Bangladesh J. Bot. **50**(1): 61-68, 2021 (March)

EVALUATION OF α-GLUCOSIDASE INHIBITION, ANTIOXIDANT AND ANTIBACTERIAL EFFECTS OF *GYMNEMA SYLVESTRE* R. BR.

TAHSEEN GHOUS, KULSOOM AKHTAR[1], SAIQA ANDLEEB*[2], SAIRA KHIZAR[1], SHAUKAT ALI[3], ROZINA GHULAM MUSTAFA[2], IRSA SHAFIQUE[2] AND ANUM NASEER[2]

Department of Chemistry, Mirpur University of Science and Technology (MUST) Azad Jammu and Kashmir, Mirpur-10250, Pakistan

Keywords: α-glucosidase antidiabetic, Antioxidant, Antibacterial, *Gymnema sylvestre*

Abstract

The results of antioxidant assays with *Gymnema sylvestre* leaf extracts revealed that methanol, acetone, and aqueous extracts possessed maximum DPPH (83, 83, and 75%) and ABTS scavenging potential (85, 71, and 82%). Chloroform possessed maximum total flavonoid (24.6 mg/100 ml) and total iron contents (435.3 ± 0.0125 mg $FeSO_4$ E/G). The extracts of n-hexane, ethyl acetate, and chloroform of *Gymnema sylvestre* leaves displayed an IC_{50} value of 170.2, 44.4, 131.6 µg/ml, respectively in α- glucosidase inhibition assay. Methanol and aqueous extracts showed maximum inhibition of *E. ammni* (17 ± 2.0 and 19.6 ± 0.57 mm), *E. coli* (15.3 ± 0.57 and 17.6 ± 0.57 mm), and *S. aureus* (16.6 ± 1.52 and 19.3 ± 1.15 mm). It may be concluded that all potential effects of *G. sylvestre* leaf extracts were due to the presence of phytochemical constituents such as flavonoids, steroids, quinones, phenols, triterpenoids, tannins, alkaloids, and saponins. Present findings suggest that these extracts present a satisfactory source for the preparation of antioxidant and antidiabetic medicines.

Introduction

Diabetes is categorized by hyperglycemia due to a decrease in insulin excretion, action, or both (Gandhi and Sasikumar 2012). Long-lasting impairment and failure of many organs, particularly nerves, heart, kidneys, eyes, and blood vessels are due to prolonged hyperglycemia. The beginning and progression of diabetes-related complications may partially mediate due to the maximum production of free radicals and minor antioxidant defense (Reinmuth-Selzle *et al*. 2017). So, the use of antioxidants in diabetic patients can be helpful to sustain their level in the body as well as to cure diabetic problems (Iwai 2008).

WHO has reported that almost 3% of the world's population is suffering from diabetes and the prevalence is estimated to increase twofold by the year 2025 i.e, 6.3% (Abdalla and Ibhrahim 2012). For the treatment of diabetes, obesity, and hyperlipemia are the drug design targets which serve as inhibitors of the enzymes glucosidase and alpha-amylase (Dej-adisai *et al*. 2017). In developing countries where maximum people have inadequate resources and do not have access to current treatment, plants have long been used for the cure of diabetes. Much of the work has been done to determine their effects and biological properties, due to the possible significance of these inhibitors in plant physiology, animal and human nourishment (Nithya *et al*. 2014).

During the last two decades, herbal medicines have been progressively used all over the world because plants are less toxic and are safer than manufactured drugs. Fifty percent of the recent drugs are of a natural product origin and play a significant role in drug development (Reinmuth-Selzle *et al*. 2017). The ethnobotanical community has a significant interest in plants possessing

*Author for correspondence: <drsaiqa@gmail.com>. [1]Department of Chemistry, University of Azad Jammu and Kashmir, Muzaffarabad, 13100, Pakistan. [2]Microbial Biotechnology laboratory, Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, 13100, Pakistan. [3]Department of Zoology, Government College University, Lahore, Pakistan.

antidiabetic activities. In many plant species, bioactive constituents are present which are isolated for nonstop use as drugs and pharmacological agents (Aljohi *et al*. 2016). In the current research, *Gymnema sylvestre* was selected to explore its antibacterial, antioxidant, and antidiabetic activities.

Materials and Methods

Gymnema sylvestre R. BR. leaves were collected from Muzaffarabad Azad Jammu and Kashmir, Pakistan, and was identified by an Ethno-botanist, Department of Botany, University of Azad Jammu & Kashmir, Pakistan. The leaves were rinsed with running tap water to remove dust, air-dried at room temperature (20 ± 2°C), and were crushed into a fine powder. Hundred gram of fine powder were soaked in 250 ml of solvents including n-hexane, ethyl acetate, chloroform, acetone, methanol, and water, respectively for one week. Periodical shaking was carried out and was filtered. The filtrates were evaporated under vacuum at 40°C using a rotary evaporator. All extracts were weighed and the percentage yield of the extracts was calculated as: % yield = obtained mass of extract/actual mass of sample powder *100.

Phytochemical screening was carried out for all the extracts following the standard methods (Audu *et al.* 2007, Parekh and Chand 2008, Obasi *et al.* 2010). Phenolic contents were determined using the Folin-Ciocalteu reagent method (Zhou and Yu 2006). The phenolic contents in extracts were expressed in terms of gallic acid equivalent (µg of GA/g of extract). Estimation of total flavonoid contents of extracts was quantified by the method illustrated by Zou *et al*. (2004). Total flavonoid contents in extracts were also expressed as Rutin equivalents (RE) milligrams per gram of extract (mg/g).

$ABTS^{+}$ free radical scavenging activity was carried out to evaluate the antioxidant potential of plant extracts according to the method described by Re *et al*. (1999). DPPH antioxidant potential was also measured (Braca *et al*. 2001). Fe^{+2} chelating activity was investigated by the method described by Dinis *et al*. (1994). The chelating activity of the extracts was calculated as: Chelating rate (%) = Ao-Ai/Ao*100. The Fe^{+3} reducing power of the extracts was determined and the absorbance was measured at 700 nm (Oyaizu 1986).

The inhibition of α-glucosidase activity was determined following Dewi *et al*. (2007). Percentage inhibition (I) was calculated as IAG % = (Ac – As)/Ac*100, where Ac is the absorbance of the control and As is the absorbance of the sample.

For antibacterial activity test, seven bacterial pathogens were used *viz., Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium, Shigella flexneri, Enterobacter amnigenus* were taken from Microbial Biotechnology Laboratory, Department of Zoology, the University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. Antibacterial activity was assessed by the agar well diffusion method (Rios *et al*. 1988). The results of the sensitivity tests were expressed as (0) for no sensitivity or resistance, *(1 - 5 mm) for low sensitivity, **(>5 - 10 mm) for moderate sensitivity, and ***(>10 - 25 mm) for high sensitivity. Sensitivity test of antibiotics {aminoglycosides (Streptomycin 10 µg/ml), kanamycin 10 µg/ml, penicillin's (ampicillin 10 µg/ml, penicillin G 10 µg/ml), tetracyclines (tetracycline 10 µg/ml), and fluoroquinolones (ciprofloxacin 10 µg/ml, nalidixic acid 5 µg/ml), and chloramphenicol 10 µg/ml} against all tested bacterial strains was assessed by agar disc diffusion method and used as positive control (Bauer *et al*. 1966). Each experiment was repeated in triplicate and the standard deviation from absolute data was calculated: (http://easycalculation. com/statistics/standard-deviation. php).

Results and Discussion

The per cent yield of extracts was higher in aqueous (41), followed by methanol (12.1) > *n*-hexane (4.7) > ethyl acetate (3.58) > acetone (3.46) > chloroform (2.25), respectively (Fig. 1). The preliminary phytochemical screening revealed the presence of phytochemical constituents such as terpenoids, diterpenoids, flavonoids, tannins, phenols, and saponins, mostly in aqueous, methanol, acetone, and chloroform extracts of *G. sylvestre* leaves (Table 1). Glycosides reduce hypertension and terpenoids are good antioxidant and anti-cancer agents (Ali *et al.* 2008). Terpenoids and diterpenes are significant in chloroform, methanolic, aqueous, acetone, and *n*-hexane extracts, but are completely absent in ethyl acetate extract. Steroids are completely absent in aqueous and *n*-hexane extracts but are maximum in methanolic and chloroform extracts, and effectively present in ethyl acetate and acetone extracts. Xanthoproteins and amino acids are maximum in aqueous

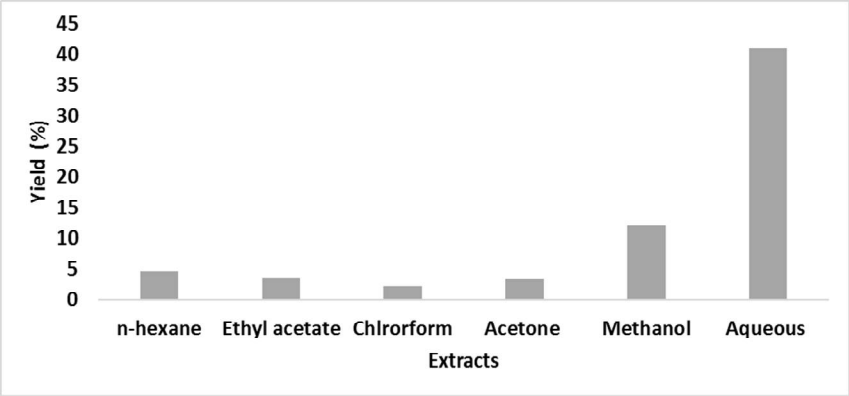

Fig. 1. The total percentage yield of extracts of *G. sylvestre* leaves.

and methanolic extracts and minimum in acetone extract. In all extracts except for methanol, carbohydrates and carboxylic acids are not present (Table 1). Saponins are maximum in methanolic, aqueous, and ethyl acetate extracts while adequately present in chloroform, *n*-hexane, and acetone extracts. Saponins show hemolytic, anti-inflammatory, and antimicrobial effects and are widely used in Chinese medicine (Liu and Henkel 2002). Phenolic compounds are abundantly present in plants and have anti-aging, anti-inflammation, anti-carcinogen activities as well as cardiovascular protection. Phenols are generally present in all types of plants and have antioxidant activity (Chandrashekharaiah *et al.* 2013), reduce lipid oxidation and improve the nutritious quality of food (Wojdyło *et al*. 2007). The maximum total phenolic contents were observed in acetone (0.33 mg/100 ml) and methanol (0.20 mg/100 ml) while minor in ethyl acetate (0.15 mg/100 ml), chloroform (0.177 mg/100 ml) and aqueous (0.06 mg/100 ml) extracts (Fig. 2). Flavonoids are significant polyphenols because they show the broad spectrum of biological activities like anti-inflammatory, antioxidant, vasorelaxant, antimicrobial, antiviral, anticarcinogenic, antimutagenic) (Guo *et al*. 2011). The maximum total flavonoid contents were estimated in chloroform (24.6 mg/100 ml), ethyl acetate (20.53 mg/100 ml), and *n*-hexane (19.11 mg/100 ml), as shown in Fig. 2. Flavonoids reduce the chances of cancer and cell damage (Okwu and Josiah 2006). Their concentration of flavonoids is higher in aqueous, methanolic, and acetone extracts, and is lower in *n*-hexane (Singh *et al*. 2007).

Currently, significant attention has been established for the screening of natural antioxidants to determine them from natural sources that may offer beneficial potential (Kim and Kim 2003). Plants with greater donating ability indicate greater DPPH free radical scavenging activity (Saha

et al. 2008). Methanol, aqueous, and acetone extracts of *G. sylvestre* showed the highest DPPH radical scavenging activity 83, 83, and 75%. It may be said that the scavenging potential effect was increased from methanol to *n*-hexane. Similarly, $ABTS^{+}$ decolorization assay showed maximum antioxidant potential found in methanol, acetone, and aqueous extracts and 85, 71, and 82%, respectively (Fig. 2).

Table 1. Phytochemical analysis of various extracts of *G. sylvestre* leaves.

Extracts → Phytochemical tests ↓	Aqueous	Methanol	Acetone	Chloroform	E. acetate	Hexane
Alkaloid	-	++	++	-	+	-
Quinone	+++	+++	+	+	-	-
Cardiac glycoside	-	-	-	-	-	-
Terpenoid	+++	+++	+++	+++	-	++
Diterpenoid	+++	+++	+++	+++	-	++
Steroid	-	++	+	++	+	-
Xanthoprotien	+++	+++	+	-	-	-
Flavonoid	+++	+++	+++	++	++	+
Tanin	+	+++	++	+++	++	+
Phenol	+	+++	++	+++	++	+
Carbohydrate	-	+	-	-	-	-
Carboxylic acid	-	+	-	-	-	-
Saponin	+++	+++	++	++	+++	++
Resin	++	++	-	-	-	+
Amino acid	++	++	+	-	-	-
Phytosterol	-	+	+	++	+	-

+ low quantity, ++ moderate quantity,+++ high quantity.

The results exposed that all the extracts contained a significant amount of Fe^{+2} (Fig. 2). Results showed that chloroform contains maximum iron content (435.3 ± 0.0125 mg $FeSO_4$ E/g), adequate amount found in ethyl acetate and acetone extract (297.3 ± 0.0360 mg $FeSO_4$ E/g and 350 ± 0.0917 mg $FeSO_4$ E/g), but minimum in methanol, n-hexane and aqueous extracts (116.5 ± 0.007, 95.5 ± 0.0608, 96.65 ± 0.0511 mg $FeSO_4$ E/g) of *G. sylvestre* leaves (Fig. 2).

Diabetes mellitus is a prolonged syndrome of metabolism due to a complete or relative lack of insulin. It is categorized by hyperglycemia in the postprandial or fasting state, and its complicated form is improved by ketosis and protein wasting (Ahamad and Naquvi 2011), and is also associated with several complications like peripheral vascular insufficiencies, neuropathy, and retinopathy. Many therapeutic drugs (miglitol, acarbose, and voglibose) are good inhibitors of α-glucosidase but the major side effects of these medicines are flatulence, bloating, meteorism, abdominal distention, and probably diarrhea (Kimmel and Inzucchi 2005). Natural products can also decrease postprandial hyperglycemia with slight antagonistic effects as they contain a minimum inhibitory effect for α- amylase and strong inhibition of α-glucosidase (Kim *et al*. 2009). However, exploration of maximum active and harmless hypoglycemic compounds from plants has sustained to be a significant area of dynamic study. Many polyphenolic compounds, triterpenoids, tannins, flavonoids, saponins, alkaloids have been tested for the hypoglycemic effect (Metwally *et al*. 2010).

Yeast alpha-glucosidase inhibitory activity of *G. sylvestre* leaf extracts was also examined in the present work. In the absence of an inhibitor, the enzyme-substrate complex absorbs light greater than in the presence of inhibitors. *In vitro* results showed that n-hexane and chloroform extracts showed an appreciable and greater alpha-glucosidase inhibitory effect, while ethyl acetate showed the least inhibition (Table 2). The extracts of *n*-hexane, ethyl acetate, and chloroform of *Gymnema sylvestre* leaves displayed an IC_{50} value of 170.2, 44.4, 131.6 µg/ml, respectively in α-glucosidase inhibition assay (Table 2). The present study revealed that *G. sylvestre* leaves could be beneficial in controlling postprandial hyperglycemia. Present results are consistent with the previously reported studies (Kim *et al*. 2009, Mayur *et al*. 2010, Shai *et al*. 2010).

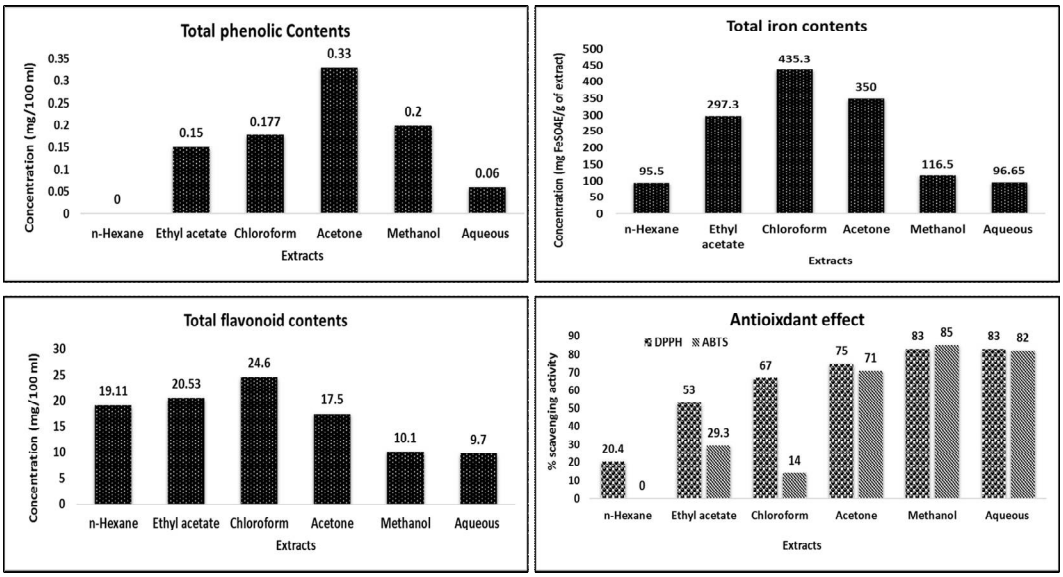

Fig. 2. Total phenolic, total flavonoid, total iron contents, and antioxidant potential of various extracts of *G. sylvestre* leaves.

Many phytochemicals possess good antimicrobial activities (Sule *et al*. 2011). *G. sylvestre* leaf extracts showed *in vitro* inhibition of bacterial growth, as they contain flavonoids, steroids, quinones, phenols, triterpenoids, tannins, alkaloids, and saponins. Such compounds can act singly or in the mixture to prevent bacterial growth and deliberated the strong antibacterial activity. Results revealed that maximum antibacterial effect was shown by the methanol and aqueous extracts whereas *n*-hexane extract showed resistance against all the tested microorganisms (Table 3). Methanol and aqueous extracts exposed the high degree of antimicrobial activity against *E. ammni* (17 ± 2.0 and 19.6 ± 0.57 mm), *S. typhimurium* (14.6 ± 0.57 and 15 ± 0.0 mm), *S. pyogenes* (11.6 ± 0.57 and 12.6 ± 0.57 mm), *S. flexneri* (16.3 ± 1.52 and 15.3 ± 1.15 mm), *E. coli* (15.3 ± 0.57 and 17.6 ± 0.57 mm), and *S. aureus* (16.6 ± 1.52 and 19.3 ± 1.15 mm). Whereas acetone extract showed significant antimicrobial activity against *S. flexneri, E. ammni,* and *S. aureus* and least with *E. coli, P. aeruginosa, S. typhimurium, S. pyogenes*. Chloroform and ethyl acetate found to be less effective. Chloroform extract displayed mild to adequate activity against *E. coli* and *S. typhimurium* while ethyl acetate showed moderate antimicrobial activity against *S. flexneri, E. ammni,* and *S. aureus* and showed resistance against other microbes.

A relationship between the extract solubility and antimicrobial activity of various fractions was observed. This recommends that in successive extraction, in polar solvent maximum antimicrobial compounds were soluble in methanol and aqueous extracts exhibited the strong antimicrobial activity followed by the acetone, chloroform, and ethyl acetate extracts. Results indicate that major antimicrobial compounds are polar as shown by the maximum degree of antimicrobial the activity of aqueous and methanolic extracts. Saumendu *et al*. (2010) reported

Table 2. Percent inhibition of yeast α-glucosidase by hexane, ethyl acetate, chloroform, and water extracts of *G. sylvestre* at varying concentrations.

Conc. (µg/ml)	% inhibition by *n*-Hexane	IC_{50} (µg/ml) *n*-Hexane	% inhibition by ethyl acetate	IC_{50} (µg/ml) ethyl acetate	% inhibition by chloroform	IC_{50} (µg/ml) chloroform
15.625	3.0625	170.2	1.187	44.4	2.0625	131.6
31.25	6.125		2.375		4.125	
61.5	12.25		4.75		8.25	
125	24.5		9.5		16.5	
250	62		27		51	

Table 3. Inhibition zone of *G. sylvestre* extracts against bacterial pathogens.

Extracts	Pathogens used						
	Escherichia coli	*Shigella flexneri*	*Enteroc-cocus ammni*	*Salmonella typhimu-rium*	*Strepto-coccus pyogenes*	*Pseudo-monas aeruginosa*	*Staphlo-coccus aureus*
n-hexane	0.0 ± 0.0	0.0 ± 0.00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Ethyl acetate	0.0 ± 0.0	1.6 ± 0.57	1.5 ± 0.70	0.0 ± 0.0	5.0 ± 1.0	0.0 ± 0.0	8.3 ± 1.15
Chloroform	5.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	7.3 ± 1.52	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Acetone	6.0 ± 0.0	11 ± 1.0	9.3 ± 0.57	8.0 ± 1.0	3.6 ± 1.52	8.3 ± 1.52	10.3 ± 1.52
Methanol	15.3 ± 0.57	16.3 ± 1.52	17 ± 2.0	14.6 ± 0.57	11.6 ± 0.57	8.3 ± 3.78	16.6 ± 1.52
Aqueous	17.6 ± 0.57	15.3 ± 1.15	19.6 ± 0.57	15 ± 0.0	12.6 ± 0.57	7.3 ± 0.57	19.3 ± 1.15

that different extracts of *G. sylvestre* displayed a zone of inhibition against *B. subtilis, S. aureus* but not against *E. coli* however, in the present study it is perceived that aqueous, methanol, acetone, and chloroform extracts showed a zone of inhibition against *E. coli*. Results recommend that these extracts can be helpful to treat diarrhea, pyogenic and urinary tract infections, and septicemia.

The present finding recommends that *n*-hexane and chloroform extracts of *G. sylvestre* may be beneficial for preventing diabetes complications, as chloroform and *n*-hexane extracts showed the significant inhibitory potential of alpha-glucosidase. Further research is needed to characterize the bioactive compound accountable for the observed activities.

References

Ahamad J and Naquvi KJ 2011. Review on role of natural alpha-glucosidase inhibitors for management of diabetes mellitus. Int. J. Biomed. Res. **2**(6): 374-380.

Ali SS, Kasoju N, Luthra A Singh A, Sharanabasav H, Sah A and Bora U 2008. Indian medicinal herbs as sources of antioxidants. Food Res. Intern. **41**(1): 1-15.

Aljohi A, Matou-Nasri S and Ahmed N 2016. Antiglycation and antioxidant properties of *Momordica charantia*. PloS one **11**(8): 0159985.

Audu SA, Mohammed I and Kaita HA 2007. Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life Sci. J. **4**(4): 75-79.

Bauer AW, Kirby WMM, Sherris JC and Turck M 1996. Antibiotic susceptibility testing by standardized single disk method. Amer. J. Clin. Pathol. **36**: 493-6.

Braca A, Tommasi ND, Bari LD, Pizza M, Politi and Morelli I 2001. Antioxidant principles from *Bouhinia terapotensis*. J. Nat. Prod. **64**: 892-895.

Chandrashekharaiah KS, Bolaki MN, Sanjay GS, Bathija A, Murthy VK, Narayanaswamy M and Swamy NR 2013. Anti-oxidant and anti-hyperglycemic properties of methanolic extracts of medicinal plants. Biosci. Biotech. Res. Asia **10**(2): 607-612.

Dej-adisai S, Pitakbut T and Wattanapiromsakul C 2017. Alpha-glucosidase inhibitory activity and phytochemical investigation of *Borassus flabellifer* Linn. Afr. J. Pharma. Pharm. **11**(3): 45-52.

Dewi RT, Iskandar YM, Hanafi M, Kardono L, Angelina M, Dewijanti ID and Banjarnahor SD 2007. Inhibitory effect of Koji *Aspergillus terreus* on α-glucosidase activity and postprandial hyperglycemia. Pak. J. Biol. Sci. **10**(18): 3131-3135.

Dinis TC, Madeira VM and Almeida LM 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophy. **315**(1): 161-169.

Gandhi GR and Sasikumar P 2012. Antidiabetic effect of *Merremia emarginata* Burm. F. in streptozotocin induced diabetic rats. Asi. Pac. J. Tropical Biomed. **2**(4): 281-286.

Guo T, Wei L, Sun J, Hou Cl and Fan L 2011. Antioxidant activities of extract and fractions from *Tuber indicum* Cooke & Massee. Food Chem. **127**(4): 1634-1640.

Iwai K 2008. Antidiabetic and antioxidant effects of polyphenols in brown alga *Ecklonia stolonifera* in genetically diabetic KK-Ay mice. Plant Foods Human Nut. **63**(4): 163.

Kim GN, Shin JG and Jang HD 2009. Antioxidant and antidiabetic activity of Dangyuja (*Citrus grandis* Osbeck) extract treated with *Aspergillus saitoi*. Food Chem. **117**(1): 35-41.

Kim HY and Kim K 2003. Protein glycation inhibitory and antioxidative activities of some plant extracts in vitro. J. Agri. Food Chem. **51**(6): 1586-1591.

Kimmel B and Inzucchi SE 2005. Oral agents for type 2 diabetes: An update. Clin. Diab. **23**(2): 64-76.

Liu J and Henkel T 2002. Traditional Chinese medicine (TCM): Are polyphenols and saponins the key ingredients triggering biological activities? Curr. Med. Chem. **9**(15): 1483-1485.

M Abdalla MA and Ibhrahim MS 2012. Antidiabetic effects of fenugreek (*Trigonella foenum-graecum*) seeds in the domestic rabbit (*Oryctolagus cuniculus*). Res. J. Med. Plant **6**: 449-455.

Mayur B, Sancheti S, Shruti S and Sung-Yum S 2010. Antioxidant and glucosidase inhibitory properties of *Carpesium abrotanoides* L. J. Med. Plants Res. **4(**15): 1547-1553.

Metwally A, Omar A, Harraz F and El Sohafy S 2010. Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. Pharmacognosy Magazine **6**(23): 212.

Nithya TG, Divagar M and Juliet L 2014. Evaluation of *in vitro* antidiabetic activity of Seendhil herbal formulation. Asian J. Pharm. Clin. Res. **1**(7): 91-93.

Obasi NL, Egbuonu ACC, Ukoha PO and Ejikeme PM 2010. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. Afri. J. Pure Appl. Chem. **4**(9): 206-212.

Okwu DE and Josiah C 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J. Biotechnol. **5**(4): 357-361.

Oyaizu M 1986. Studies on products of browning reaction. The Jap. J. Nut. Diet. **44**(6): 307-315.

Parekh J and Chands S 2008. Phytochemical screening of some plants from western regions of India. Plant Arch. **8**: 657-662.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. **26**(9): 1231-1237.

Reinmuth-Selzle K, Kampf CJ, Lucas K, Lang-Yona N, Fröhlich-Nowoisky J, Shiraiwa M, Lakey PS, Lai S, Liu F and Kunert AT 2017. Air pollution and climate change effects on allergies in the anthropocene: Abundance, interaction, and modification of allergens and adjuvants. Envir. Sci. Technol. **51**(8): 4119-4141.

Saha M, Hasan S, Akter R, Hossain M, Alam M, Alam M and Mazumder M 2008. *In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. Bangl. J. Vet. Med. **6**(2): 197-202.

Saumendu DR, Sarkar K, Dipankar S, Singh T and Prabha B 2010. *In vitro* antibiotic activity of various extracts of *Gymnema sylvestre*. Int. J. Pharma. Res. Dev. **2**: 1–3,

Seeley HW and Van Demark PJ 1962. Microbes in action. A laboratory manual of microbiology. San Francisco, Freeman.

Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale A, Boaduo N and Eloff JN 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. South Afr. J. Bot. **76**(3): 465-470.

Singh R, Singh S, Kumar S and Arora S 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chem. Toxicol. **45**(7): 1216-1223.

Sule A, Ahmed QU, Samah OA, Omar MN, Hassan NM, Kamal LZM and Yarmo MA 2011. Bioassay guided isolation of antibacterial compounds from *Andrographis paniculata*. Ame. J. Appl. Sci. **8**(6): 525-534.

Wojdyło A, Oszmiański J and Czemerys R 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. **105**(3): 940-949.

Zhou K and Yu L 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT-Food Sci. Tech. **39**: 1155-1162.

Zou Y, Lu Y and Wei D 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. J. Agri. Food Chem. **52**(16): 5032-5039.

(Manuscript received on 13 June, 2019; revised on 3 April, 2020)