Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.119

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *DIOSCOREA BULBIFERA* L. FRUITS

M. Mohanty[1, 2], R. Choudhury[1], S. Kumar[1] and R. Maggirwar[3*]
[1]Biodiversity & Conservation Lab., Ambika Prasad Research Foundation, India,
[2]School of Applied Sciences, Centurion University of Technology & Management (CUTM), Odisha, India
[3]PG Dept of Botany Shri Shivaji Science College, Amravati (MS) India
*E-mail: dr.krekha@yahoo.com

(Date of Receiving-05-12-2020; Date of Acceptance-15-03-2021)

ABSTRACT

Dioscorea bulbifera (Pita aalu) is a medicinal plant belongs to family *Dioscoreaceae*, used to cure various diseases like malaria, diarrhoea, diabetes, dysentery, cancer, piles and skin infections. We designed the present study to analyse phytochemical and antibacterial activity of *D. bulbifera* fruit so as to conform its edible property. For this we followed phytochemical screening of the fruit extracts and found that it is consist of tannin, saponin, phenols, steroids, terpenoids and some carbohydrates in high concentration. The antibacterial activity of aqueous extract of the fruit against *Streptococcus mutans* pathogen was determined by using agar well diffusion, disc diffusion and broth dilution assay. From the results it was confirmed that the aqueous extract of the fruits of *D. bulbifera* showed high inhibition zone at 100µg/ml is 19 mm in agar well diffusion, 14 mm in disc diffusion assay. The minimum inhibitory concentratiom (MIC) of extract of *D. bulbifera* arranged in respect to the parts from 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500µg/ml and inoculums control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to absence of bacteria. Presence of primary and secondary metabolites indicates the nutraceutical value of *D. bulbifera* fruits. So the fruits of *D. bulbifera* can be used as a tool against antimicrobial resistance (AMR).

Keywords: Agar well diffusion, Antibacterial, Disc diffusion, Medicinal values, MIC, Phytochemistry.

INTRODUCTION

The common name of *D. bulbifera* is air potato, air yam, bitter yam and cheeky yam (Shajeela *et al.,* 2011). It is usually found in India, Maldives, China, Japan and Indonesia. It is the most edible food crop of West Africa. These are climbing, perennial, erect herbs or shrubs. Stems are woody about 10 m long. Leaves are simple, opposite, lobed, attractive, alternate, small, pale green and arising from leaf axis. It is about 20 cm long and reticulate venation is found. Stems are herbaceous, arises from the underground tubers. Toxic substances and alkaloid dioscorine present in the aerial bulb. Flowers are generally small, minute, dioecious and very rarely bisexual (Tindall 1983). Male and female flower occurred in different plants (Wagner *et al.,* 1999; Miller 2003). *D. bulbifera* is generally edible plants containing tubers, bulbils and fruits (Adama *et al.,* 2017). West Africa is known as the native place of *D. bulbifera*. It was introduced by Department of Agriculture, United State in 1967. In East Indies, the plant was reported as "naturalized and cultivated" by A. H. R. Grisebach (Grisebach 1864). It can be found more in moist forest, hardwood forest and urban forest but less in pinelands (Moriwasa 1999). Nowadays it is used as the source of food for commercial distribution (Bhandari & Kawabata 2005). *D. bulbifera* is generally cultivated in tropical, sub-tropical and moist containing area. The plant is cultivated in low temperature of about 20°C to 30°C and it can also grow in 12°C to 38°C. Loamy soil and high organic materials are best for the cultivation of *D. bulbifera* (Martin 1974; Wilkin 2001). Sexual reproduction is very rare in this plant and occurred by seeds and asexual reproduction occurred by bulbils (Miller 2003). *D. bulbifera* fruits are found during June to October. Fruits are capsulated, 3-winged, 2.5 m long. These are very light in weight and green in color. When fruits become dry, their colour changes to light brown. Fruits normally develop from the twining stem. Major types of nutrients present in the *D. bulbifera* are carbohydrates, ascorbic acid, amino acid, cystein, argenine, histidine and glutamic acid. The tuber is rich in protein, hence can be used as food (Arinathan *et al.,* 2009). The phytochemistry of *D. bulbifera* varies with their geographical distribution. Due to presence of antioxidants, it gives energy to the body and is antagonistic to diabetes and cancer. Some non-enzymatic antioxidants are found in *D. bulbifera* like Vitamin-E and Vitamin-C (Suriyavathana 2011). Flavonoids are extracted from the rhizome of the plant using 75% of ethyl acetate (Gao *et al.,* 2002). Steroid and saponin is extracted by using 95% of the ethanolic extract (Liu *et al.,* 2010). Diterpenoids are extracted from the methanolic extract of bulbils (Kuete *et al.,* 2012). It also used in the field of nanobiotechnology and gold nanoanisotrops (Ghosh *et al.,* 2012; Kokni *et al.,* 2016). This plant has a special type of phytochemistry in which reducing agents like ascorbic acid, citric acid, phenolics and capping agents like starch, saponin *etc.* are used in the reduction, stabilization and evolution of the shape of nanoparticles (Nwoke 2019). *D. bulbifera* is commonly used as food in the tropical region. The tubers are roasted and cooked as vegetables and it can be taken as food by pigs. Bulbils are the best food for the diabetic patients (Karnick 1969) and root powder is the best medicine for tuberculosis. For the typhoid patients bulbils are the best

precaution to prevent the disease (Abhyankar & Upadhyay 2011). It is also used to compose birth control pills due to presence of diosgenin *i.e.* a steroidal saponon (Oboh *et al.,* 2001). It is also utilized for the treatment of parasitic and fungal infections. Leaves are used to treat against pink-eye. Dioscorine, a neurotoxin, has been found in tubers, alkaloids are detected in leaves, saponin, diosgenin like other pharmacologically active substances are found in *D. bulbifera* (Burkill 1985).

MATERIALS AND METHODS

The *D. bulbifera* plant species was selected as per availability and consumption rate among the rural and tribal communities of study area (Khordha). The experimental plant species of the region were identified following flora's books (Kumar & Jena 2017) and published article (Kumar *et al.,* 2013; Haines 2006; Saxena *et al.,* 1994; Kumar *et al.,* 2012).

Preparation of plant extracts

Soxhlet method was adopted to obtain the plant extracts. The fruits of *D. bulbifera* was collected and dried at room temperature under shade and powdered after drying them by using mechanical devices. The powdered fruit extracts of the plant was kept in thimble and extraction was carried out using the Soxhlet apparatus. The residue was collected and left for air drying and dried crude extracts were stored in refrigerator for further experimental work (Tiwari & Das 2011; Harborne 1998; Sofowora 1993).

Table 1: Phytochemical screening in different extracts of fruits of *D. bulbifera.*

D.bulbifera fruit extract	saponin	Tannin	Phenolic compound	terpenoids	steroids
Aqueous	+ve	+ve	+ve	-ve	-ve
Ethanol	-ve	+ve	-ve	-ve	-ve
Methanol	-ve	+ve	-ve	-ve	-ve
Acetone	-ve	+ve	-ve	-ve	-ve
N-hexane	-ve	-ve	-ve	-ve	-ve

Table 2: Estimation of Minimum Inhibitory Concentration (MIC)

Bacteria	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
S. mutans	Growth	No Growth	No Growth	No Growth	No Growth
Broth	No growth	No growth	No growth	No growth	No growth
Inoculum (Bacterial broth)	Growth	Growth	Growth	Growth	Growth

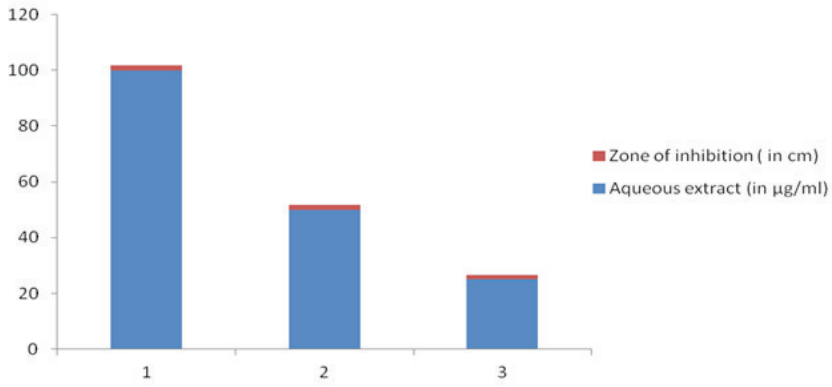

Figure 1: Antibacterial activity of *D. bulbifera* extract against *S. mutans* (Agar well diffusion assay of aqueous extract) (mean values; n=3)

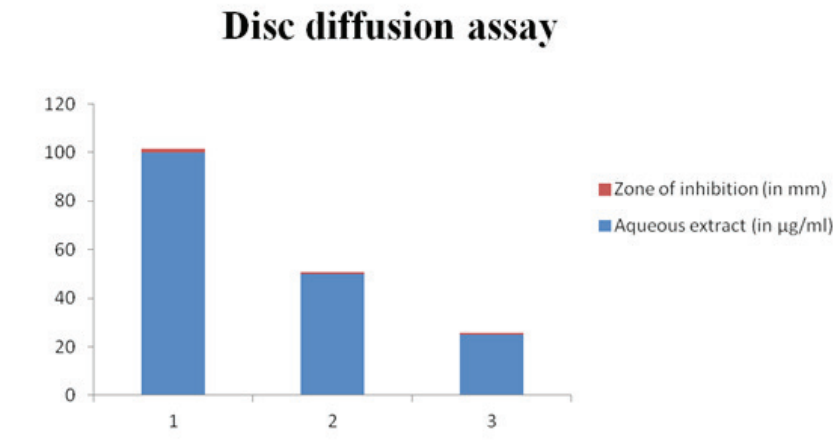

Figure 2: Disc diffusion assay (mean values; n=3)

Test for Tannin

0.5 g of dried powder sample was boiled in 10 ml of distilled water and filtered with whatman 42 filter paper. 2 ml of filtrate solution was taken in a test tube and 3 to 5 drops of 0.1% of ferric chloride solution was added. The brownish green or blue black colouration indicated the presence of tannin.

Test for Saponin

0.5 g of dried powder sample was boiled in 10 ml of distilled water and filtered with whatman 42 filter paper. 5 ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponin.

Test for Phenolic compounds

0.5 g of extract was treated with 3 to 5 drops of 1% ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

Test for Terpenoids

6 ml of extract was mixed in 2.5 ml of chloroform and 3 ml of concentrated sulphuric acid was added. A radish brown colouration of interface indicated the presence of terpenoids.

Test for Steroids

2 ml of plant extract was dissolved in 5 ml of chloroform and then 5 ml of concentrated sulphuric acid was added. Formation of two phases (upper red and lower yellow with green fluorescence) indicated the presence of steroids.

Antibacterial activity

The fruit extract of *D. bulbifera* plant was screened for antibacterial activity against a Gram- positive bacteria *S. mutans* (MTCC 497). The MTCC (Microbial Type Culture Collection) bacterial strain was collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was done using slight modification of standard methods of Agar Well Diffusion assay (Irshad 2012), Disc Diffusion method (Yasin *et al.,* 2013; Zaidan *et al.,* 2005; Thompson *et al.,* 2013) and Broth Dilution assay (MIC) (Wiegand *et al.,* 2008; Deaker *et al.,* 2011).

Media

Nutrient broth was used to maintain broth culture. The constituents of the nutrient broth included 0.5 g NaCl, 0.5 g peptone and 0.3 g beef per 100 ml. An additional 1.5 g of agarwas added to make up the nutrient agar medium (Deaker *et al.,* 2011).

Agar Well Diffusion Assay

Agar well diffusion asssay was followed to test the antibacterial activity of fruit extracts of *D. bulbifera* against a bacterial strain. Nutrient agar plates were prepared as per manufacturer's instructions. 100 µL of nutrient broth cultures of the test microbes prepared a day before, were poured on the plates uniformly and a lawn culture was prepared using a sterile spreader in a laminar hood. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100% DMSO (two fold serial dilution were made in amount of 100 µL per well ranged from 0.5-2.0 mg/ml). 100 µL of samples were added by sterile syringes into the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 hrs. Plates were incubated at 35±2°C for 18-24 hrs. Kanamycin and Ampicillin served as standard antibiotics control. Triplicates were maintained and the experiment was repeated thrice. For each replicates the readings (diameter of zone of inhibition of mm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded (Irshad *et al.,* 2012).

Disc Diffusion Assay

Antibacterial activity using Disc diffusion assay was done using the 6 mm of disc prepared from whatman filter paper. Each extracts were dissolved in dimethyl sulfoxide. The sets of three dilutions (0.5, 1.0, 2.0 mg/ml) of crude extracts and standard drugs were prepared. 6 mm of discs was kept in the drugs for 12 hr before placing to the agar plates. The zones of growth inhibition around the disc were measured after 18 to 24 hrs of incubation at 37°C for bacteria. The sensitivities of microbial species to the fruit extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disc) on the agar surface around the disc and values less than 8 mm were considered as not active against microorganisms (Yasin *et al.,* 2013; Zaidan *et al.,* 2005; Thompson *et al.,* 2013; Wiegand *et al.,* 2008).

Preparation of working slant

Stock culture of MTCC 3906 are maintained at 4°C on slants of semi-solid media containing 1.5 % of agar-agar, 0.3 % beef extract and 0.5 % peptone. Active working cultures for experiments were prepared by transferring a loopful of culture mass from the stock. Slants were incubated for 24 h at 36 ±1.0ºC.

Broth preparation

Colonies of prepared slants of MTCC 3906 are picked off using sterile loop and inoculated in sterile conditions in autoclaves cool liquid both medium containing 0.3 % of beef extract and 0.5 % peptone. The broth was incubated for 24 hrs at 36 ±1.0ºC until there was visible growth indicated by turbidity standard (Deaker *et al.,* 2011).

Swabbing and Inoculation of drugs

Swabbing with autoclaved cotton swab was done using broth strain on petri plates. Wells (6 mm) were made using sterile borer for Agar cup well method. Stock solutions of samples were prepared in 100 % DMSO and twofold serial dilutions were made in amount of 100 µL/well ranged from 0.5, 1.0, 2.0 mg/ml. 100 µl of samples were added by sterile syringes in to the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 hr. Only the solvent (DMSO) was poured in to the wells in another set of plates as part of negative control (Amanda et al. 2012). The positive control set consisted of standard antibiotics Kanamycin. For the Disc diffusion assay, only swabbing was done using sterile swab. The discs of respective aforesaid concentration were placed on media. Both petri plates (for Agar well diffusion & Disc diffusion method) were incubated at 36 ± 1.0º C for 18 hrs. Zones of inhibition free microbial growth appeared around each well and disc in the form of clear rings which confirmed the antibacterial activity of the respective samples. Those samples which did not have any inhibitory effect on the microbe did not form any clear ring. In this way the antibacterial activity of the samples was confirmed. Triplicates were maintained and the experiment was repeated thrice. For each replication the readings (zone of inhibition) were taken and the mean values were recorded (Rose *et al.,* 1939).

Data analysis

Mean and SD (standard deviation) was calculated taking triplicate values of zone of inhibition (mm for agar well diffusion assay; mm for disc diffusion of samples using Excel, Microsoft Corporation-2010, US).

MIC using Broth Dilution Assay

The fruit extracts of *D. bulbifera* plant was screened for their antibacterial activity. Antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method. Selected colonies of aforesaid bacteria were picked off to a fresh isolation plate and inoculated into the corresponding tubes containing 5 ml of trypticase soy broth. The broth was incubated for 8 ± 1 hrs at 35 ± 2°C until there was visible growth. Mc Farland No.5 standard and PBS (Phosphate buffer saline) were used to adjust the turbidity to get 10^5 cfu/m (Wiegand *et al.,* 2008; Deaker *et al.,* 2011).

Data Interpretation

After incubation, the tubes showing no visible growth after 8 hrs till 12 hrs were considered to be inhibition of bacteria which represent MIC values of a respective concentration. Triplicates were maintained and the experiment was repeated thrice, for each replicates. The readings were taken as foresaid.

RESULTS AND DISCUSSION

Phytochemical screening of *D. bulbifera* fruits in different extract showed that tannin is most prevalently present in it as compared with other secondary metabolites. Aqueous extract of *D. bulbifera* shows best result in phytochemical screening (Table 1).

The aqueous extract of the fruits of *D. bulbife*ra shows high inhibition zone at 100µg/ml is 19 mm (Figure 1) in agar well diffusion and 14 mm in disc diffusion assay (Figure 2). The MIC of extract of *D. bulbifera* arranged in respect to the parts from 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, 500µg/ml and inoculums control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to absence of bacteria (Table 2).

CONCLUSION

Adequate Food and medicines are burning issues in world. Growing population and anti-microbial resistance create a need to screen new nutraceuticals having food and medicinal values. Keeping this in view, present study has designed and as per traditional potentials, analysis was carried out. Results concluded that the fruits of *D. bulbifera* are rich with primary and secondary metabolites. Hence, it might be a potential nutraceutical for future.

REFERENCES

Abhyankar, R.K. and R. Upadhyay (2011) Ethnomedicinal studies of tubers of Hoshangabad. MP *Bulletin of Environment, Pharmacology and Life Sciences*, 1(1): 57-59.

Adama, I., J.V.K. Afun, M.B. Mochiah, M. Owusu-Akyaw and W.A. Overholt (2017) Survey and Exclusion Study to Explore for Natural Enemies in Ghana for the Control of Aerial Yam (*Dioscorea bulbifera* L.) in Florida, USA. *Journal of Experimental Agriculture International*, 1-11.

Arinathan, V, V.R. Mohan and A. Maruthupandian (2009). Nutritional and anti-nutritional attributes of some underutilized tubers. Tropical and Subtropical Agroecosystems, 10(2): 273-278.

Bhandari, M.R. and J. Kawabata (2005) Bitterness and toxicity in wild Yam (*Dioscorea* spp.) tubers of Nepal. *Plant Foods for Human Nutrition,* 60(3): 129-135.

Burkill H.M. (1985). The useful plants of West Tropical Africa. Royal Botanic Gardens, 1.

Deaker, R., M.L. Kecskés, M.T. Rose, K. Amprayn, K. Ganisan, T.K.C. Tran and I.R. Kennedy (2011) Practical methods for the quality control of inoculant biofertilisers. *Australian Centre for International Agricultural Research*.

Gao, H., M. Kuroyanagi, L. Wu, N. Kawahara, T. Yasuno and Y. Nakamura (2002) Antitumor-promoting constituents from *Dioscorea bulbifera* L. in JB6 mouse epidermal cells. *Biological and Pharmaceutical Bulletin*, 25(9): 1241-1243.

Ghosh, S., S. Patil, M. Ahire, R. Kitture, S. Kale, K. Pardesi and B.A. Chopade (2012). Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. International Journal of Nanomedicine, 7: 483.

Grisebach, A. (1864) Flora of the British West Indian Islands. L. Reeve & Company, 1.

Haines, H.H. (2006) The botany of Bihar and Orissa. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Harborne, A.J. (1998) Phytochemical methods a guide to modern techniques of plant analysis. *Springer Science & business media*.

Irshad, S., M. Mahmood and F. Perveen. In vitro antibacterial activities of three medicinal plants using agar well diffusion method. *Research Journal of Biological Science*, 2(1): 1-8.

Karnick, C.R. (1969) Dioscorea (yams)-The food of the slaves, with potentials for newer drugs: A review. *Quarterly Journal of Crude Drug Research*, 9(2): 1372-1391.

Kokni, F.K., H.A. Solanki and D.D. Patel (2016) Study of ethnomedicinal plants and its documentation of waghai forest, gujarat. *Life Science Leaflets*, 81: 11.

Kuete, V., R. Betrandteponno, A.T. Mbaveng, L.A. Tapondjou, J.J. Meyer and L. Barboni (2012) Antibacterial activities of the extracts, fractions and compounds from *Dioscorea bulbifera*. *BMC Complementary and Alternative Medicine*, 12: 228.

Kumar, S. and P.K. Jena (2017). Tools from Biodiversity: Wild Nutraceutical Plants. In Mathematical Advances towards Sustainable Environmental Systems. *Springer*, Cham, 181-213.

Kumar, S., A.K. Parida and P.K. Jena (2013) Ethno-Medico-Biology of Bān-Aālu (*Dioscorea* species): A neglected tuber crops of Odisha, India. *International Journal of Pharmaceutical and Life Sciences*, 4(12): 3143-3150.

Kumar, S., P.K. Jena and P.K. Tripathy (2012) Study of wild edible plants among tribal groups of Simlipal Biosphere Reserve Forest, Odisha, India; with special reference to Dioscorea species. *International Journal of Biological*

Technology, 3(1): 11-19.

Liu L., Y.S. Dong and Z.L. Xiu (2010) Three-liquid-phase extraction of diosgenin and steroidal saponins from fermentation of *Dioscorea zingibernsis* CH Wright. Process Biochemistry, 45(5): 752-756.

Martín, F.W. (1974) Tropical yams and their potential. Part 2. *Dioscorea bulbifera*. Agriculture Handbook, United States Department of Agriculture, 466.

Miller, J.H. (2003) Nonnative Invasive Plants of Southern Forests: A Field Guide to Identification and Control. Southern Research Station, 62.

Miller, J.H. (2003) Nonnative Invasive Plants of Southern Forests: A Field Guide to Identification and Control. Southern Research Station, 62

Moriwasa, T.L. (1999) Weed notes: *Dioscorea bulbifera*, *D. alata*, *D. sansibarnesis* California, USA. The Nature Conservancy, Wild land Invasive Species Programme.

Nwoke, U.D. (2019) Pharmacological and Antioxidant Properties of a Hydromethanol Extract of *Dioscorea Bulbifera* (Air Potato). Doctoral dissertation, Federal University of Technology, Owerri.

Oboh, G., M.M. Ekperigin and A.A. Akindahunsi (2001) Studies on the steroid hormone precursors of two tropical wild yams (*Dioscorea bulbifera* and *D. manganotiana*). Abdus Salam Internatinal Centre for Theoretical Physics, 114.

Rose, S.B. and R.E. Miller (1939) Studies with the agar cup-plate method: I. A standardized agar cup-plate technique. *Journal of bacteriology*, 38(5): 525.

Saxena, H.O. and M. Brahmam (1994) The flora of Orissa. Regional Research Laboratory; Orissa Forest Development Corporation, 1: 437-439.

Shajeela, P.S., V.R. Mohan, L.L. Jesudas and P.T. Soris (2011) Antifertility acitivity of ethanol extract of *Dioscorea esculenta* (L.) Schott on male albino rats. *International Journal of Pharm Tech Research* CODEN, 3: 946-954.

Sofowora, A. (1993) Phytochemical screening of medicinal plants and traditional medicine in Africa. Ibadan: Spectrum Books Ltd.

Suriyavathana, M. and S. Indupriya (2011) Screening of antioxidant potentials in *Dioscorea bulbifera*. *International Journal of Pharmceutical and Life Sciences*, 2(4): 661-664.

Thompson, A., D. Meah, N. Ahmed, R. Conniff-Jenkins, E. Chileshe, C.O. Phillips and P.E. Row (2013) Comparison of the antibacterial activity of essential oils and extracts of medicinal and culinary herbs to investigate potential new treatments for irritable bowel syndrome. *BMC complementary and alternative medicine,* 13(1): 1-19.

Tindall, H.D. (1983) Vegetables in the Tropics. MacMillan, Oxford, 0-333: 24268-8.

Tiwari, R.K.S. and K. Das (2011) Inhibitory effect of cow urine based plant extracts against Rhizoctonia solani causing sheath blight of rice. *Indian Phytopathology*, 64(3): 265.

Wagner, W.L., D.R. Herbst and S.H. Sohmer (1999) Manual of the Flowering Plants of Hawai'i. University of Hawai'i and Bishop Museum Press, 1-2.

Wiegand, I., K. Hilpert and R.E (2008) Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature protocols*, 3(2): 163.

Wilkin, P. (2001) *Dioscoreaceae* of south-central Africa. Kew Bulletin, 361-404.

Yasin, A., F.A. Minhas and M. Zubair. Antibacterial screening of leaves of wild *Viburnum nervosum* and *V. foetens* of Azad Kashmir.

Zaidan, M.R., A. Noor Rain, A.R. Badrul, A. Adlin, A. Norazah and I. Zakiah (2005) In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Tropical biomedicine*, 22(2): 165-170.