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Evaluation of edible herb *Solanum tuberosum* L. to be used as nutraceuticals

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ABSTRACT

The study was designed to explore nutraceuticals from the edible herb *Solanum tuberosum* L. through investigation of antioxidant, antihyperglycemic and antibacterial activities. Antioxidant activity was accomplished using DPPH free radical scavenging and reducing power assay. Oral glucose tolerance test was used to measure antihyperglycemic activity. At last, antibacterial activity was evaluated by disc diffusion method. Phytochemical assay of the plant extract indicated the presence of therapeutically active phytoconstituents. Total polyphenol, flavonoids and tannin contents were 27mg GAE/g, 22mg QE/g and 14mg GAE/g of dried extract respectively. DPPH radical scavenging and reducing power of *S. tuberosum* were comparable to standard antioxidant. A well-built relationship was observed between polyphenol, flavonoid and antioxidant activity of extract. *S. tuberosum* predominantly ($p<0.05$) reduced blood glucose level at 400mg/kg in glucose induced hyperglycemic mice and revealed maximum inhibition against *Enterococcus faecalis* in the antibacterial assay. Due to these beneficial effects these edible herbs may be a good source of nutraceuticals.

Keywords: Antioxidant components, DPPH radical scavenging, Antihyperglycemic, Antibacterial, Nutraceuticals.

INTRODUCTION

Nutraceuticals provide basic nutrition and modify physiologic and pathologic conditions for the benefit of the recipient. Herbs may be the source of functional food due to its significant nutritional components and health-promoting ingredients. Herbs, as well as herbal medicines, are in great demand both in developed and developing countries to promote wellness and for healing purpose. Free radicals are one of the major barriers to maintain a healthy life. Free radicals and reactive agents convertible to free radicals are produced as the part of defense mechanism and the by- products of conventional cellular aerobic metabolism. They act as signaling and regulatory agents at physiological level but hazardous oxidants at pathologic level. At latter, they are responsible for certain life-threatening diseases [1]. By mopping up the free radicals antioxidants prevent radicals induced disorders. Recently food scientists and nutrition specialists have agreed that food antioxidants contribute to the maintenance of good health [2]. Major antioxidants come from foods are vitamins and minerals, trace elements as well as different secondary metabolites especially polyphenols. Fresh or processed foods of edible herbs provide a balanced source of vitamins and minerals. Vitamins directly react with reactive oxygen species and also increase the activities of antioxidant enzymes. Antioxidants also increase the shelf life of food and maintain nutrition level. Reactive oxygen species are the major causes of insulin resistance and diabetes [3]. Several hypoglycemic compounds possess antioxidant properties [4]. Dietary antioxidants from herbs may be an alternative way of prevention or treatment of diabetes and other fatal diseases. Among the South Asian countries, Bangladesh is a rich source of herbs that are consumed as vegetables. *S. tuberosum* of family Solanaceae is an annual or perennial edible herb. The

leaves of it are used to treat spasm, prevent premature aging and heart diseases, diuretic, blood pressure lowering effect, protection against colon cancer, inflammation of the prostate and diabetes in folklore medicine [5]. Though starch is predominantly present in the tuber, it also contains chlorogenic acid, neo-chlorogenic acid, 4-O-caffeoylquinic acid, 3, 5-dicaffeoylquinic acids, L-tyrosine, caffeic acid, scopolin, ferulic acid, anthocyanins, carotenoids and vitamins and minerals [6]. The tuber has reported various pharmacological activities [7]. Therefore, the present study was undertaken to find out natural source of nutraceuticals throw determination of the antioxidant components such as total phenolic, flavonoids and tannin content, antioxidant activity by 2, 2-Diphenyl-1-picryldydrazyl (DPPH) radical scavenging and reducing power assay, correlation of investigated antioxidant components and antioxidant activities and also oral glucose tolerance test and possible antibacterial activity.

MATERIALS AND METHODS

Chemicals and reagents: 2, 2-Diphenyl-1-picryldydrazyl (DPPH·), ascorbic acid, gallic acid, quercetin, butylated hydroxytoluene were purchased from Merck, Germany. Folin-Ciocalteau reagent was purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Glibenclamide hydrochloride was obtained from Square Pharmaceuticals Ltd, Bangladesh. Including the solvents used, all other chemicals, were of analytical grade.

Plant materials and extraction: *S. tuberosum* leaves were collected locally and identified by Bangladesh national herbarium (Acc. No. 38635). After shade drying the plant materials were grinded by a mechanical grinder (Capacitor start motor, Wuhu motor factory, China). Coarsely grinded powder was macerated in 95% ethanol. After filtration and evaporation of solvent, the yield of the extract was 2.7% w/w *S. tuberosum*.

Experimental animals: Swiss-albino mice of 4-5 weeks age and weighing 20-25g were procured from Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. The mice were housed in polypropylene cages under pathogen free condition at an ambient temperature of 24 ± 1^0C; 12 h light/dark cycle with 55 ± 5 % controlled relative humidity. The animals were allowed standard pellet diet and water *ad libitum*.

Test microorganisms: In the antibacterial assay, total twelve pathogenic bacterial strains were used. 6 of them were Gram positive i.e. *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pyogens, Streptococcus agalactiae, Enterococcus faecalis* and six were Gram negative namely *Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Shigella boydii, Escherichia coli* and *Vibrio cholera.* All of the strains were collected from Microbiology Lab of Pharmacy Discipline, Khulna University, Khulna, Bangladesh.

Identification of phytochemical constituents: Different standard qualitative chemical test were carried out to identify different phytochemical constituents such as carbohydrates, alkaloids, glycosides, phenolic compounds, flavonoids, tannins, steroids, gum, saponins and acidic compounds [8].

Estimation of antioxidant components

Total polyphenol content: The total polyphenol content of the extracts was determined by the modified Folin-Ciocalteu method [9]. An aliquot of 0.5 ml extract solution was mixed with 5 ml 1:10 v/v Folin-Ciocalteu reagent. Then 4ml 7.5% w/v sodium carbonate was added to the mixture. The mixture was allowed to incubate in a thermostat at 40^0C for 30 minutes and the absorbance was measured at 765 nm using Shimadzu UV visible spectrophotometer (Model 1800, Japan). The standard calibration curve was prepared using different concentrations (0-1mg/ml) of gallic acid. Based on the measured absorbance of the extract, Gallic Acid Equivalent (GAE) was read (mg/ml) from the calibration line and then total polyphenol content value was expressed in terms of mg of gallic acid equivalent per gram of dry extract.

Total flavonoids content: A well-known and simple aluminum chloride colorimetric method was used to determine the total flavonoids content in the extracts [2]. In 1ml extract solution, 4ml distilled water and 0.3ml 5% w/v sodium nitrate was sequentially mixed. Five minutes later, 0.3ml 10 % w/v aluminum chloride was added to the mixture with continuous shaking. At the sixth minute 2ml 1M sodium hydroxide was added and the volume was adjusted to 10ml. After that, absorbance was taken at 510 nm. For this assay quercetin was used for standard calibration curve. From the calibration curve, total content of flavonoids was expressed as mg quercetin equivalent (QE) per gram of dry plant extract.

Total tannin content: Total tannin in the extracts was determined as reported by Amorim *et al*., 2008. 0.1 ml of the extract solution was added to 7.5 ml distilled water. Then 0.5ml Folin-Ciocalteu reagent and 1ml 35% w/v sodium carbonate were sequentially added to the mixture and finally the

volume was adjusted to l0ml. Before taking absorbance at 725nm the mixture was allowed to stand at ambient room temperature for 30minutes. Gallic acid was used as standard to serve the calibration curve from where quantity of total tannin was estimated and expressed as mg gallic acid equivalent per gram of dry mass.

Evaluation of Antioxidant activity

DPPH free radical Scavenging Assay: DPPH (2, 2-Diphenyl-1-pycrylhydrazyl), a stable and commercially available organic nitric free radical scavenger, was used for qualitative and quantitative estimation of antioxidant activity of the extracts.

Qualitative Analysis: Qualitative free radical antioxidant activity was assayed based on Thin Layer Chromatography (TLC) technique. TLC technique is simple, easy, cheap, rapid, versatile and effective primary tool for identification of antioxidant activity. The extract was spotted on silica gel 60 G F_{254} precoated aluminum plates and the chromatogram was developed in different solvent system. After application of 2mg/ml alcoholic DPPH, presence of white spots on pink background of the TLC plates indicates the presence of antioxidant activity of experimental extract.

Quantitative analysis: The *in vitro* antiradical activity was quantitatively estimated according to the method of Sharma *et al*., 2009 with slight modification. At first, stock solution (1024µg/ml) of the extract was prepared. Then serial dilution was carried out to obtain the desired concentration series (512, 256, 128, 64, 32, 16, 8, 4, 2, 1 µg/mL). 1ml solution from each concentration was added to the 3ml of immediately prepared 0.004% w/v DPPH solution. The mixture was allowed to stand in dark place for 30minutes at ambient room temperature. After incubation, the presence of DPPH in each concentration of the sample was detected at 517nm. One of the natural antioxidants, ascorbic acid was used as standard. The antiradical activity of the extract and standard was calculated using the formula: % discoloration = $[(Abs_0-Abs_1)/Abs_0] \times 100$; where Abs_0 is the absorbance of control and Abs_1 is the absorbance of extract or standard. Concentration of extract providing 50% discoloration of DPPH solution (IC_{50} value), potential indicator of antiradical activity, was estimated from the obtained data. Ascorbic acid equivalent antioxidant capacity (AEAC) was also calculated.

Reducing power assay: The reducing power of extracts was determined by the method of Oyaizu *et al*., 1986 with modifications. At first stock solution of the extract was prepared. Dilution was carried out to obtain the desired concentrations. 2.5ml phosphate buffer (0.2mol/L; PH 6.6) and 2.5ml 1% w/v potassium ferricyanide were subsequently added to 1ml various concentrations of extract solution. The resultant mixture was incubated for 20 minutes at 50°C in a thermostat. After cooling at room temperature, to stop the reaction 2.5ml 10% w/v trichloroacetic acid was added to the mixture. The mixture was centrifuged at 3000 rpm. for 10minutes. 2.5ml aliquot of supernatant was mixed with 2.5ml distilled water and 0.50ml 0.1% w/v ferric chloride with continuous shaking. 10minutes later the absorbance of the resultant chromospheres was measured at 700nm. Butylated Hydroxy Toluene (BHT) was used to compare the reducing power of the extract. Increased absorbance indicates increased reducing power of extract.

Oral glucose tolerance test: Oral glucose tolerance test was conducted by the slight modification of the method followed by Djilani *et al*., 2011. In our investigation overnight fasted mice were divided into 5 groups each containing 5 in number. Mice of Group I were kept as control and group II received glibenclamide (5mg/kg, orally) as reference drug. Mice form groups III to V were treated with extract (400 mg/kg, orally). Thirty minutes later, glucose (10 g/kg) was orally loaded to all mice. After administration of the extract, blood samples were collected from tail vein prior to (0 min) and at 1st, 2nd and 3rd hour. By using a glucometer (EZ Smart-168, Tyson Bioresearch Inc, Taiwan), the blood glucose levels were taken.

Antibacterial Activity: Antibacterial activity of extract was determined by disc diffusion method Ahmed *et al*., 2003. The antibacterial activity of the extracts was observed at the dose of 200µg/disc, 300µg/disc and 500µg/disc and kanamycin (30µg/disc) was used as standard antibacterial agent. A suspension of the test organisms was added to sterile nutrient agar medium at 45°C and poured into sterile Petri dishes to solidify. Blank disc filled with different doses of sample extract, control and also the standard kanamycin disc were placed in such a way that possible chance of overlapping of zone of inhibition can be prevented. After that at least 120 minutes was allowed to diffuse the drug into the agar. Then the Petridishes were incubated at 37^0C. At the end of incubation period of twenty four hours the observed zone of inhibition was measured in millimeters (mm).

Statistical analyses: Microsoft Excel is used for Statistical analyses. Moreover, Regression analyses were used to signify the relationship between the

antioxidant activities and antioxidant components. Statistical significance of oral glucose tolerance test was estimated by student's t-test. All values are expressed as mean ± SD of three parallel measurements.

RESULTS

Phytochemical Screening: The crude extract was subjected for phytochemical screening and results were presented in table 1.

Antioxidant components: The levels of antioxidant components obtained from the extract were presented in Table 2.

Antioxidant activity: The antioxidant activities obtained from the extract were presented in Table 3.

Oral glucose tolerance test: Effects of the extract on oral glucose tolerance test in normal control mice were presented in table 4.

***Antibacterial activity*:** The antibacterial activity of the tested extract against the tested twelve microorganisms was shown in the Table 5.

Table 1: Results of phytochemical screening

Phytochemical Constituents											
Samples	Carbohydrate	Alkaloids	Glycosides	Phenolic compounds	Flavonoids	Tannins	Steroids	Protein &Amino acids	Saponin	Gum	Acidic compounds
S. tubreosum	+	+	+	+	+	+	-	+	+	+	+

(+) Indicates the presence & (-) Indicates the absence of chemical constituents

Table 2: Levels of total phenolic content (TPC), total flavonoids content (TFC) and total tannin content (TTC) of the extract.

Plants	Total Phenolic Content [a] (mg GAE/g extract)	Total Flavonoids Content [a] (mg QE/g extract)	Total Tannin Content [a] (mg GAE/g extract)
S. tubreosum	27.01 ± 0.25	21.05 ± 2.11	11.97 ± 0.86

[a] Values expressed as mean ± SD of triplicate measurements

Table 3: Antioxidant activity analysis of the extract by DPPH radical-scavenging and reducing power experiments

Samples	**DPPH Scavenging activity [a]**		**Reducing power [a]**
	(IC_{50} in µg/ml)	AEAC (mg AA/100 g)	(absorbance at 1 mg/ml)
S. tuberosum	267.11 ± 0.95	5.49 ± 0.11	0.136 ± 0.09
Ascorbic acid*	14.70 ± 0.11	-	-
BHT*	-	-	1.472± 0.008

**Antioxidant standards; [a] Values expressed as mean ± SD of triplicate measurements*

Table 4: Effect of extract on oral glucose tolerance test

Groups	Treatment	Blood glucose level [a] (mmol/L)			
		0 h	1st h	3rd h	5th h
I	Control (10ml/kg)	6.28 ± 1.42	21.01 ± 3.16	8.6 ± 0.59	5.53 ± 0.08
II	Glibenclamide (5mg/kg)	6.44 ±1.32	14.54 ± 3.62	3.94 ± 1.28	2.66 ± 0.95
III	*S. tuberosum*(400mg/kg)	6.02 ± 2.17	18.32 ± 2.46*	6.74 ± 1.60*	4.48 ± 1.68*

[a] Values represent as mean ± SD, n = 5, p<0.05 compared to control

Table 5: Average zone of inhibition (mm) against different bacterial strains by extract

Microorganisms	*S. tuberosum*		
	200 µg	300 µg	500 µg
S. aureus	7.71±0.2	13.54±0.5	15.70±0.12
S. epidermidis	6.74±0.5	10.07±0.07	10.28±0.5
S. saprophyticus	9.74±0.25	13.60±0.5	16.11±0.21
S. pyogens	7.00±0.1	7.94±0.04	10.50±0.07
S. agalactiae	10.76±0.13	13.75±0.11	14.20±0.51
E. faecalis	12.98±0.09	18.06±0.27	18.64±0.11
S. dysenteriae	0	0	8.00±0.05
E. coli	7.39±0.08	8.58±0.16	9.52±0.08
S. flexneri	0	0	0
S. sonnei	11.22±0.12	11.97±0.08	12.02±0.05
S. boydii	0	0	8.67±0.05
V. cholera	0	6.48±0.17	10.08±0.1

DISCUSSION

Plant is a biosynthetic laboratory. It contains several non-nutritive chemicals known as phytochemical constituents. Among these constituents' phenolic compounds, flavonoids, tannins and alkaloids are the most valuable for therapeutic activity. So, identification of the nature of the compounds present in extracts is essential to evaluate the biological activity of the extracts. Phytochemical studies revealed the presence of therapeutically important phytochemical constituents that's why the experimental extracts may be responsible for different therapeutic response in the biological system.

Antioxidant components: Based on the results of phytochemical screening extract give positive result in phenolic compounds, flavonoids and tannin tests that why they were taken to estimate the total phenolic content as well as flavonoids and tannin.

The most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds. Due to presence of hydroxyl groups, conjugated ring structures and carboxylic groups phenolic compounds are capable to act as free radical scavenger, hydrogen donors, singlet and triplet oxygen quenchers, metal chelator, lipid peroxidator and also exert multiple biological responses [15,16]. *S. tuberosum* more efficiently reduces the phosphomolybdic acid of Folin-Ciocalteu reagent in basic condition and formed intense blue color complex of reduced molybdenum. The present findings were comparable to previous study [17]. The flavonoids content of the investigated plants is of interest when evaluating its antioxidant properties as flavonoids have been claimed as high level natural antioxidant due to their strong antioxidant properties. In aluminum chloride colorimetric method flavonoids form acid stable complexes with aluminum chloride. Based on the complex formed *S. tuberosum* contains flavonoids content. Although phenolic compounds have been related to antioxidant activity, in peroxyl radicals quenching tannins are 15-30 times more potent than normal phenolics. That's why in the present study it has emphasized on tannins in determining the antioxidant activity of the extracts. Tannin content was present in *S. tuberosum.*

Antioxidant activity: Antioxidants act by different mechanisms. As each assay is specific for particular reaction system, no single assay is suitable to determine the precise mechanism of action of antioxidants. So it is prudent to use different assays to determine antioxidant activities.

The most appropriate, classic, easy, fast and highly sensitive way to evaluate the antioxidant activity of the natural sources as well as single compound is the use of DPPH free radical scavenging method [18]. DPPH is unaffected by side reaction [19]. As DPPH or the similar compounds are absent in the physiological system but major disadvantage is the steric obstruct provided by it. Smaller the antioxidant molecules greater the antioxidant activity due to greater accessibility to the reaction site of the DPPH free radical. *S. tuberosum* showed better scavenging activity. Though sufficient antioxidant components are present, reduced accessibility of the components

to the reaction site may be the possible causes of the lower free radical scavenging activity.

Reducing power measure the reduction ability of reductants which was evaluated by the reduction of ferricyanide complex/Fe (III) to the ferrous/Fe (II) form. The reducing power of a compound may therefore serve as a significant indicator of its potential antioxidant activity [20]. Although a reductant is not necessarily an antioxidant, an antioxidant is commonly a reductant. It assumed that reductants and antioxidants both are responsible for ferric reducing capacity of the extract.

Correlation: The antioxidant potential of *S. tuberosum* depends on the selected method, concentration and physicochemical properties of components. A comparison of the correlation coefficient (R^2) is better than the quantity of antioxidant components to evaluate the antioxidant property.

In our study, a moderate correlation (R^2 = 0.669) between phenolic compounds and flavonoids has been found. This could be explained by the presence of -OH groups in some none phenolic compounds such as proteins and amino acids, carbohyrate, saponin and many vitamins [21]. that are reactive to Folin-Ciocalteu reagent. Total phenol content showed strong (R^2 = 0.900) and (R^2 = 0.976) positive correlation to DPPH radical scavenging and reducing power assay respectively. On the other hand there was strong (R^2 = 0.918) positive correlation to DPPH scavenging assay and (R^2 = 0.803) correlation with reducing power assay in terms of flavonoids content level. Total tannins content was negligibly correlated to DPPH radical scavenging (R^2= 0.052) and also to reducing power assay (R^2 = 0.003). Finally, it assumes that natural flavonoids are responsible for antioxidant activities of the extracts.

Oral glucose tolerance test: Oral administration of glucose is used to approximate how quickly glucose is cleared from the blood. In present investigation, oral glucose tolerance test has revealed the glucose tolerance capability of all the tested extracts. After loading glucose extracts effectively reduce peak plasma glucose level. Extract showed maximum effect at first hour and with increasing the time the effect was not so much significant. Many secondary metabolites such as polysaccharides, alkaloids, amino acids, flavonoids, phenolics, coumarins, inorganic ions and guanidines are reported to have glucose lowering activity [22]. There is a relation of antioxidant and antidiabetic activity of several compounds. Mixture of compounds including the antioxidants of the experimental herbs may be responsible for interfering glucose absorption from gut, stimulating glycogenesis in liver or increasing secretion or sensitivity of insulin and consequent glucose tolerance.

Antibacterial activity: The phytochemicals of polyphenols, flavonoids, tannins, terpenoids, saponin, and alkaloids show antimicrobial activity [23]. In the crude extract of *S. tuberosum*, *Shigella flexneri* was insensitive but from the other lowest sensitive ones *Shigella dysenteriae* and *Shigella boydii* were sensitive only at highest dose. And all of the three doses the maximum zone of inhibition were observed against *Enterococcus faecalis*.

The result of the disc diffusion antimicrobial activity test also showed that the extract was more active to Gram positive bacteria in comparison of Gram negative bacteria. These differences in sensitivity of the extract may be due to the differences in presence of bioactive compounds and also the antimicrobial agents may be large in size, complex and charged molecule so it is difficult for the agents to pass through the porins in the outer membrane of the Gram-negative bacteria consequently lower activity against Gram-negative bacteria.

CONCLUSION

The findings of the research support the traditional uses of the edible herb *S. tuberosum*. These provide nutritional benefit and also antioxidant, antihyperglycemic and antibacterial activities. Due to these beneficial effects these edible herbs may be good source of nutraceuticals. However, further studies will be essential to isolate the active compounds responsible for different pharmacological activities from the leaves of *S. tuberosum*.

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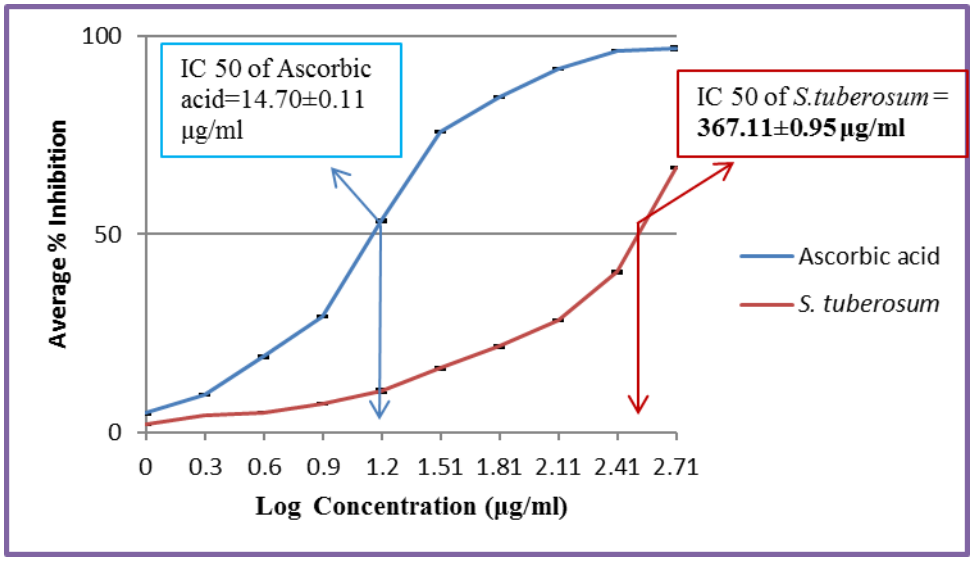

Figure 1: Comparison of DPPH radical scavenging activity (IC_{50} value) of standard and sample

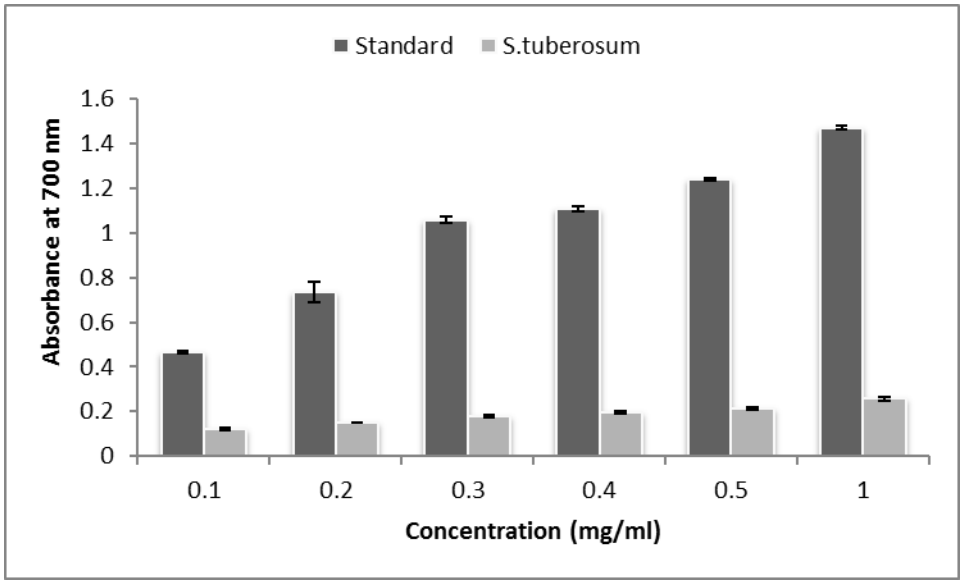

Figure 2: Comparison of reducing power assay of BHT and *S. tuberosum*

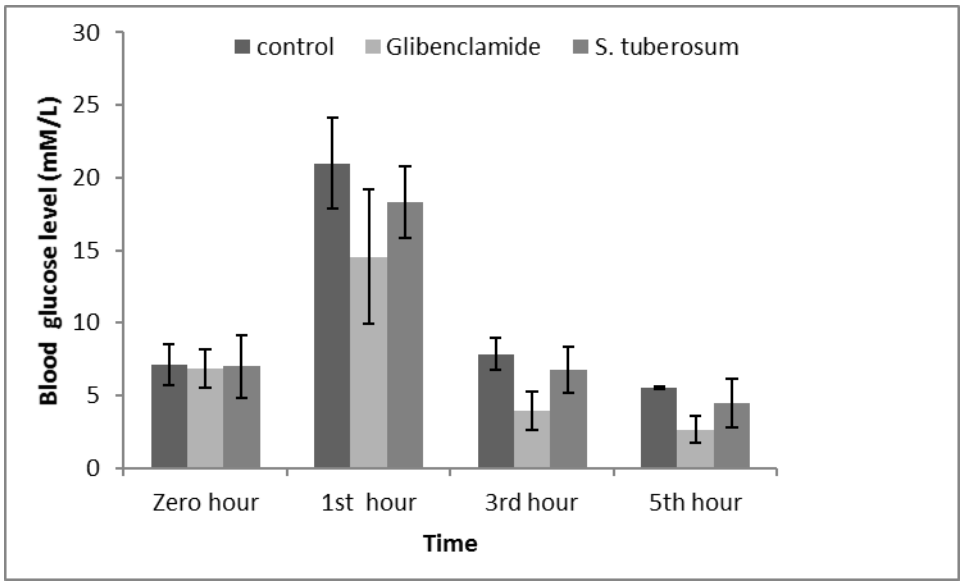

Figure 3: Effect of the *S. tuberosum* on antihyperglycemic activity test in normal control mice.

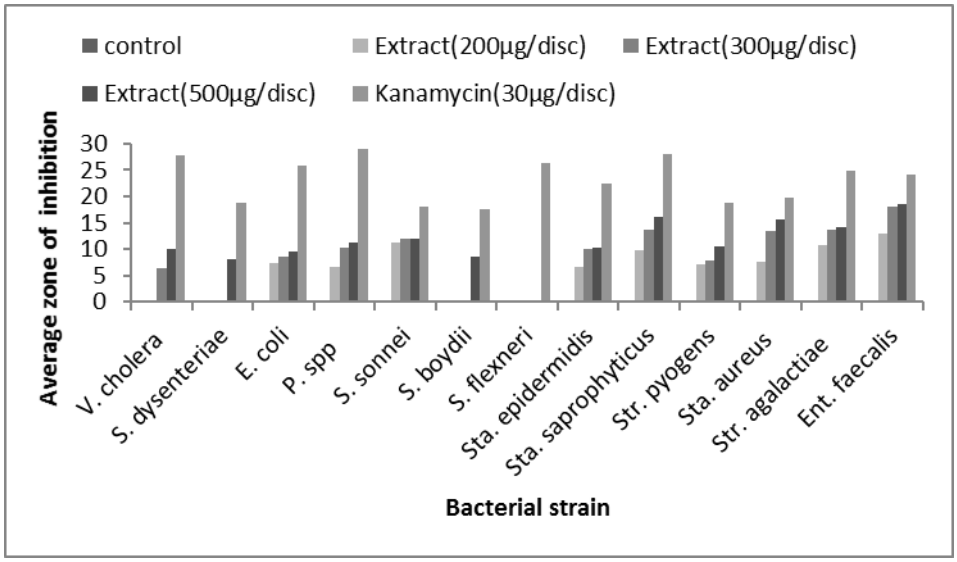

Figure 4: Antibacterial activity of *S. tuberosum* at different doses against gram positive and gram negative bacteria in comparison of kanamycin.

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