# In Vitro Antidiabetic, Free Radical Quenching Effect and Phytochemical Profiling of Shankhpushpi With Special Reference To Chitrakoot Region

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**ABSTRACT**

Traditionally medicinal plants and herbs have always been a potential source to cure various diseases as well as in the fields of agriculture, veterinary etc. Several phyto-chemical and pharmacological investigation have clearly shown multidisciplinary usages of these plant derived medicine. Development of organic phyto-pharmaceutical agents is very interesting creation in field of medicinal phyto-chemistry and these phyto- agents are being used to treat various chronic diseases with the help of essential bioactive compounds. In the present research tenure represents the *in vitro* quenching of radicals (DPPH/ABTS) & enzyme (α glucosidase) by 80% hydro-methanolic extract of Shankhpushpi(*Convolvulus pluricaulis*), also have been analyzed for their total polyphenolic and flavanoidic content. These study points out that Shankhpushpi have potent antiradical and antidiabetic agents.

**KEY WORDS :**Shankhpushpi, medicinal plant, phytochemical, antidiabetic activity.

## INTRODUCTION

The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind health in all parts of the world (Malviya et al.,2010). Hyperglycemia is state of higher blood glucose level in body. It is cause by insulin not to activity of key anabolic hormone known as insulin, which is secreted in response to increased blood glucose level for control post prandial hyperglycemia (Bhowmik et al., 2009). The acquiescence of disturbed glucose and insulin metabolism respond in development of type 2 diabetes mellitus and cardiovascular disease, has given rise to the concept of the metabolic syndrome(Pandeya et al.,2013). Pancreatic/intestinal enzymes including α-amylase/α-glucosidase play a key action in glucose metabolism catalyze hydrolyzing dietary starch and other polysaccharides which then breakdown to monosachharides like glucose and fructose prior to absorption in whole blood stream.

Glucose homeostasis is the solution for the treatment of Diabetes(Kaskoos RA., 2013). An effective antidiabetic compound should posses both antihyperglycemic and free radicals scavenging properties, with minimal or no side effects(Griffiths D W & Moseley G 1980).Acarbose-like drugs, competitive inhibitors of α-glucosidase present in the epithelium of small intestine, have been established to reduces postprandial digestion, and improve glycemic index without promoting insulin secretion in NIDDM patients. But this type of medication has serious side/adverse effects(Suvarchala Reddy., 2010 and Verma et al., 2012).

In diabetic patients oxidative stress caused by uncontrolled glucose metabolism. The potentially reactive derivatives of oxygen and nitrogen radicals continuously produced in cells, are detoxified by free radical scavengers and antioxidants(Tripathi et al., 2014). Hence mitigation of oxidative stress or α-glucosidase activity play a vital role to prevent diabetes mellitus and its compilations. A compound with free radical quenching effect combined with inhibitory activities against α-glucosidase may be a more effective antidiabetic agents, with minimal or no side effect with minimal or no side effects (Griffiths D W & Moseley G., 1980).

*Convolvulus Pluricaulis* Choisy. also known as Shankhpushpi belongs to family convolvulaceae, is a perennial herb that seems like morning glory. All parts of the herb are known to possess therapeutic benefits. The plant is used locally in Indian and Chinese medicine to cure various diseases. It is an important herb in Ayurveda for the therapy of chronic cough, sleeplessness, epilepsy, hallucinations, anxiety, liver disease, microbial disease, cytotoxic & viral diseases, central nervous system disease and its crude extract or isolated bioactive compound exhibited in vitro & in vivo pharmacological effect like CNS depression tranquillizing, antidepressant, antistress, neurodegenerative, antiamnesic, antioxidant, anticatatonic activity etc (Agarwa et al., 2014).

Current research was designed to investigate phytochemical profile of Shankhpushpi (leaves, stem, flower & root) and this study also undertaken to evaluate free radical quenching activity and α-glucosidase inhibitory effects of separated parts of shakhpushpi.

## MATERIAL AND METHOD

### Sample collection of Plant Materials

Different parts including Leaves, Stem, Flower & Root of Shankhpushpi (*Convolvulus Pluricaulis*)were collected in March 2017 from Chitrakoot region and identified. All plant parts were collected, washed with fresh water and dried under

shade at room temperature separately. Selected parts were powdered and stored separately in sterile and air tight container for further research.

**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) 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.

**Preparation of Plant extracts**

100 mg powdered sample of plant parts were extracted with 10 ml HPLC grade 80% hydro-methanol through open air reflux process at 40°C for 6 hours till dried than make the volume again 10 ml with hydro-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 dissolve with 10 ml with DMSO and preserved at 4-5°C for further process.

**Phytochemical profiling (Primary screening)**

Qualitative estimation of phytochemicals in selected parts of Shankhpushpi under study were carried out in different organic solvent extracts (double distilled $H_2O$, Methanol & Petroleum Ether), which were prepared by using standard method of Tripathi et al., 2017. The extracted samples were screened for carbohydrates, alkaloids, flavonoids, proteins resin, anthocyanin & betacyanin, saponin, steroid & phytosterol, tannin, galactotannin, starch, glycoside, phenol, phlobatannin, terpenoid using our previous reported methods (Tripathi et al., 2017).

**Determination of Total Polyphenolic Content**:

Total polyphenolic content of extracts of plant leaves was measured using Folin-Ciocalteu reagent method, adopted as it is described by Tripathi et al., 2014. 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 $Na_2CO_3$ (20% w/v) incubated at 45ºC for 60 min absorbance was measured spectrophotometrically at 765nm (Synergy $H_4$ multimode micro plate reader, biotek instrument, inc Winoosci, VT,USA).quantification was performed with respect to the standard curve of Catechol. (y= 0.018X+0.045, $R^2$ =0.998) results were expressed as milligram of catechol equivalent per ml of extract (Tripathi et al., 2014).

**Determination of Total Flavonoid contents:**

Basic principle of aluminum chloride method: The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C4 keto group and both C3 & C5 hydroxyl group of flavones and flavonols. In addition it also forms acid liable with ortho-dihydroxyl groups in the A- or B- ring of flavonoids. Standard quercetin solutions of various concentrations were used to build up the calibration curve.Total flavonoid in the plant extracts, in brief, 50 µl of sample, followed by 50µl of $AlCl_3.6H_2O$ in ethanol and 25µl Sodium acetate, solution added. The absorbance at 430nm was taken (BioTek$^{synergyH4}$ multi-mode microplate reader, BioTek Instruments, Inc Winooski, VT, USA), after 2.5 h of incubation at $20^0$ C. Total flavonoid contents were calculated with respect to the standard curve of the flavonoid quercetin dehydrate (Y=0.017x+0.025, $R^2$=0.941). Results were expressed as micrograms of quercetin dehydrate equivalents (QE) per ml of the extract (Shoib A. B. & Shahid A. M., 2015).

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**Determination of Total Flavonol contents:**

Flavonol content in the sample (100 times diluted with methanol) was measured by mixing equal volume of plant extract with 2% $AlCl_3.6H_2O$ in a 96 well plate. Absorbance was recorded at 420 nm spectrophotometrically (BioTek$^{synergyH4}$ multi-mode microplate reader, BioTek Instruments, Inc Winooski, VT, USA). Flavonol contents in the extracts were determined with respect to the standard curve of the flavonoid quercetin (Y=0.017x+0.025, $R^2$=0.941). Results were expressed as micrograms of quercetin equivalents (QE) per ml of the extract (Miliauskas G. et al, 2004).

**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., 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 decolonization was recorded at 517 nm on a Biotek Synergy $H_4$ 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= 0.840x + 10.97, $R^2$= 0.968). Results were expressed as milligrams of ascorbic acid equivalent per ml of the extract (Tripathi et al., 2013).

**ABTS free radical scavenging assay**

Method of ABTS was adopted from Pandeya et al., 2016. This assay is based on the ability of different substances to scavenge 2-2' azinobis (ethylbenzthiazoline-6- sulfonic acid) or ABTS radical cation. In its radical form, ABTS has a characteristic absorbance at 734nm which disappears after its reduction by an antiradical compound. Reduction of blue-green

ABTS radical coloured solution by hydrogen donating antioxidant is measured by the suppression of its characteristic long wave (734nm) absorption spectrum. The ability to test samples to scavenge ABTS radical cation was compared to ascorbic acid standard. The ABTS radical cation was regenerated by mixing 7Mm stock solution with 2.45 Mm potassium persulphate and incubating for 18 hours in dark at room temperature until reaction was complete and absorbance of ABTS cation solution was 0.637 (± 0.02) by diluting water at room temperature then 20 µl of test sample with different concentrations were mixed with 180 µl of ABTS solution and absorbance was measured at 734 nm after 5 minutes. Quantification was performed with respect to the standard curve of ascorbic acid (Y=0.755x+17.50, $R^2$=0.985). The ABTS scavenging capacity of the test samples was compared with ascorbic acid and percentage inhibition calculated as:

**ABTS radical scavenging activity (%)** = $\frac{(A_0 - A_1)}{A_0} \times \mathbf{100}$

Where $A_0$= Absorbance of control, $A_1$ = Absorbance of sample. All the determinations were performed in triplicates (n=3). (Pandeya et al., 2016)

**Determination of α-Glucosidase Inhibition activity**

Method for determination of α-Glucosidase were adopted from Tripathi. et al., 2013. Rat intestinal acetone powder (Sigma chemicals,USA) was sonicated properly in normal saline (100:1 w/v) and after centrifugation at 3000 rpm × 30 mins the supernatant was treated as crude intestinal α-glucosidase. 50 µl microliters of test samples dissolved in DMSO ( 5mg /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 spectrophotometerically (Synergy $H_4$ multimode micro plate reader, biotek instrument, inc. Winoosci, VT, USA). All the samples were run in triplicate and acarbose was taken as standard refrence compound. Severaral dilutions of primary solution (5mg/ml DMSO) were made and assayed accordingly to obtain concentration of the test sample required to inhibit 50 % activity ($IC_{50}$) of the enzyme. Quantification was performed with respect to the standard curve of acarabose (Y= 0.638x+11.94, $R^2$= 0.974) results were expressed as milligram of Acarbose equivalent per ml of extract. Percent α-glucosidase inhibition was calculated as using following equation:

**Percent α-Glucosidase inhibition (%) = $A_0$-$A_1$/$A_0$×100**

Where $A_0$ was the absorbance of the control and $A_1$ was the absorbance in the presence of the sample.(Tripathi. et al., 2013)

**Statistical Analysis**

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

**RESULT**

Herbal medicines are being used since prehistoric times which are widely accepted in advance therapeutics and their utilization stimulated the trend of using of natural products in treatment of various diseases. For present study different parts of Shankhpushpiwere collected from the local area of Chitrakoot (University campus, Rajulla farms & Deendayal Research Institute) according to their therapeutic value and seasonal availability. It was make sure that plants were healthy and uninfected.

Chitrakoot is a holy place for Hindu pilgrimage, which is situated in the northern region of Satna district of Madhya Pradesh. It has a very rich wealth of medicinal plants, which has also been described in our epics like Ramayana.

वस्वौकसारां नलीनीमतीत्यैवोत्तरान् कुरून्।

पर्वतश्चित्रकूटोऽसो बहुमूलफलोदकः।।26।। (26) Valmiki Ramayana

निशि भान्त्यचलेन्द्रस्य हुताशनशिखा इव।

औषध्यः स्वप्रभालक्ष्म्या भ्राजमानाः सहस्त्रशः।।21।। (21) Valmiki Ramayana

According to Valmiki Ramayana, Chitrakoot is a beautiful and sacred place where different types of herbs, shrubs, trees and climbers bearing variety of fruits, flowers and roots are available. Great saint Valmiki and Goswami Tulsidas illustrated a comprehensive account of biodiversity in their texts Ramayana and Ramcharitmanas respectively (Tripathi et al., 2015).

According to Maharshi Valmiki, Kamadgiri hill is rich in diverse plants. These are Aam (*Mangifera indica*), Jamun (*Syzygium cumini*), Asna (*Lagerstroemia indica*), Lodh (*Symplocos racemosa*), Chironji (*Buchanania lanzan*), Kathl (*Artocarpus heterophyllus*), Dhawa (*Anogeissus lantifolia*), Dhak (*Butea monosperma*), Ankol (*Allangium salvifolium*), Bhavya (*Dillenia indica*), Tinsa (*Ougenia oogeinensis*), Bel (*Aegle marmelos*), Tendu (*Diospyros melanoxylon*), Bans (*Dendrocalamus strictus*), Kasmri (*Gmelina arborea*), Neem (*Azadirachta indica*), Sakhua (*Shorea robusta*), Barun (*Crateva unilocularis*), Mahua (*Madhuka longifolia*), Tilak (*Wendlandia exerta*), Ber (*Zizyphus mauritiana*), Anola (*Phyllantus emblica*), Kadamb (*Anthocephalus chinensis*), Bent (*Calamus rotung*), Indrajau (*Holarrhena pubescens*), Bijak (*Punika granatum*) and Neebu (*Citrus aurantifolia*) with other flowering fruiting and shade giving trees (Sikarwar, 2011).

In addition, Chitrakoot has a rich wealth of medicinal plants too i.e. *Tinospora cordifolia, Gymnema sylvstre, Achyranthes aspera, Urginea indica, Curculigo orchioides, Dioscorea bulbifera, Desmodium gangeticum, Coccinia grandis, Cordia macleodii, Litsea glutinosa, Oroxylum indicum, Steriospermum suaveolens, Pterocarpus marsupium, Terminalia arjuna, T. Bellirica, T. Chebula, Actinopteris radiate, Cyperus rotundus, Vernonia cinerea, Sida cordifolia, Ampelocissus latifolia, Peristrophe paniculata, Cassia tora, Diplocyclos palmatus, Tridex procumbens, Phyllanthus fraternus, Elytraria acaulis,*

*Solanum nigrum, Termnus labialis, Vitex negundo, Abutilon indicum, Cocculus hirsutus, Hemidesmus indicus, Enicostemma hyssopifolium, Boerhavia diffusa, Solanum virginianum, Helicteres isora, Aegle marmelos, Allangium salvifolium* etc (Sikarwar, 2011).

Qualitative estimation phytochemicals of extracts of leaves, Stem, Flower & Root of *Convolvulus pluricaulis* was reported in Table.1. For phytochemical screening, water, methanol and petroleum ether extracts were used. Preliminary phytochemical tests indicate the presence or absence of various phytoconstituents in a given plant sample. The presence of carbohydrate Protein Phenol alkaloids, tannins, flavonoids, steroids and reducing sugars was observed in all the selected plants parts extracts except phlobatannins, & terpenoids all these bioactive compounds were found to be present in almost all the studied samples.

**Table 1: Phytochemical Screening of Shankhpushpi**

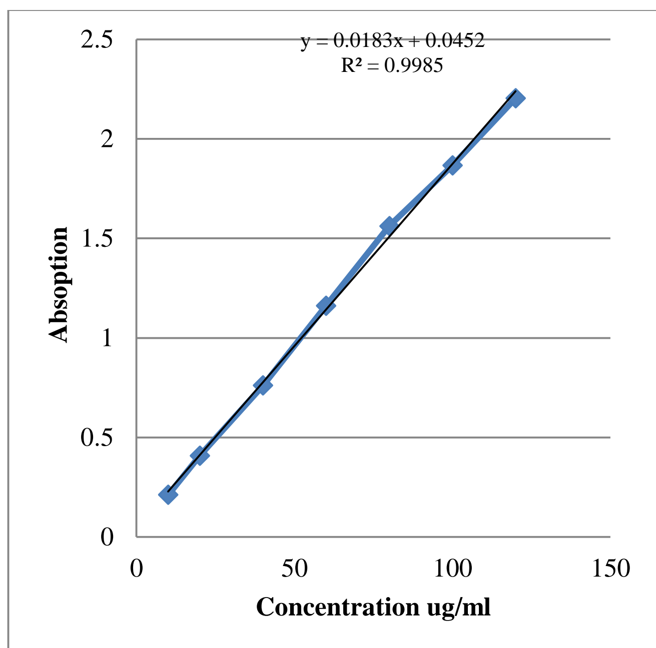
| S. No. | Phytochemicals | leaves | | | Stem | | | flower | | | root | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | W | M | PE | W | M | PE | W | M | PE | W | M | PE |
| 1. | Carbohydrate | + | + | - | + | - | - | + | + | - | - | - | - |
| 2. | Alkaloid | + | + | + | + | + | + | + | + | + | + | + | + |
| 3. | Flavonoid | + | + | + | + | + | - | + | - | - | - | - | - |
| 4. | Protein | - | - | + | + | + | - | - | - | - | - | - | - |
| 5. | Resin | + | + | + | + | + | - | + | + | + | + | - | + |
| 6. | Anthocyanin | + | - | - | + | - | - | - | - | - | - | - | - |
| 7. | Saponin | + | - | - | + | - | - | + | - | - | + | - | - |
| 8. | Steroid | - | - | - | - | - | - | + | + | + | - | - | + |
| 9. | Tannin | + | + | + | + | + | + | + | + | + | + | + | + |
| 10. | Starch | + | - | - | - | - | - | - | - | - | - | - | - |
| 11. | Glycoside | - | + | + | - | - | + | + | + | + | - | - | - |
| 12. | Phenol | + | + | + | + | + | + | + | + | + | - | + | + |
| 13. | Phlobatanin | - | - | - | - | - | - | - | - | - | - | - | - |
| 14. | Terpenoid | + | - | - | - | - | - | + | - | - | - | - | - |

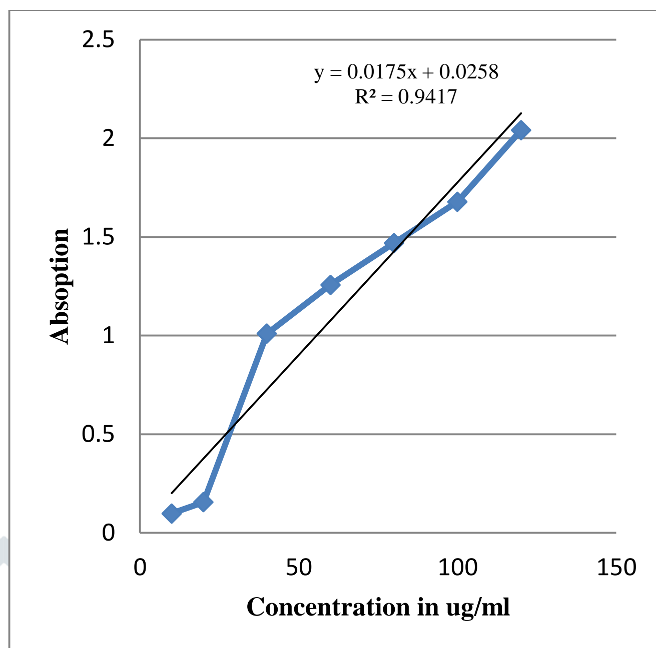
W = water extract, PE = Petroleum ether extract, M = Methanolic extract

Presence of TPC in plant extract was based on the absorbance of selected plant parts and Folin-Ciocalteau reagent mixture at 725 nm. Since Folin-Ciocalteau assay measures all the phenolics, the choice of catechol as standard is based on the availability of a stable and pure chemical.

**Table 2: Represent Total Polyphenolic content (TPC)**

| S. No. | Name of Plant's Part | TPC equivalent to Catechol µg/ml ± SD | TFC equivalent to Qurecitine µg/ml ± SD | TFLC equivalent to Qurecitine µg/ml ± SD |
|---|---|---|---|---|
| 1 | Leaf | 7.28±0.0002 | 4.35±0.001 | 1.17±0.002 |
| 2 | Stem | 28.72±0.001 | 7.5±0.004 | 0.64±0.001 |
| 3 | Flower | **38.22**±0.0004 | **31.470**±0.0002 | **6.1**±0.001 |
| 4 | Root | 5.72±0.002 | 3.7±0.004 | 0.412±0.003 |




**Graph 1 : Standard Curve Of Catechol For Estimation Of TPC**

**Graph 2 : Standard Curve Of Querecitine For Estimation Of TFC & TFLC**

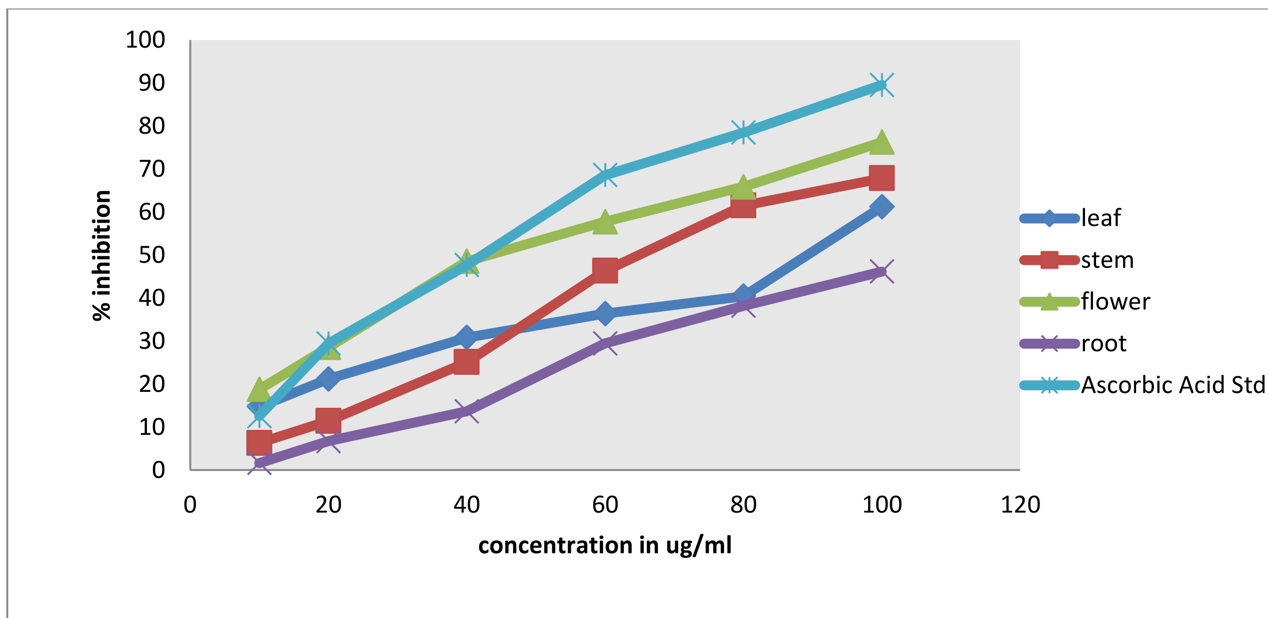
Graph-1 shows the standard curve of catechol, which is used for the quantification of total polyphenolic contents equivalent to catechol µg/ml in methanolic extracts of selected plant samples. Table-1 presents the concentration of total polyphenolic content in catechol equivalent µg/ml. In the present investigation concentration of TPC of plants were ranged from 5.72±0.002µg/ml to 38.22±0.0004 µg/ml. Highest concentration of polyphenols was shown by Flower's hydro-methanolic extract (38.22±0.0004 µg/ml), while in stem, it was found to be 28.72±0.001µg/ml. Presence of polyphenolic contents in all the sample was observed in our preliminary phytochemical screening studies. All the selected plat parts showed positive results for the test for phenols.The amount of total flavonoid & total flavonol compounds that are present in same table 1. A standard curve was plotted to quantify Favonoid/flavonolic compound was reported equivalent to Qurcetin µg/ml in methanolic extract of selected plant samples, as the concentration of the extract increases, the mean absorbance value also increases.The amount of TFC & TFLC exhibited by hydr-methanolic flower's extract 31.470±0.0002 µg/ml & 6.1±0.001 µg/ml ) was higher compared with other selected plant parts extracts Graph 2 represent the standard curve of querecitin.

DPPH& ABTS scavenging activity (% inhibition) are shown in respectably table 3 & 4 and statistical results of the data are shown in graph 3& 5. Graph 4 shows the standard curve of ascorbic acid. This test is based on the ability of the stable 1, 1'- diphenyl 2- picryl hydrazyl (DPPH) and ABTS free radical to decolorize in the presence of free radicals. In our investigation DPPH & ABTS radical scavenging assay protocol, ascorbic acid was used as reference standard, 80% methanol was used a blank, while DPPH & ABTS solution without sample served as a control. Percentage inhibition was calculated and a curve between % inhibition and concentrations were plotted. Linear regression analysis was carried out for calculating the inhibition concentration of sample required to scavenge DPPH & ABTS radicals by 50% ($IC_{50}$ value). $IC_{50}$ value of standard ascorbic acid is calculated to be 46.464µg/ml. Table -6 shows the $IC_{50}$ values of all the samples. When compared to standard ascorbic acid with selected plant parts exhibited good DPPH & ABTS scavenging activity, in terms of $IC_{50}$ values. Highest DPPH & ABTS radical scavenging activity was shown by flower's hydromethanolic extracts ($IC_{50}$ = 52.850 µg/ml & 47.6µg/ml).The total phenolic content for 50, 100 and 200 µg/ml extracts was 0.2092, 0.2380 and 0.3608 mg GAE/ gram. Free radicals quenching activity of methanolic extract of *Convolvulus pluricaulis* and the standard was found to be highest at 100ug/ml which was 52.56% and 93.48% respectively, reported by Balaji et al., 2014.

**Table 3: % inhibition of DPPH scavenging activity of 80%hydro- methanolic extracts of Shankhpushpi; Leaves, Stem, Flower, Root and ascorbic acid**

| S. No. | Conc. in | ( % of free radical (DPPH) Scavenging) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ascorbic | Error±SD | leaf | Error± | stem | Error± | flower | Error± | root | Error±SD |

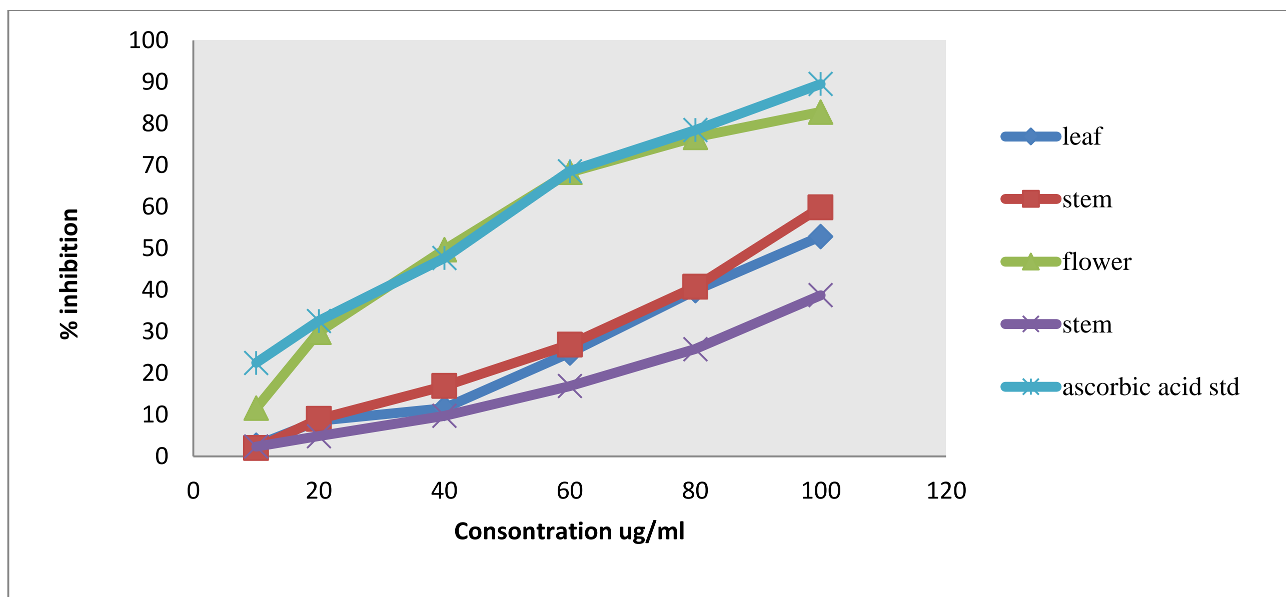
| | μg/ml | Acid | | | SD | | SD | | SD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 12.56 | 0.0004 | 14.74 | 0.006 | 6.32 | 0.0002 | 18.76 | 0.000 | 1.65 | 0.0007 |
| 2 | 20 | 29.45 | 0.003 | 21.2 | 0.000 | 11.43 | 0.0004 | 28.56 | 0.0002 | 6.77 | 0.0003 |
| 3 | 40 | 47.68 | 0.0003 | 30.82 | 0.0000 | 25.112 | 0.005 | 48.57 | 0.0001 | 13.69 | 0.0002 |
| 4 | 60 | 68.59 | 0.002 | 36.39 | 0.0000 | 46.32 | 0.0006 | 57.756 | 0.0006 | 29.473 | 0.0002 |
| 5 | 80 | 78.47 | 0.002 | 40.45 | 0.0005 | 61.503 | 0.0026 | 65.867 | 0.0004 | 38.195 | 0.001 |
| 6 | 100 | 89.54 | 0.001 | 61.2 | 0.0004 | 67.82 | 0.0006 | 76.25 | 0.0004 | 46.165 | 0.000 |




**Graph 3: Representing graph between % of free rdical (dpph) scavenging and concentration.**

**Table 4: %inhibition of ABTS scavenging activity of 80% hydro- methanolic extracts of Shankhpushpi; Leaves, Stem, Flower, Root and ascorbic acid**

| S.No. | Conc. in μg/ml | ( % of free radical (ABTS) Scavenging) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ascorbic Acid | Error± SD | leaf | Error± SD | stem | Error± SD | flower | Error± SD | root | Error±SD |
| 1 | 10 | 12.56 | 0.0003 | 2.67 | 0.001 | 1.98 | 0.002 | 11.58 | 0.000 | 2.35 | 0.0002 |
| 2 | 20 | 29.45 | 0.000 | 8.56 | 0.0002 | 8.97 | 0.0012 | 29.87 | 0.0002 | 4.87 | 0.0004 |
| 3 | 40 | 47.68 | 0.000 | 11.57 | 0.0004 | 16.9 | 0.0004 | 49.67 | 0.0002 | 9.76 | 0.0004 |
| 4 | 60 | 68.59 | 0.0004 | 24.87 | 0.0004 | 26.87 | 0.0004 | 68.29 | 0.0007 | 16.9 | 0.0006 |
| 5 | 80 | 78.47 | 0.0002 | 39.86 | 0.0005 | 40.76 | 0.004 | 76.73 | 0.0006 | 25.79 | 0.0005 |
| 6 | 100 | 89.54 | 0.0004 | 52.87 | 0.000 | 59.87 | 0.0002 | 82.8 | 0.0002 | 38.76 | 0.0002 |



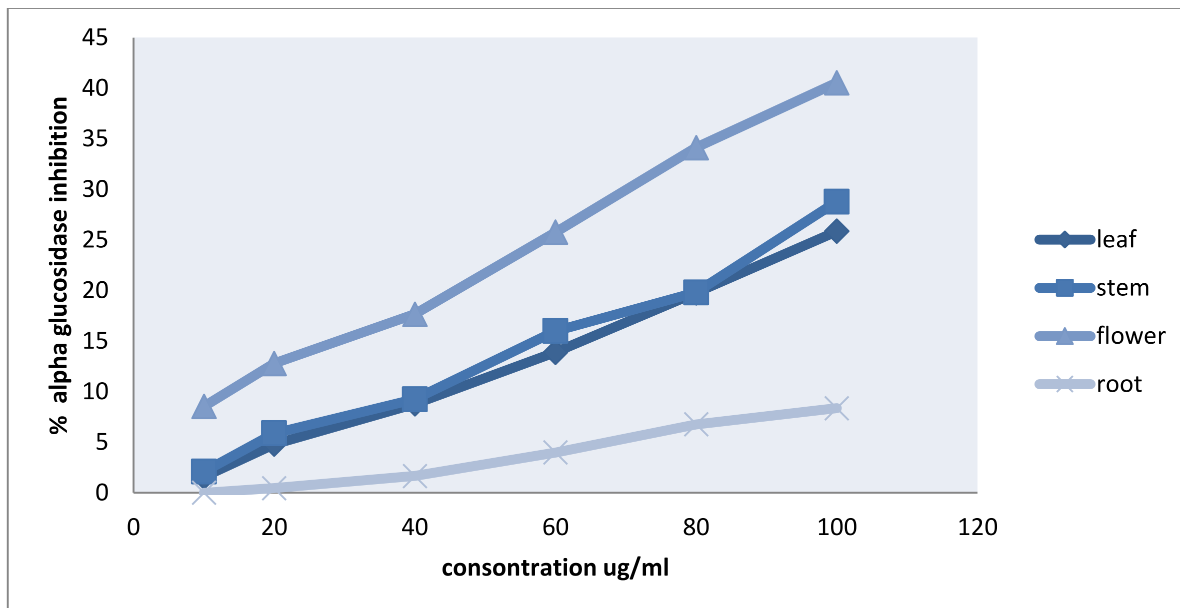

**Graph 4: Representing graph between % of ABTS scavenging and concentration.**

Alpha glucosidase inhibition activity are shown in table 5. Graph 6 presents the statistical analysis of data. Graph 5 shows the standard curve of acarbose. Glycosidases widely spread in microorganisms, plants and animals, are a very important class of enzymes. The hydrolysis of glucosidic linkage catalyze by these types of enzyme, thereby degrading oligosaccharides and glycoconjugates. Among these glycosidases, alpha glucosidase is able to catalyze the cleavage of glycosidic bonds involving terminal glucose connected at the site of cleavage through a linkage at the anomeric center.Glycosidases are involved in a number of principle biological processes such as digestion, biosynthesis of glycoprotein, lysosomal catabolism of glycoproteins and lysosomal catabolism of glucocenjugates, which are related to many metabolic disorders such as diabetes, obesity, glycosphingolipid lysosomal storage disease, HIV and tumors (Sancheti S et al., 2014). Several alpha glucosidase and alpha amylase inhibitors have been isolated from plants to act as replacement drugs with increased potency and lesser deleterious effects, than existing synthetic drugs (Kazeem et al 2013). Delay of carbohydrate absorption with a plant based alpha glucosidase inhibitor offers a prospective therapeutic approach for the management of type II diabetes mellitus(Reddy N.V.L.S, 2010).

In our experiments, reference standard acarbose showed an $IC_{50}$ value of 59.65 µg/ml (table 6). Compared to acarbose, some of the extracts exhibited very effective inhibition, and highest was observed in flower's hydromethanolic extract ( $IC_{50}$ value is127.55µg/ml).

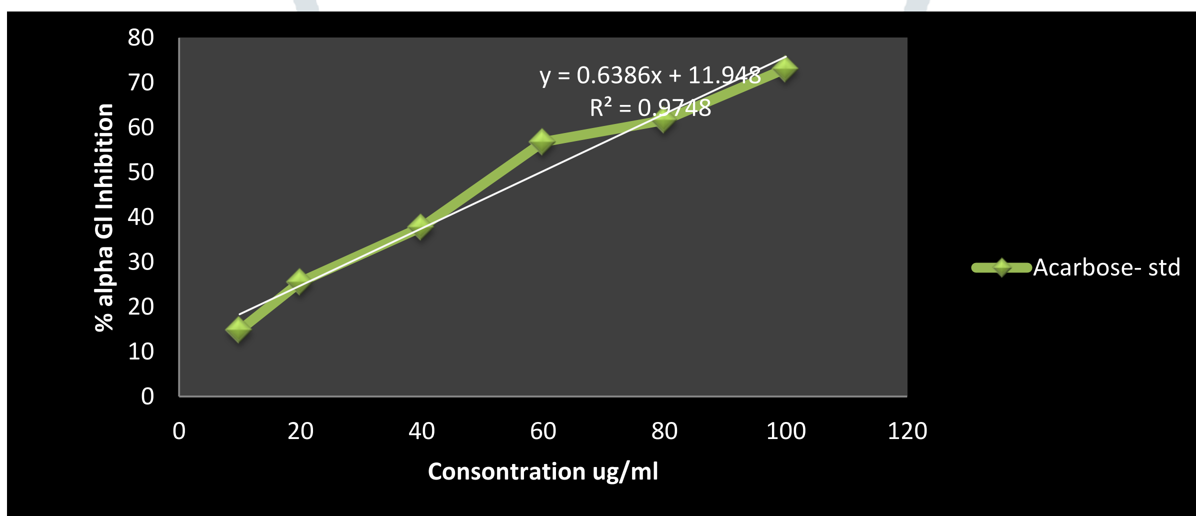
**Table 5: % inhibition of Alpha GI scavenging activity of 80% hydro- methanolic extracts of Shankhpushpi; Leaves, Stem, Flower, Root and Acarbose**

| S. No. | Conc. in µg/ml | ( % of alpha Glucosidase enzyme Scavenging) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Acarbose | Error±SD | leaf | Error± SD | stem | Error± SD | flower | Error± SD | root | Error±SD |
| 1 | 10 | 14.834 | 0.004 | 1.57 | 0.000 | 2.077 | 0.000 | 8.533 | 0.0004 | 0 | 0.0004 |
| 2 | 20 | 25.56 | 0.0003 | 4.8 | 0.000 | 5.87 | 0.001 | 12.78 | 0.000 | 0.45 | 0.000 |
| 3 | 40 | 37.764 | 0.0024 | 8.78 | 0.0006 | 9.245 | 0.000 | 17.654 | 0.000 | 1.65 | 0.0006 |
| 4 | 60 | 56.74 | 0.0004 | 13.895 | 0.0004 | 15.987 | 0.000 | 25.765 | 0.0007 | 3.98 | 0.0004 |
| 5 | 80 | 61.65 | 0.000 | 19.765 | 0.000 | 19.8 | 0.002 | 34.12 | 0.000 | 6.76 | 0.000 |
| 6 | 100 | 73.1 | 0.0000 | 25.86 | 0.000 | 28.78 | 0.0004 | 40.54 | 0.000 | 8.354 | 0.0007 |




**Graph 5: Representing graph between % of alpha Glucosidase enzyme inhibition and concentration of selected samples**

.




**Graph 6: Representing graph between % of alpha Glucosidase enzyme inhibition and concentration of acarbose as standard.**

**Table 6: $IC_{50}$ values of samples in µg/ml equivalent to ascorbic acid in DPPH**

| S.N. | Selected plant's part | $IC_{50}$ Value ( µg/ml ± SD) | | |
|---|---|---|---|---|
| | | DPPH | ABTS | Alpha GI |
| 1 | Leaf | 86.62±0.0003 | 99.75±0.0004 | 193.91±0.0001 |
| 2 | Stem | 70.151±0.002 | 91.31±0.002 | 181.62±0.004 |
| 3 | Flower | **52.850±0.001** | **46.67±0.001** | **126.71±0.0024** |
| 4 | Root | 105.095±0.0004 | 137.6±0.001 | 531±0.0004 |

## CONCLUSION

It can be concluded that hydro-methanolic solvent is a more efficient solvent in extracting phyto-constituents. TPC, TFC & TFLC of the hydro methanolic extract of *Convolvulus pluricaulis,*point out that it may account for its free radical scavenging (DPPH &ABTS) and also antidiabetic characteristics. Best antidiabetic and free radical scavenger agents present in flower of shankhpushpi, because of presence of highest amount of phytochemicals in comparison of other selected parts.

## REFERENCES

1. Malviya N., Jain S. and Malviya S., (2010). Antidiabetic potential of medicinal plants. acta poloniae pharmaceutica ñ drug research, Vol 67 (2 ): 113-118.
2. Bhowmik A., Khan L. Ali, Akhter M. and Rokeya B., (2009). Studies on the Antidiabetic Effects of *Mangifera indica* Stem-Barks and Leaves on Nondiabetic, Type 1 and Type 2 Diabetic Model Rats.Bangladesh Journal of Pharma-cology, Vol 4 (2): 110-114.
3. Pandeya K. B., Tripathi I. P., Mishra M. K., Dwivedi N., Pardhi Y., Kamal A., Gupta P., Dwivedi N., Mishra C., (2013). A Critical Review on Traditional Herbal Drugs: An Emerging Alternative Drug for Diabetes.International Journal of Organic Chemistry, 3: 1-22.
4. Kaskoos R. A., (2013). In-vitro α-Glucosidase Inhibition and Antioxidant Activity of Methanolic Extract ofCentaurea calcitrapa from Iraq. Ameri J of Essential Oils and Nat Prod,1(1): 122-125.
5. Griffiths D. W. & Moseley G., (1980), The effect of diets containing field beans of high or low polyphenolic content onthe activity of digestive enzymes in the intestines of rats. J Sci Food Agric, 31**:** 255-259.
6. Suvarchala Reddy N. V. L. , Anarthe Sneha J. and Raghavendra N. M., (2010).*In Vitro* Antioxidant and Antidiabetic activity of *Asystasia gangetica* (Chinese Violet) Linn. (Acanthaceae). International Journal of Research in Pharmaceutical and Biomedical Science, Vol 1 (2): 72-75.
7. Verma S., Sinha R., Kumar P.,Amin F., Jain J., and Tanwar S., (2012).Study of *Convolvulus pluricaulis* for Antioxidant and Anticonvulsant Activity. Central Nervous System Agents in Medicinal Chemistry, 12: 55-59.
8. Tripathi I P.,Mishra M. K., Tripathi R., Mishra C., Pandey K. B. 2014.free radical scavenging activity and total polyphenolic content of selected traditional plant of solanaceae family, Proceedings of the 3rd World Conference on Applied Sciences, Engineering and Technology, WCSET 2014: 047-050.
9. Griffiths D. W.& Moseley G., (1980). The effect of diets containing field beans of high or low polyphenolic content onthe activity of digestive enzymes in the intestines of rats. J Sci Food Agric,Vol 31**:** 255-259.
10. Agarwa P., Sharma B., Fatima A., Jain S. K., (2014). An update on Ayurvedic herb Convolvulus pluricaulis Choisy.Asian Pac J Trop Biomed; 4(3): 245-252.
11. Tripathi I.P., Tripathi R. and Tiwari A., (2017). Investigation of Biologicaly Active Phytoconstituents present in selected Plants Material of Verbenaceae, Lamiaceae and Fabaceae family.International Journal of Multidisciplinary and Current Research, Vol 5: 31-37.
12. Tripathi I. P., Mishra M. K.,Mishra C., Tripathi R., Kamal A., Tripathi P., Shukla V. P., Gangele R., and Pandeya K. B., (2013).Assessment of Antioxidant and Total Polyphenolic Content of Some Plants of Euphorbiaceae Family. Indian Journal of Applied Research, 3 (10): 1-4.
13. Tripathi I.P., Mishra M. K., Tripathi R., Mishra C., Kamal A.,Shastri L., Dwivedi A., Shukla U. K.and Pandeya K. B.,(2014). Synthesis, Spectral, Electrochemical analysis and Screening for α- Glucosidase inhibition of some complexes of cobalt (II) and Ethylenediamine, *Research Journal of Chemical Sciences*, 4 (6): 1-6.
14. Balaji K., Hean K. C., Ravichandran K. and Shikarwar M., (2014).*In-Vitro* Evaluation of Antioxidant Activity and Total Phenolic Content of Methanolic Extract of *Convolvulus pluricaulis.* RJPBCS 5(6): 959-964.
15. Sancheti S., Sancheti S., Lee S-H, Lee J-E. And Seo S-Y., (2011). Screening of Korean medicinal plant extracts for α-glucosidase inhibitory activity. Iranian journal of pharmaceutical research, Vol 10(2): 261-264.
16. Kazeem M.I., Ogunbiyi J.V. and Asnafa A.OT., (2013). In vitro studies on the inhibition of alpha amylase and alpha glusidase by leafextracts of Picralima nitida (Staph).'Tropical journal of pharmaceutical research, Vol.12(5): 719-725.
17. Reddy N.V.L.S., Anarthe S.J. and Raghavendra N.M., (2010). In vitro antioxidant and antidiabetic activity of Asystasia gangetica (Chinese violet) Linn. (Acanthaceae).'International journalof research in pharmaceutical and biomedical sciences. Vol 1(2): 72-75.
18. Pandeya K. B., Tripathi I. P., Mishra M. K. and Jaiswal N., (2016). Antiradical and antioxidant properties of some oxovanadium (IV) complexes of I-amino acids. Journal of biological sciences and medicine, 2, 4(2016): 38-47.
19. Tripathi I. P., Tripathi R., Mishra C., Mishra M. K., (2015). Quantification of total polyphenolic contents & antifree radical potential of selected traditional plants of Chitrakoot region. Life sci inter res j, Vol 2: 16-19.
20. Sikarwar R. L. S., (2011), Chitrakoot forests: A treasure of cultural and biological diversity, National conference on forest biodiversity: earth's living treasure, 22nd May: 62-67.
21. Shoib A. B. & Shahid A. M., (2015), Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume, *Journal of Taibah University for Science***,** Vol 9: 449–454.
22. Miliauskas G., Venskutonis P.R, Beek van T.A, (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts, *Food Chemistry*, Vol 85: 231–237.