

ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITIES OF CORN STEEP LIQUOR ANTI-DIABETIC HERB EXTRACTS

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Abstract

Traditionally, Corn-steep fermenting liquor (CSL) has been used to prepare infusion for the management of chronic diseases, especially diabetes mellitus. Antioxidants and antimicrobials have gained attention in this regard due to the role of free radicals and infections in the etiology and complication of the disease. The present study investigated the antioxidant and antimicrobial properties of CSL extracts of *Citrullus colocynthis* (CC), *Curculigo pilosa* (CP) and *Gladiolus psittacinus* (GP). DPPH scavenging activity, Hydrogen peroxide scavenging activity, Iron chelating activity, total reducing power, total flavonoids and total phenolics were determined using standard methods while antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiellae pneumoniae* was determined using agar well diffusion method. CP gave the highest total phenolic content (86.84mg GAE/g extract) while the total flavonoid content of GP was the highest (55.95mg QUE/g extract) in comparison with other extracts. The IC₅₀ values based on Iron chelating (72µg/ml), DPPH (174µg/ml) for CP and Hydrogen peroxide (95µg/ml) for GP were lower, indicating good antioxidant potential. Also, CP exhibited the highest reducing power in a concentration dependent manner. CC inhibited *S. aureus* (12mm), *E. coli* (14mm), *S. typhi* (14mm) and *K. pneumoniae* (14mm) while CP inhibited *S. aureus* (12mm), *S. typhi* (12mm) and *K. pneumoniae* (14mm). GP only inhibited *S. aureus* (12mm) and *K. pneumoniae* (12mm). All the extracts were inactive against *B. subtilis*. The results of this study showed that CSL extracts of CC, CP and GP possess antioxidant and antimicrobial properties thereby justifying their use in folk medicine for the management of diabetes mellitus.

Keywords: Antioxidant, Antimicrobial, Corn-steep fermenting liquor, *Citrullus colocynthis*, *Gladiolus psittacinus*, *Curculigo pilosa*

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1. INTRODUCTION

Many chronic diseases are caused by oxidative stress which occurs due to imbalance in generation and scavenging of free radicals (Hazra *et al.*, 2008). The role of free radical in disease pathology is well established and is known to be involved in many systemic disorders in human beings such as diabetes, atherosclerosis, aging, and neurodegeneration (Harman, 1998). Antioxidants play a role in scavenging of these free radicals and eventually in prevention of diseases caused by them. The human body protects itself from free radicals by various enzymic and non-enzymic antioxidants, sometimes these protective mechanisms are disrupted by various pathological conditions and there is need for antioxidant supplements (Hazra *et al.*, 2008).

Diabetes is associated with an increased propensity for infections and factors contributing to infections in diabetics include: defective phagocytic capabilities of polymorphonuclear leukocytes (PMN) and ketoacidosis (Joshi and Mahajan 2003). Despite the advances in diabetes management and availability of newer insulin, infections still accounts for much of the death in diabetics. Studies on plants used in folkloric medicine have indicated the presence of phytochemicals such as saponins, phenolics, flavonoids, tannins, coumerins and terpenoids with varying degree of antioxidative potential (Saeed *et al.*, 2012). Though synthetic antioxidants are available, recently attention has been shifted to natural antioxidant and antimicrobial sources due to toxicity concern (Ebrahimzadeh *et al.*, 2008). Although the

toxicity profiles of most medicinal plants have not been scientifically investigated, it is generally accepted that plant-derived medicines are safer than synthetic medicines (Oluyemi *et al.*, 2007).

Citrullus colocynthis (CC) also known as bitter apple is a desert plant of the family *Cucurbitaceae*. It is a desert viny plant from tropical Asia and Africa and it is naturally adapted to arid environments (Jayaraman and Christina, 2008). It resembles a common watermelon (*Citrullus lanatus*) vine but bears small, hard fruits with a bitter pulp. Traditionally the fruit is used in the treatment and management of diarrhea, constipation, diabetes, edema, fever, jaundice and bacterial infections. Its hydro-ethanol extract of the pulp has been reported to show antidiabetic effect (Dallak *et al.*, 2009), and its antimicrobial activity has also been reported (Gurudeeban *et al.*, 2011; Bnyan *et al.*, 2013; Mehta *et al.*, 2013).

Circuligo pilosa (CP) belongs to the family of *Hypoxidaceae* and is an herbaceous plant with stout, erect rhizomes bearing a cluster of grass-like leaves to 60 cm long and flower shoots to 20 cm at the end of the dry season. The rhizomes of this plant possess medicinal properties and are used as food. In the Southwestern Nigeria, *C. pilosa* is used as a purgative and in treatment and management of hernia, infertility, diabetes, genital infections and sexually transmitted infections especially gonorrhoea. It is also traditionally used in the production of infant food and sorghum beer in West Africa (Sofodiya *et al.*, 2011), its anticandidal property has also been reported (Gbadamosi and Egunyomi, 2010).

Gladiolus psittacinus (GP) belongs to the family of *Iridaceae* and it is commonly called Sword lily or Maid of the mist. Traditionally it is used as remedy for cold, dysentery, asthma, gonorrhoea and intestinal parasites (Adjanohoun *et al.*, 1991), also it is used in some parts of Nigeria to manage diabetes (Abo *et al.*, 2008) and the methanol extract of its corm has been reported to lower blood glucose sugar in

alloxan-induced diabetic rats (Adediwura and Kio, 2008).

Corn steep liquor is a waste product of wet corn milling. It is an important component of growth media and use for culturing of organisms. It is an important solvent in traditional medicine of Yoruba tribe of Nigeria, where it is used to form infusion in treatment and management of typhoid, malaria, diarrhea and diabetes. Despite the wide uses of corn steep liquor in making various infusions for the treatment of diseases traditionally, there is paucity of scientific information to justify its use. The aim of this present study is to evaluate the *in vitro* antioxidant and antimicrobial activity of three medicinal infusions prepared using corn steep liquor.

2. RESULTS AND DISCUSSION

2.1 Preparation of corn steep liquor: 1000g of dried-corn was soaked in 3L hot water (100°C) for 72 hr, after 72hr the soaked corn was milled and filtered using muslin cloth. The filtrate was allowed to settle for 24hrs, the supernatant was decanted and this is “Corn steep liquor”

2.2.1 Plant materials: *Citrullus colocynthis* fruit, *Circuligo pilosa* rhizome, and *Gladiolus psittacinus* corm were purchased from Bodija market, Ibadan and authenticated in Botany Department, University of Ibadan.

2.2.2 Extraction: *Citrullus colocynthis* pulp was freeze dried while *Circuligo pilosa* rhizome, and *Gladiolus psittacinus* corm were air-dried, the dried materials were grinded using electric blender. 100g of powdered material(s) were extracted with 1.5litre of corn steep liquor. After 72hr, the extracts were filtered using muslin cloth and the filtrates were concentrated using rotary evaporator. The extracts were kept in refrigerator until use.

2.2.3 Test Micro-organisms: Six clinical-isolated microorganisms namely *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiellae pneumoniae* obtained from University College Hospital (UCH), Ibadan were used.

2.3 Hydrogen peroxide scavenging activity:

The ability to scavenge hydrogen peroxide was estimated according to the method of Ruch *et al.* 1989. Extract (100-600µg/mL) were added to 3.0mL hydrogen peroxide (40mM) prepared in phosphate buffer (50mM, pH 7.4) and the absorbance were measured at 230nm after 10 min incubation at room temperature against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging ability is calculated as follows:

Hydrogen peroxide scavenging activity = $\frac{\text{(Abs control - Abs sample)}}{\text{(Abs control)}} \times 100$

2.4 Total phenolics: The total phenolics content was determined by the spectrophotometric method (Kim *et al.*, 2003). 1ml of extract (1µg/ml) was mixed with 1ml of Folin-ciocalteu phenol reagent. After 5min, 10ml of 7% of Na₂CO₃ solution was added to the mixture followed by addition of 13ml of distilled water and mixed thoroughly. The mixture was kept in the dark for 90min at 25⁰C after which the absorbance was read at 750nm. Total phenolic content was evaluated from a Gallic acid standard curve and expressed as Gallic acid (GAE) equivalent/ g extract.

2.5 Total flavonoid: Total flavonoid was estimated spectrophotometrically using the earlier method of Zhishen *et al.*, 1999 as modified by Talukdar, 2013. 0.5ml of 2% ethanolic AlCl₃ (aluminium chloride) solution was added to 0.5 ml of extract (1µg/ml). After 45min incubation at room temperature, the absorbance of the reaction mixture was measured at 420 nm. Total phenolic content was evaluated from a Quercetin standard curve and expressed as Quercetin (QUE) equivalent/ g extract

2.6 Reducing power: Reducing power was estimated according to the method of Oyaizu, 1986. 1ml of extract (100µg/ml-1000µg/ml) was mixed with 0.5ml phosphate buffer (0.2M, pH 6.6) and 0.5ml potassium ferricyanide (0.1%), followed by incubation at 50⁰C for 20 min. After which 0.5ml of TCA (10%) was added to terminate the reaction. Upper portion of the solution (1ml) was mixed with 1ml

distilled water and 0.1ml of FeCl₃ solution (0.01) was added. The reaction mixture was allowed to stand 10 min at room temperature before absorbance was read at 700nm. BHA was used as standard. A higher absorbance of the reaction mixture indicated greater reducing power.

2.7 DPPH radical scavenging activity: DPPH radical scavenging activity was estimated according to the method of Wettasinghe and Shahidi, 2000. 1ml extract (100µg/ml-1000µg/ml) was added to 3.9ml of DPPH (0.025g/l prepared in methanol). The samples were shaken and allowed to stand in dark room for 35min and absorbance was read at 517nm. DPPH scavenging ability was calculated from DPPH radical scavenging activity = $\frac{\text{(Abs control - Abs sample)}}{\text{(Abs control)}} \times 100$ DPPH solution without extract serves as control. Ascorbic acid was used as standard.

2.8 Metal chelating activity: The chelation of ferrous ions was estimated using the method of Dinis *et al.*, 1994 with slight modifications. 0.5 mL ferrous chloride (0.2 mM) was added to 0.2mL ferrozine (5mM). The reaction was started by the addition of 0.1ml (20µg/ml-100µg/ml) of the extract and incubated at room temperature for 10 min and the absorbance was measured at 562 nm. EDTA was used as a positive control

Metal chelation ability = $\frac{\text{(Abs control - Abs sample)}}{\text{(Abs control)}} \times 100$

2.9 Estimation of antimicrobial activity: The stock microorganisms were inoculated into sterile nutrient broth and incubated at 37⁰c for (18-24hr), the innoculum suspension were standardized to give a dilution of 1:100 of the stock micro organisms, 0.2ml were taken into the prepared sterile nutrient agar, then aseptically poured into sterile petri dishes. Using a sterile cork borer of 8mm diameter, wells were made according to the number of graded concentrations of the extracts. In each well, the different graded concentration of the sample were applied. The dishes were allowed to stay on the bench for 2 hours to allow pre-diffusion and then incubated at 37⁰C for 24 hours. Gentamicin and distilled water were

used as positive and negative control respectively.

Statistical Analysis: All data are given as the mean \pm SD of three measurements. The significance of the differences between the means of the samples were established by the analysis of variance ($P < 0.05$). IC_{50} was evaluated from Microsoft excel (2007) and graphs were drawn with graph pad prism 6.

3. RESULTS AND DISCUSSION

3.1 DPPH radical scavenging activity

DPPH is a stable free radical which dissolve in methanol to give a characteristic absorption band at 517-520nm. This assay shows the ability of the extracts to convert the DPPH to its reduced form Diphenylpicrylhydrazine with the loss of its violet colour. When a solution of DPPH is mixed with that of a sample that can donate a hydrogen atom, it is converted to its reduced form with the loss of this violet colour (Alam *et al.*, 2013). Although all our extracts showed scavenging activity on DPPH radical in the order: CP > GP > CC, their activities were found to be lower than that of the standard (AsA) (Figure1)

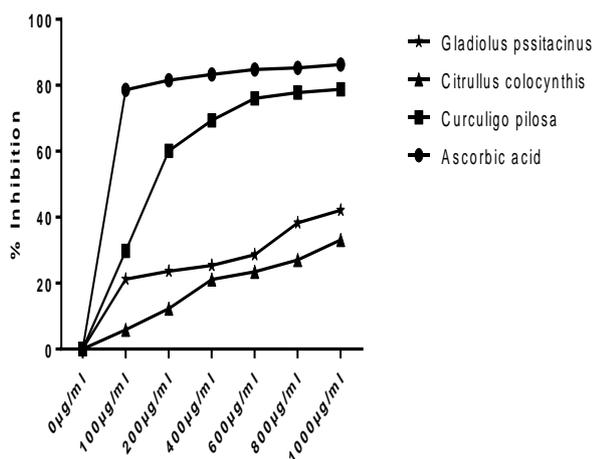


Fig. 1: DPPH radical scavenging activity of CSL extracts of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus*. Data are expressed as mean (n=3)

3.2 Total phenolics

Phenolic compounds are powerful chain breaking antioxidants (Shahidi and Wansundeara, 1992) and they are also an important plant metabolite because of their antioxidative capacity (Hatano *et al.*, 1989). In CSL extracts of *Citrullus colocynthis*, *Gladiolus psittacinus* and *Curculigo pilosa*, the phenolics content were 29.74mg/GAE, 47.08mg/GAE and 84.96mg/GAE equivalent respectively (Figure 2).

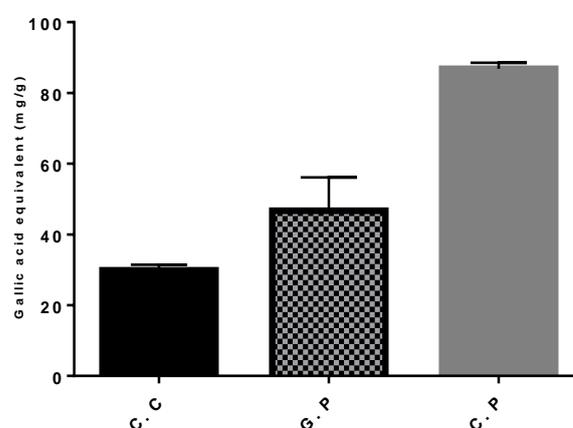


Fig. 2: Total phenolics content of CSL extract of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus*. Data are expressed as mean + SD (n=3)

3.3 Total flavonoid

Flavonoids are polyphenolic compounds, which exhibit several health effects such as antioxidant, anti-inflammatory, antiviral and anticancer (Umamaheswari and Chatterjee, 2008).

The antioxidative potentials of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions and inhibition of enzymes responsible for free radical generation (Benavente-Garcia *et al.*, 1997). The flavonoid contents of CSL extract of *Citrullus colocynthis*, *Gladiolus psittacinus* and *Curculigo pilosa* were 34.5mg/QUE, 55.9mg/QUE and 36.97mg/QUE equivalent respectively Figure 3.

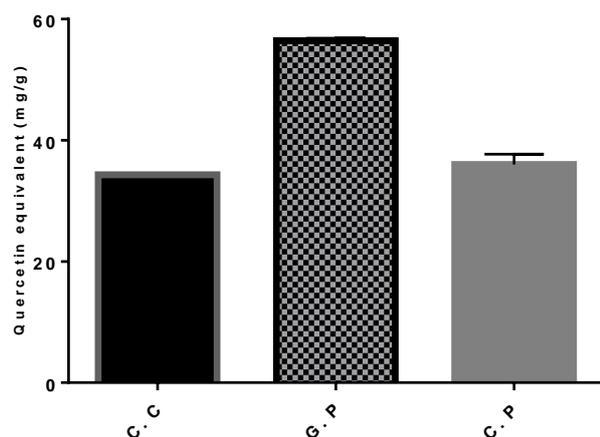


Fig. 3: Total flavonoids content of CSL extract of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus*. Data are expressed as mean + SD (n=3)

3.4 Reducing power

Reducing power activity is usually used to evaluate the ability of natural antioxidants to donate electrons (Dorman *et al.*, 2003). It is based on ability of antioxidant to form coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, the presence of antioxidants in the extract would result in the reduction of Fe^{3+} to Fe^{2+} upon donation of an electron. Reducing power is monitored by measuring the formation of Perl's Prussian blue at 700nm (Ebrahimzadeh *et al.*, 2008).

The extracts showed concentration-dependent curve for reducing power (Figure 4), all the extracts exhibited a remarkable reducing potential but they are far lesser than that of the standard antioxidant (Butylated-hydroxyanisole).

The reducing capacity of a compound has always served as a significant indicator of potential antioxidant activity of an extract (Hazra *et al.*, 2008) figure 4.

3.5 Iron chelating capacity

Figure 5 shows the result of iron chelating capacity of the extracts.

All our extracts exhibited an appreciable iron chelating capacity, the activity follow the same trend as DPPH. Ferrozine can form a complex with a red colour by chelating with Fe^{2+} , The

complex formation is disrupted in the presence of other chelating agents with reduction in the intensity of red colour of the complex. Estimation of the colour reduction determines the chelating activity of the extract (Soler-Rivas *et al.*, 2000).

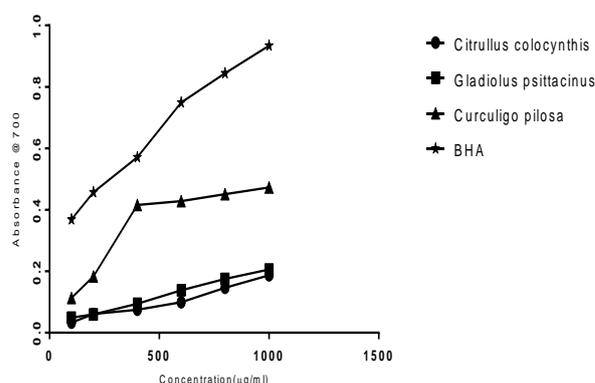


Fig. 4: Reducing power of CSL extracts of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus*. Data are expressed as mean (n=3)

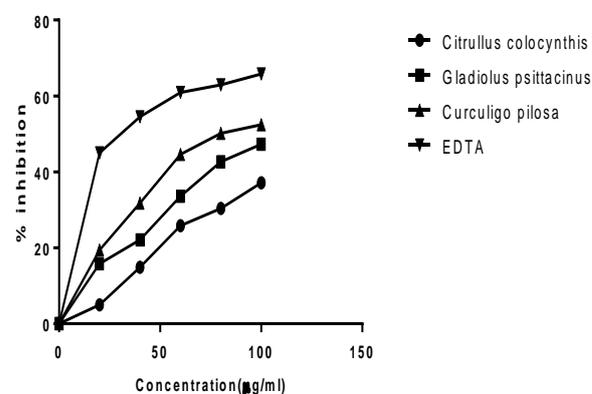


Fig. 5: Iron chelating capacity of CSL extracts of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus*. Data are expressed as mean (n=3)

Report has shown that the chelating agents which forms σ bond with a metal are effective as antioxidants, because they cause reduction of the redox potential thereby stabilizing the oxidized form of the metal ion (Jamuna *et al.*, 2012). The IC_{50} of radical scavenging and iron chelating of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus* is shown in Table 1.

Table 1: IC₅₀ of radical scavenging and iron chelating of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus* (µg/ml).

	CC	CP	GP	AsA	EDTA
DPPH	1389±8.88 ^d	174±7.0 ^b	1209±3.60 ^c	12±1.73 ^a	-
Hydrogen peroxide	142±5.56 ^d	110±3.61 ^c	95±2.65 ^b	29±4.51 ^a	-
Iron chelating	120±1.73 ^d	72±1.53 ^b	94±4.51 ^c	-	23±2.0 ^a

Data are expressed as mean + SD (n=3). Means followed by the same letter within the row are not significantly different (P<0.05).

Table 2: Antibacterial activity of corn steep liquor of *Citrullus colocynthis*, *Gladiolus psittacinus* and *Curculigo pilosa*

Extracts	Conc.(mg/ml)	S.a	E.coli	B.sub	Ps.a	Kleb	Sal
CC	100	12	14	-	-	14	14
	50	10	10	-	-	12	12
	25	-	-	-	-	10	10
	12.5	-	-	-	-	-	-
GP	100	12	-	-	-	12	-
	50	10	-	-	-	10	-
	25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
CP	100	12	-	-	-	14	10
	50	10	-	-	-	10	10
	25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
Distilled water		-	-	-	-	-	-
Gentamicin	(10µg/ml)	38	36	38	40	38	36

S. a = *Staphylococcus aureus*, Ps. a = *Pseudomonas aeruginosa*, E. coli = *Escherichia coli*, Sal. = *Salmonella typhi*, B. sub = *Bacillus subtilis* and Kleb. = *Klebsiellae pneumoniae*. Data are expressed as mean (n=3)

3.6 Hydrogen peroxide scavenging activity

The scavenging effect of the extracts on hydrogen peroxide were dose dependent (100-600µg/ml) and is in order of G.P> C.C> C.P as shown in fig. 6.

Scavenging of H₂O₂ by the extracts may be attributed to their phenolics, which can donate electrons to H₂O₂, thus converting it to water (Shahriar *et al.*, 2012).

3.7 Antimicrobial activity

The result showed that the all extracts were able to inhibit *Staphylococcus aureus* and

Klebsiellae pneumoniae, CC and CP inhibited *Salmonella typhi* while *Escherichia coli* was inhibited by CC alone at 0mg/ml. *Bacillus subtilis* and *Pseudomonas aeruginosa* were not inhibited by the extracts at any concentration (Table 2).

These observations demonstrated that the Corn steep liquor extracts of *Citrullus colocynthis* pulp, *Curculigo pilosa* rhizome, and *Gladiolus psittacinus* corm possess good antimicrobial activity.

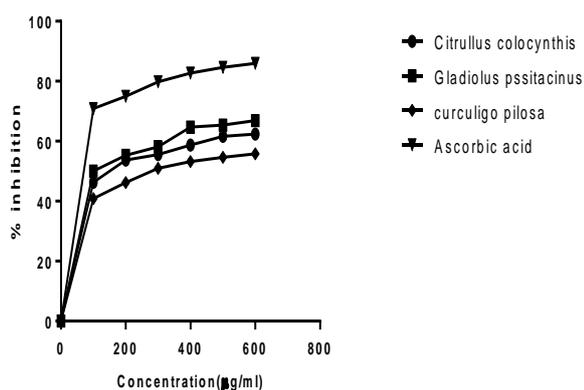


Fig. 6: Hydrogen scavenging activity of CSL extracts of *Citrullus colocynthis*, *Curculigo pilosa*, and *Gladiolus psittacinus*. Data are expressed as mean (n=3)

4. CONCLUSIONS

The study provides a scientific rationale for the traditional use of Corn steep liquor extracts of *Citrullus colocynthis* pulp, *Curculigo pilosa* rhizome, and *Gladiolus psittacinus* corm in the management and treatment of diabetes mellitus. However *in vivo* studies are still needed to fully understand the activities of the extracts before clinical use.

5. REFERENCES

- [1] Abo, K.A., Fred-Jaiyesimi, A.A., Jaiyesimi, A.E.A (2008): Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *Journal of ethnopharmacology* **115**: 67–71.
- [2] Adediwura, F., Kio, A. (2008). Antidiabetic activity of *gladiolus psittacinus* in alloxan induced diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicine* **5**: 154 – 157.
- [3] Adjanohoun, E, Ahyi, M.R.A., Ake-Assi, L., Elewude, J.A., Dramane, K., Fadoju, S.O., Gbile, Z.O., Goudole, E., Johnson, C.L.A., Keita, A., Morakinyo, O., Ojewole, J.A.O., Olatunji, A. O., Sofowora, E.A..(1991). Traditional medicine and pharmacopoeia. Contribution to ethnobotanical floristic studies in Western Nigeria. Nigeria: Pub. Organization of African Unity, Scientific Technical and Research Commission Lagos; 420p.
- [4] Alam, M.N., Bristi, N.J., Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal* **21**: 143–152.
- [5] Benavente-Garcia, O., Castillo, J., Marin, F.R., Ortuno, A., Del-Rio, J.A. (1997). Uses and properties of Citrus flavonoids. *Journal of Agricultural and Food Chemistry* **45**: 4505-4515.S
- [6] Hazra, B., Biswas, S., Mandal, N. (2008). Antioxidant and free radical scavenging activity of *Spondias pinnata*. *Journal of Biological Medicine* **8**: 63-67.
- [7] Brynan, I., Hamid, H., Mufeed, E., (2013). Antibacterial activity of *Citrullus Colocynthis* against different types of bacteria. *Advances in Life Science and Technology* **7**: 48-51.
- [8] Dallak, M., Al-Khateeb, M., Al-Hashem, F., Bashir, N., Abbas, M., Elessa, R. (2009). *In vivo*, acute, normo-hypoglycemic, antihyperglycemic, insulinotropic actions of orally administered ethanol extract of *Citrullus colocynthis* (L.) schrab pulp. *American Journal of Biochemistry and Biotechnology*. **5**:118–125.
- [9] Dinis, T.C.P., Madeira, V.M.C., Almeida, L.M. (1994). Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Archives of Biochemistry and Biophysics* **315**: 161–169.
- [10] Dorman, H.J.D., Peltoketo, A., Hiltunen, R., Tikkanen, M.J. (2003). Characterisation of the antioxidant properties of deodourisation aqueous extracts from selected *Lamiaceae*
- [11] Ebrahimzadeh, M.A., Pourmorad, F., Hafezi, S. (2008). Antioxidant activities of Iranian Corn Silk. *Turkish Journal of Biology* **32**: 43-49.
- [12] Gbadamosi, I.T., Egunyomi, A. (2010). Phytochemical screening and *in vitro* anti-candidal activity of extracts and essential oil of *Curculigo pilosa* (Schum and Thonn) Engl. Hypoxidaceae. *African Journal of Biotechnology* **9**: 1236-1240.
- [13] Gurudeeban, S., Ramanathan, T., Satyavani, K., Dhinesh, T. (2011). Antimicrobial effect of coastal medicinal plant *Citrullus colocynthis* against pathogenic microorganisms. *African Journal of Pure and Applied Chemistry*. **5**:119-122.
- [14] Harman, D. (1998). Free radical theory of aging. Current status. Amsterdam: Elsevier; p 3–7.
- [15] Hatano, T., Edamatsu, R., Mori, A. (1989). Effect of interaction of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chemical and Pharmaceutical Bulletin* **37**: 2016-2021.
- [16] Jamuna, S., Paulsamy, S., Karthik, K. (2012). Screening of *in vitro* antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae) *Journal of Applied Pharmaceutical Science* **2**: 149-154.
- [17] Jayaraman, R., Christina, A.J.M. (2008). Evaluation of *Citrullus colocynthis* fruits on *in vitro* antioxidant activity and *in vivo* DEN/PB induced

- hepatotoxicity. *International Journal of Applied Research in Natural Products* **6**: 1-9.
- [18] Kim, D.O., Jeong, S.W., Lee, C.Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* **81**: 321–326.
- [19] Mehta, A., Garima, S., Sumita, K., Meenakshi, S., Kothari, S.L. (2013). Antimycobacterial activity of *Citrullus colocynthis* (L.) Schrad against drug sensitive and drug resistant *Mycobacterium tuberculosis* and MOTT clinical isolates. *Journal of Ethnopharmacology* **149**: 195–200.
- [20] Saeed, N., Khan, M.R., Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine* **12**: 221.
- [21] Shahriar, M., Akhter, S., Hossain, M.I., Haque, M.A., Bhuiyan, M.A. (2012). Evaluation of in vitro antioxidant activity of bark extracts of *Terminalia arjuna*. *Journal of Medicinal Plants Research* **6**: 5286-5298.
- [22] Sofidiya, M.O., Oduwole, B., Bamgbade, E., Odukoya, O., Adenekan, S. (2011). Nutritional composition and antioxidant activities of *Curculigo pilosa* (Hypoxidaceae) rhizome. *African Journal of Biotechnology* **10**: 17275-17281.
- [23] Joshi, N., Mahajan, M. (2003). Infections and diabetes. In *Textbook of Diabetes*. Pickup JC, Williams G, Publ (Eds). Blackwell Publishing, 3rd edition. pp. 40.1-40.16.
- [24] Oluyemi, K.A., Okwuonu, U.C., Baxter, D.G., Oyesola, T.O. (2007). Toxic effects of methanolic extract of *Aspilia Africana* leaf on the estrous cycle and uterine tissues of Wistar rats. *International Journal of Morphology* **25**:609–614.
- [25] Oyaizu, M. (1986). Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition* **44**: 307-315.
- [26] Ruch, R.J., Cheng, S.J., Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* **10**: 1003-1007.
- [27] Shahidi, F., Wanasundara, P.K. (1992). Phenolic antioxidants. *Food Science and Nutrition* **32**: 67-103.
- [28] Soler-Rivas, C., Espin, J.C., Wichers, H.J. (2000). An easy and fast test method to compare total free radical scavenging capacity of foodstuffs. *Phytochemical Analysis* **11**: 330–338.
- [29] Talukdar, D. (2013). *In Vitro* antioxidant potential and Type II Diabetes related enzyme inhibition properties of traditionally processed legume-based food and medicinal recipes in Indian Himalayas. *Journal of Applied Pharmaceutical Science* **3**:26 – 32.
- [30] Umamaheswari, J., Chatterjee, T.K. (2008). In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicine* **5**: 61-73.
- [31] Wettasinghe, M., Shahidi, F. (2000) Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chemistry* **70**: 17–26.
- [32] Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* **64**:555-559.