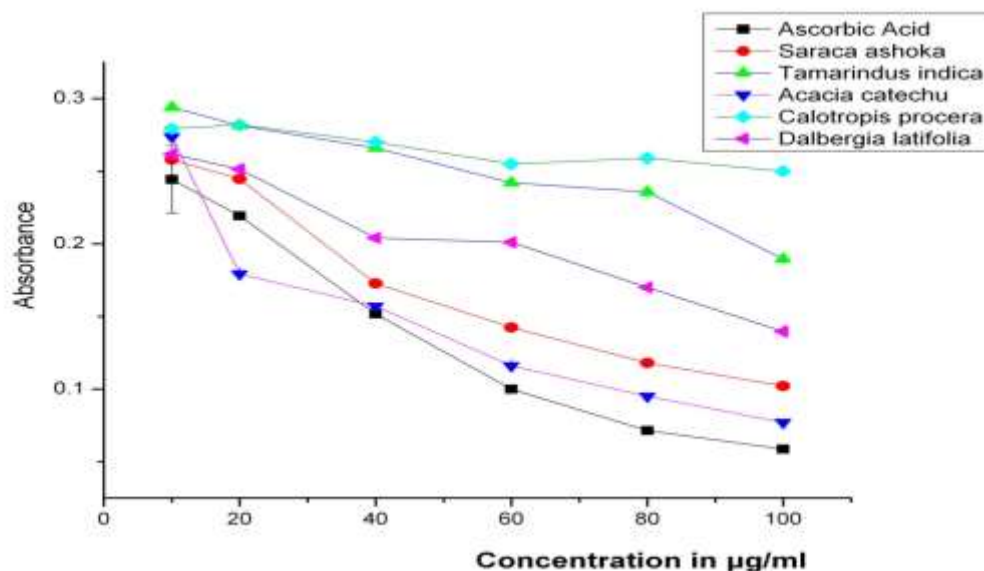
due to the simple, rapid, sensitive, and reproducible procedure⁷². Radical scavenging activity is very important due to the deleterious role of free radicals in biological systems. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. In this study DPPH scavenging assay of selected plants was compared with standard ascorbic acid. The graphical representation of the data is shown in Graph 2 & 3. Table 2 shows the IC₅₀ values of methanolic extracts of plant leaves.

Table 2- The IC₅₀ Values Of Selected Plant Extracts And Ascorbic Acid.

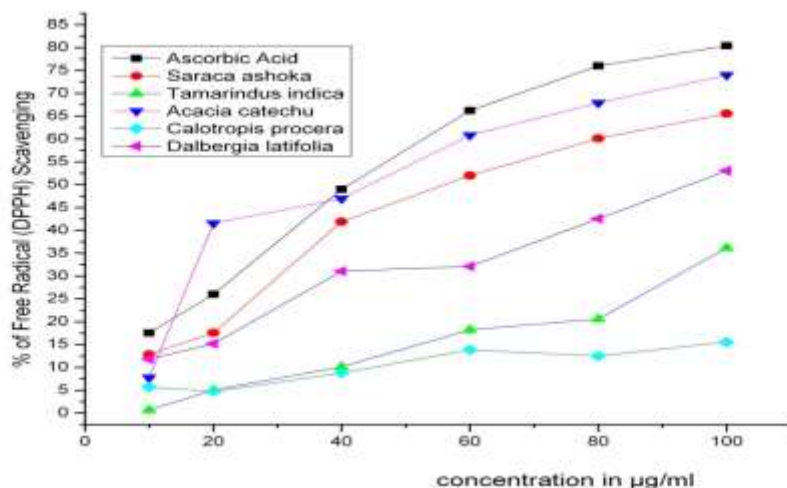
S.N.	Plants Name (Botanical name)	IC ₅₀ Value of Test samples for DPPH Radical scavenging $\mu\text{g/ml}$
1	Kattha (<i>Acacia catechu</i>)	45.336
2	Ashoka (<i>Saraca ashoka</i>)	65.336
3	Madar (<i>Calotropis procera</i>)	Nil
4	Sheesham (<i>Dalbergia latifolia</i>)	93.34
5	Emali (<i>Tamarindus indica</i>)	Nil

A. K. Pandey et. al.⁶⁹ (2011), examined the free radical scavenging activity of Ashok (*S. asoca*) bark which exhibit 4.82 mg/ml IC₅₀ value. Yizhong cai et al. 2004⁷³ estimated the antioxidant value of Kattha (*A. catechu*) seed 2674.0 trolox /10g dry weight. Khalid et al.⁶⁸ (2011) assessed the antioxidant activity of bark extract of Seesham (*D. latifolia*) which inhibit DPPH 92.10 % & IC₅₀ value was found 0.17 mg/ml.

In the present study methanolic extracts of the leaves of Kattha (*A. catechu*) and Ashoka (*S. asoca*) show better free radical scavenging activity than Seesham (*D. latifolia*). IC₅₀ value of methanolic extracts of Kattha (*A. catechu*), Ashoka (*S. asoca*) and Seesham (*D. latifolia*) is 45.336 $\mu\text{g/ml}$, 65.336 $\mu\text{g/ml}$ and 93.34 $\mu\text{g/ml}$ respectively. Kattha (*A. catechu*) demonstrates strongest scavenging activity among these five plants. But Ashoka (*S. asoca*) and Seesham (*D. latifolia*) showed mild antioxidant activity, while Emali (*T. indica*) and Madar (*C. procera*) doesn't have significant antioxidant activity.



Graph 2- Representing Graph Between Concentration (mg/ml) And Absorbance.



Graph-3 representing graph between % of free rdical (dpph) scavenging and concentration.

3.3 Analysis of α -Glucosidase inhibition activity:

Reddy et al.⁷⁴ (2010) reported in their *in vitro* studies of the methanolic extract of leaves of *Asystasia gangetica* has significant *in vitro* antioxidant and α -Glucosidase and α -Amylase enzymes inhibitory activity. The results shown in Graph 4-5. Graph 5 indicates the % inhibition of α -Glucosidase enzyme, from leaf extracts of Ashoka (*S. asoca*), Emali (*T.indica*), Kattha (*A. catechu*), Madar (*C. procera*) and Seesham (*D. latifolia*) at different concentration. The α -Glucosidase inhibition activity increased in a dose dependent manner. Acarbose used for reference standard to quantify the IC₅₀ values.

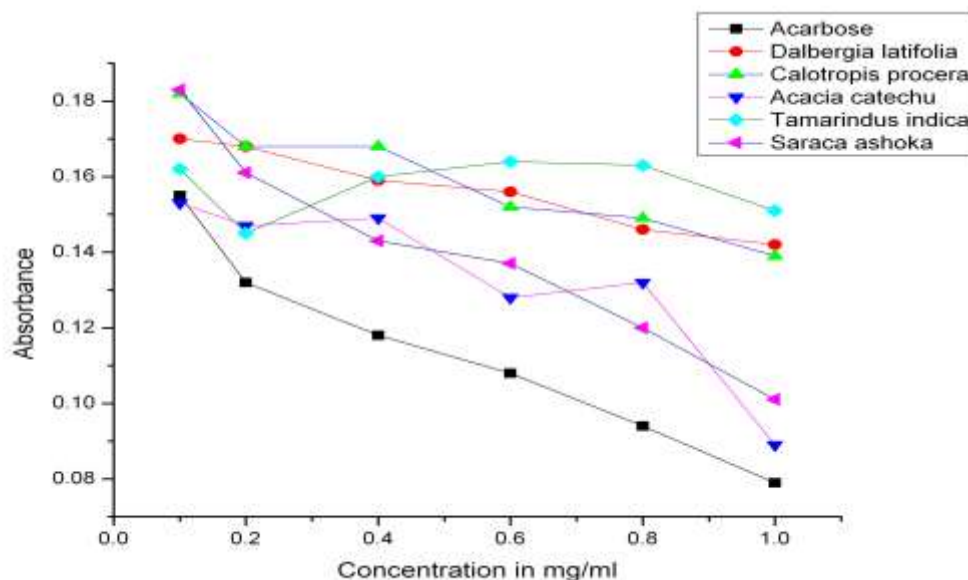
Studies have also reported that the powdered seeds of *A. catechu* exhibit antihyperglycemic action by increasing insulin secretion in non diabetic rats⁷⁵. Sunil Kumar reviewed that the bark and heartwood of *A. catechu* mainly used for the treatment of diabetes⁷⁶. Roy et al 2005⁷⁷ demonstrated that *C. procera* showed antioxidant and antihyperglycemic effect against alloxan induced diabetes in rats. Rahmatullah et al.⁷⁸ (2010) reported that administration of leaves of *C. procera* also serves as an effective way to bring blood sugar in diabetic patients under control, when given in high dose.

Heartwood extracts of *D. odorifera* confirmed a significantly potent inhibition on yeast α -Glucosidase in a dose dependent manner when p-nitrophenyl- α -D-glucopyranoside was used as a substrate *in vitro*⁷⁹. *S. asoca* flowers indicated significant inhibitory potential against α -Glucosidase and α -Amylase⁸⁰.

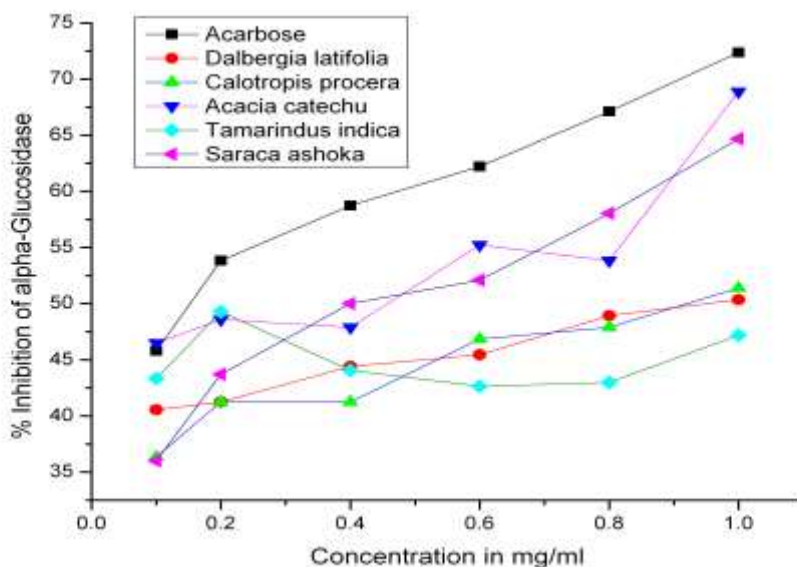
α -Glucosidase inhibition activity (IC₅₀ values) of selected plant leaves are tabulated in Table 3. The lowest IC₅₀ shows the most potent α -Glucosidase inhibition i.e. *A. catechu* (0.351 mg/ml), *S. asoca* (0.490 mg/ml), *C. procera* (0.898 mg/ml) and *D. letifolia* (0.947 mg/ml), while *T. indica* shows moderate α -Glucosidase inhibition activity.

Table 3- ic₅₀ values of acarbose and selected plant extracts.

S.No.	Plants Name (Botanical name)	IC ₅₀ Value α -Glucosidase inhibition
1	Acarbose	0.140 mg/ml
2	Kattha (<i>Acacia catechu</i>)	0.351 mg/ml
3	Ashoka (<i>Saraca ashoka</i>)	0.490 mg/ml
4	Madar (<i>Calotropis procera</i>)	0.898 mg/ml
5	Sheesham (<i>Dalbergia latifolia</i>)	0.947mg/ml
6	Emali (<i>Tamarindus indica</i>)	Nil



Graph 4- Representing Graph Between Absorbance And Concentration Of Plant Leaf Extracts In mg/ml (Inhibition Of Alpha-Glucosidase)



Graph 5- representing graph between % inhibition of α -glucosidase and concentration in mg/ml.

3.4 Statistical Analysis between Polyphenolic content and Biological activity:

Recent studies have shown that there is a positive relationship between total polyphenols and antioxidant activity⁸¹. As we gone through the various literatures regarding to this study, we concluded that polyphenolic contents and flavonoids have the potential to inhibit free radical scavenging activity and α -Glucosidase inhibition⁸².

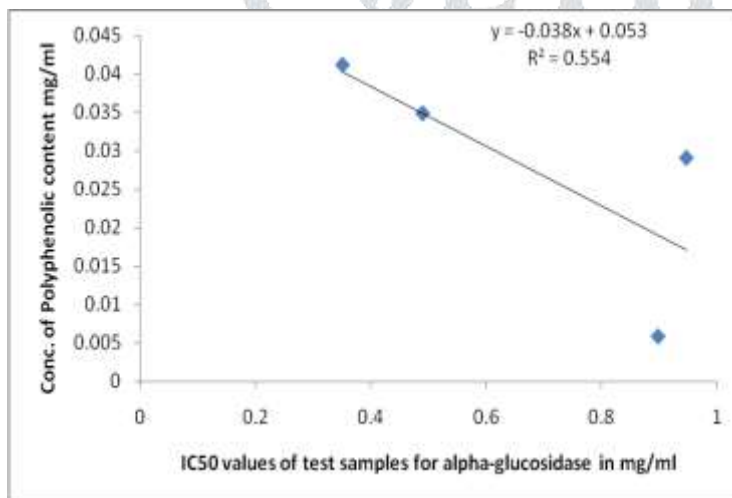
In this study, we are statically analysing the correlation between polyphenolic content and biological activity. Biological activities (Free radical scavenging and α -Glucosidase inhibition) shows dose dependent inhibition, so we are correlating the IC₅₀ values of test samples with polyphenolic contents (Table 4).

Table 4- Correlation Between Total Polyphenolic Contents And Ic₅₀ Value Of Biological Activities Of Selected Medicinal Plants (Mg/MI)

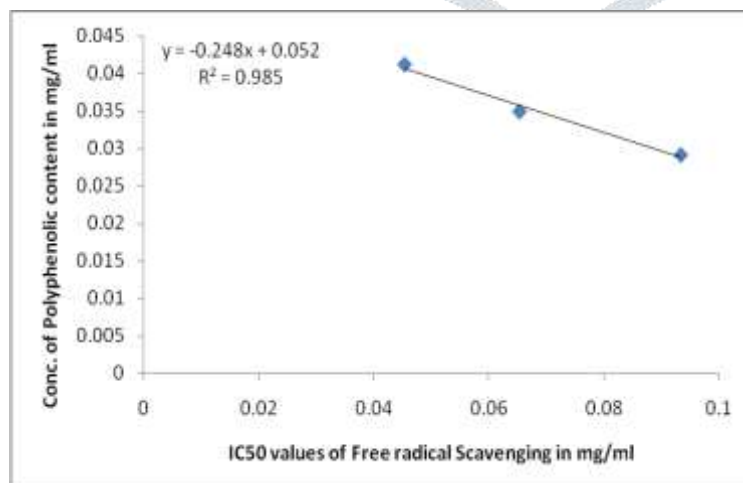
S.No.	Plants Name (Botanical name)	Total Polyphenolic Content (mg/ml)	IC ₅₀ value α -Glucosidase	IC ₅₀ value DPPH free radical
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			inhibition (mg/ml)	scavenging (mg/ml)
1	Kattha (<i>Acacia catechu</i>)	0.0412	0.351	0.0453
2	Ashoka (<i>Saraca ashoca</i>)	0.0349	0.490	0.0653
3	Seesham (<i>Dalbergia latifolia</i>)	0.02911	0.947	0.0934
4	Emali (<i>Tamarindus indica</i>)	0.0107	-	-
5	Madar (<i>Calotropis procera</i>)	.0059	0.898	-

Graph 6 presents the correlation between the polyphenolic content and α -Glucosidase inhibition. This plot clearly shows that the Pearson correlation between these two parameters are positively correlated with each other, a poor regression line having the value of $R^2 = 0.554$ indicates that this is due to various degree of polyphenolic content found in different plant extracts. *C. procera* also represents some degree of inhibition of α -Glucosidase. It may be due to presence of toxic substances.



Graph 6- correlation between polyphenolic content and ic_{50} value of α -glucosidase



Graph 7- correlation between polyphenolic content and ic_{50} value of free radical scavenging activity

Graph 7 presents the correlation between polyphenolic content and IC₅₀ free radical scavenging activity of plant extracts. This plot also shows a positive correlation between each other and linearly regression line having the value R²= 0.985 which reveals that free radical scavenging activity are dependent on the amount of polyphenolic contents directly.

IV. CONCLUSION:

The designed method (open reflection) for extracting the phytoconstituents from powdered leaves of plant is best extraction method because it consumes less amount of solvent. All the extracts were subjected to dose dependent studies to calculate IC₅₀ values of different pharmacological activities. We obtained that selected traditional plants is rich in phenolic compounds and demonstrates positive free radical scavenging activity and α -Glucosidase inhibition activity. Methanolic extracts of Kattha (*A. catechu*) leaves exhibited highest total polyphenolic contents which is directly proportional to free radical scavenging activity and also play the potential α -Glucosidase inhibitory effect. Whereas Seesham (*D. latifolia*) and Ashoka (*S. asoca*) contain total polyphenolic constituents in mid range, showed mild free radical scavenging activity and α -Glucosidase inhibition activity. Email (*T. indica*) and Madar (*C. procera*) possess lowest polyphenolic contents, showing very poor antioxidant activity. In contrast, Madar (*C. procera*) shows moderate α -Glucosidase inhibition activity which might be due to presence of some toxic substances. Through this study we are pointing out that there is a significant correlation between total polyphenols and biological activity (antioxidant activity and α -Glucosidase inhibition) of medicinal plants.

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ABBREVIATIONS:

DPPH, 2, 2-Diphenyl-1-picrylhydrazyl; FC Reagent, Folin-Ciocalteu Reagent; ROS (Reactive oxygen species); DMSO, Dimethyl sulphate; A. catechu, *Acacia catechu*; C. procera, *Calotropis Procera*; T. indica, *Tamarindus indica*; D. latifolia, *Dalbergia latifolia*; S. asoca, *Saraca asoca*.