Contents lists available at Thomson Reuters

The American Journal of Science and Medical Research

Journal homepage: http://globalsciencepg.org/ajsmr.html

Research Article

α-amylase and α-glucosidase Inhibitory Activity of Physagulin-F Isolated from *Physalis angulata* Fruits

Sateesh Pujari[1] and Estari Mamidala[2]*

[1] *CKM Arts & Science College, Desaipet, Warangal – 506 006. TS, India*
[2] *Department of Zoology, Kakatiya University, Warangal – 506 009, TS, India*

*Corresponding author:
E-mail:drestari@kakatiya.ac.in,
sateesh.pujari@gmail.com

http://dx.doi.org/10.17812/ajsmr4102
Received : 12 December 2017
Accepted; 21 January 2018
Available online : 23 February 2018

ISSN: 2377-6196 © 2017 The Authors. Published by Global Science Publishing Group. USA

ABSTRACT

Type 2 diabetes is a chronic medical condition that requires regular monitoring and treatment throughout your life. Natural compounds from plant sources have been extensively used as traditional medicines from centuries. The present study was carried out to isolate and identify the putative antidiabetic compound from the *Physalis angulata* (PA) fruit. A compound, Physagulin-F was isolated from PA fruit extract. The isolated compound Physagulin-F (50, 75, 100, 150 mg/kg) of *Physalis angulata* fruit was undertaken to evaluate the inhibitory and anti-diabetic activity against α-amylase and α-glucosidase. The compound 'Physagulin-F' showed significant concentration dependent manner antidiabetic activity. The results of this experimental study indicate that isolated compound 'Physagulin-F', possess anti-diabetic effects against α-amylase and α-glucosidase enzymes.

Keywords: *Physalis angulata*, Physagulin-F, anti-diabetic, Type 2 diabetes.

1. Introduction

Diabetic mellitus is a chronic metabolic disorder and poses a major challenge worldwide. According to Indian statistics, the current diabetic patients in India is around 40.9 million and it is expected to drastic rise up to 69.9 million by the end of 2025. Dramatically, India been emerged as diabetic capital of the world (Mohan et al., 2007; Joshi et al., 2007). Unless urgent preventive control steps are taken, it might become a biggest health problem in the running scenario. With reference to Indian Diabetes Federation (IDF) it has been estimated that every year 3.9 million deaths occurs due to diabetic mellitus and represents 6.8% of the total global mortality. In developing countries, anti-diabetic plants provide new oral anti-diabetic compounds that can counter part for high cost and side effect medicines. However, day by day the poor or non-availability of these medicines is the problematic for the people of rural back ground (Noor et al., 2008). Plant derived drugs are commonly considered as less toxic and free from hazardous effects compared to synthetically derived drugs (Valiathan, 1998). Indigenous remedies have been extensively used in the treatment of various forms of diabetes mellitus since the time of Charaka and Sushruta (6th century BC) (Grover et al., 2001). According to the data obtained from World Health Organization (WHO) 21,000 plants which were massively used in the treatment of diabetes around the world, among these, 2500 species are off Indian origin. India is bestowed for larger producer of medicinal plants with a wide diversity of agro-climatic conditions is generally called as botanical garden of the world (Sultana et al, 2008). Number of pharmacological and clinical trials conducted using these medicinal plants have reported anti-diabetic effects by repair of β- pancreatic cells of islets of Langerhans (Ahmed et al., 2010). The present study was designed to evaluate the anti-diabetic activity of isolated compound Physagulin-F of the PA fruits against STZ-induced diabetic rats. *Physalis angulata*, (a branched annual shrub) is commonly known as camapu or balaozinho in Brazil, belongs to Solanaceae (Januario et al., 2002). It is majorly distributed in tropical and subtropical regions of the world. The extracts or infusion of this plant is used in the treatment of a wide range of diseases such as asthma, hepatitis, malaria, dermatitis and rheumatism (Lin et al., 1992). Physalins (A, B, D and F) and glycosides such as Myricetin-3-Oneohesperidoside isolated from organic fractions of *Physalis angulata.*

Materials and Methods

2.1 Plant material

The fully mature PA fruits were collected in August September 2013 from the fields of Karimabad Village in Warangal District of Telangana State, India. The authenticity of the plant was carried out by Professor V.S. Raju, Taxonomist, Department of Botany, Plant Systematic laboratory, Kakatiya University, Warangal and voucher specimen was deposited in the Herbarium of the Metabolic Disorders Research Lab of the same University.

2.2 ***Preparation plant extract***

The fruits were shade dried and grinded in homogenizer in to coarse powder. The powdered material was extracted by sequential maceration method using n-hexane, chloroform, ethyl acetate, acetone and methanol (non polar to polar) solvents. Concentration of extracts was carried out by rotavaporization at their boiling points and crude was collected and stored 4°C for further use. The weight of the residual extract was measured and percent yield was calculated.
Extract yield % = W1/W2 X 100;
Where, W1 = Net wt of powder in grams after extraction and W2 = total wt of powder in grams taken for extraction.

2.3 ***Preliminary phytochemical screening***

All the solvent extracts of PA fruits were tested for the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, phenolic compounds, using standard protocols (Harbone, 1973; Rajendra Chary and Estari Mamidala, 2013; Rajendra Prasad and Estari Mamidala 2013a; Rajendra Prasad and Estari Mamidala 2013b).

2.4 ***Isolation and identification of the active compound***

i) Thin layer chromatographic studies

Thin layer chromatography (TLC) profile with other physicochemical parameter can be good tool for standardization and validation of plants. TLC profile is simple and effective method for determination of the solvent system. TLC as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample on TLC volume 1µl by using capillary at distance of 1 cm at 1 track. Basic solvent system, hexane:ethyl acetate (100:0 to 0:100) were used in TLC to select the better solvent system to run column with methanol extract.

ii) Column Chromatographic Studies

Column chromatography is a purification technique used to isolate compounds from a mixture. In column chromatography, the stationary phase is a solid adsorbent and the mobile phase is a solvent that is added to the top and flows down through the column. Separation is achieved based on the polar and non-polar interactions among the compounds, the solvent, and the solid stationary phase. Usually Silica or Alumina is used as the solid phase in order to setup the column. In this experiment, Silica was used as the solid medium for methanol extract. The column can be prepared using a column chromatography flask. Glass wool was inserted at the bottom of the flask to prevent the silica from escaping the column. The selected mobile phase (hexane:ethyl acetate-100:0 to 0:100) was continuously poured to the top with the aid of a dropper. The bottom outlet of the column was opened, allowing the eluent to flow through the column. As the eluent passed down the column, the compound fraction moved down the column. The separated fraction flowed out of the column where the different elutes were collected in separate test tubes. This was repeated until all the dissolved extract was adsorbed on to the silica gel. The collected elutes were tested in TLC up to single spot appeared.

2.5 ***Structure Elucidation:***

Based on TLC, the elute is taken further 1H NMR, 13C NMR and Mass spectral studies for structural determination.

2.7 ***α-amylase inhibitory activity***

The ability of the *P. angulate* fruit extracts and isolated compound was determined on the inhibition of alpha-amylase Kim *et al.,* (2005) with slight modification. According to this method the various concentration of isolated compounds 20 and 40 mg/ml and 0.25 µl of urinary α amylase (5 U/ml) was incubated for 15 min at 37°C in water bath. The reaction was started by the addition of 0.2 µl of 0.5% potato starch which was dissolved in 20mM phosphate buffer (pH 6.9). The tubes were incubated at 37°C for 20 minutes and followed by the addition of 2.0 ml of DNS reagent (1% 3,5-dinitrosalicylic acid, 12% sodium potassium tartrate in 0.4 M NaOH). The tubes were subjected for heating for 15 min at 100°C. The inhibition of amylase activity was determined by measuring the absorbance at 540 nm. The experiment was compared with blank control (without the extract).

2.8 ***α-glucosidase inhibitory activity***

The inhibitory effect of the isolated compounds on α glucosidase enzyme activity was determined based on the method described by Kim *et al.,* (2005) with slight modifications. The substrate solution p-nitrophynyl glucopyranoside (pNPG) was freshly prepared using 20 mM phosphate buffer of pH 6.9. Five units of α glucosidase was incubated with various concentrations of isolated compounds 20 and 40 mg/ml for 15 minutes. Three millimolar (pNPG) as a substrate was then added to start the reaction. The tubes were then incubated at 37°C for 20 minutes. The reaction was then stopped by the addition of 2 ml of 0.1 M Na_2CO_3. The yellow colored p-nitrophenol released from pNPG was measured at 400 nm. The results were expressed as percentage of the blank control.

3. Results and Discussion

The percentage of yield of extract macerated with various solvents are; Hexane (7.39 g) 3.695 %, Chloroform (6.22 g) 3.11%, Ethyl acetate (1.57g) 0.785 %, Acetone-(1.27 g) 0.65 % , Methanol (7.9 g) 3.95%. Methanol extract of *P. angulata* obtained the highest percentage yield to comparing to other solvent crude extracts (Table-1).

Table-1. Percentage Yield of crude extract of *P. angulata*

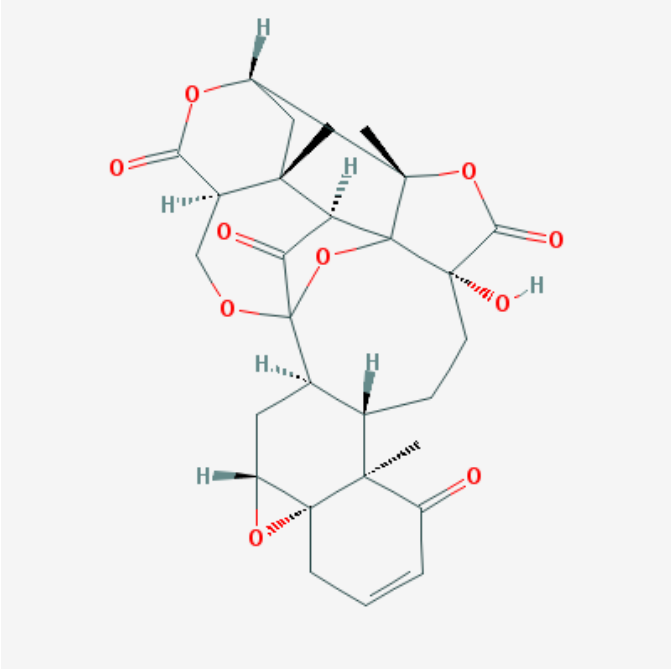
Plant	Solvent extract	Weight in grams	% yield
P. angulata (Fruit)	n-Hexane	7.39	3.695
	Chloroform	6.22	3.11
	Ethyl acetate	1.57	0.785
	Acetone	1.27	0.65
	Methanol	7.9	3.95

3.1 Isolation and Identification of the compound:

Methanol crude extract was filtered and concentrated under reduced pressure to yield a reddish brown residue. This crude extract was fractioned using silica gel (100-200 mesh) column chromatography and the components in crude extract eluted using solvents starting with hexane: ethyl acetate (100:0) and

ending with hexane : ethyl acetate (0:100). 12 fractions were yielded and designated as A1 to A 12. Among of which 1 fraction i.e. A4 which suggested possessing single compound in the crude extract. Fraction obtained from column was monitored according to the variations in composition indicated by the silica gel, 60, F254, TLC. The visualization of spots on the TLC plates was achieved by exposing TLC plates to iodine vapors after developing hexane and ethyl acetate as solvent system. The TLC pattern of A4 strongly indicates as a single compound. In general, it can be understand that, even when an active compound locates with others in the mixture, it may not be able to perform its activity because of its presence in trace amounts it may be obscured by other substances present in the mixture.

Figure-1. Structure of Physagulin-F

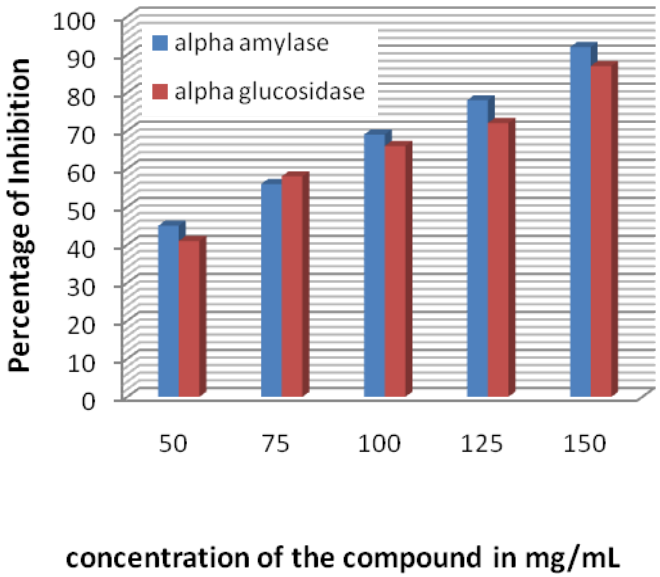


B4 fraction was designated as single compound and characterization was achieved through spectral analysis. Based on Thin layer Chromatography fractionation and 1H NMR spectral data the isolated compound is Physagulin-F belonging to the family of steroids and which possess the molecular weight $C_{28}H_{30}O_{10}$, average molecular weight is 526.538 g/mol and IUPAC name is 6-[1-(4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl)ethyl]-2,16,17-trihydroxy-7,11 dimethyl-12-oxo-5-oxapentacyclooctadec-13-en-3-ylacetate (Steroid Lactones). 1HNMR, 13CNMR data (Table-2) of the Physagulin-F isolated from fruit of *Physalis angulata* and molecular structure were shown in Figure-3.

3.2 Inhibitory activities of Physagulin-F on a-amylase and a-glucosidase

The inhibitory effects of α amylase and α-glucosidase of Physagulin-F have been found in concentration dependent manner. Physagulin-F was significantly inhibited both enzymes α amylase and α-glucosidase at 150 mg/mL. (Figure-2). Diabetes has already been become a major lifestyle disease nowadays, which requires a proper management and control. However, the use of synthetic medicine is always not suggested due to its adverse effects. Thus, an alternate source for the sought out for the safer and affordable medicine, one can opt for medicinal plants which are comparatively possess lesser side effects. Due to the economic constraints, providing modern medical healthcare is still a far-reaching goal in developing countries, especially, in India. The most commonly used drugs such as aspirin, anti-malarial and anti-cancers etc. have been originated from plant sources. It is estimated that out of 250 000 higher plants (Vinatha Naini and Estari Mamidala, 2013), less than 1% of these have been screened pharmacologically and even very few in regard to diabetic mellitus. Therefore, it is very urgent to ascertain for naturally derive drugs from herbal medicinal plants for the treatment of diabetes. The present work on antidiabetic activities of fruit methanol extract of *Physalis angulata* have been proven to be significant in both in vitro and in vivo experiment models.

Figure-2. *In vitro* anti diabetic effects of Physagulin-F **Isolated from** P. *angulata* (*Fruit*) **crude extracts.**



These study research on in vivo and *in vitro* antidiabetic activities of *Physalis angulata* fruit would become the first documentary which is being reported. These comparative studies of antidiabetic in vitro and in vivo revealed that the methanol extracts of fruit exhibited a significant relationship with percentage of enzymes and glucose levels reduction with the concentration of extract (dose dependent manner). Methanol extract dominated methanol extract in all aspects of antidiabetic studies. The further investigation process must be carried out with methanol and methanol extracts for the isolation and characterization of active principle using bioassay guided fractionation. Even though, this compound was previously reported for their isolation (Kazushi et al., 1992 and G. Krishna Et al, 2014), till today there are no scientific literature were available concerning to their biological activities, thus in continuity to isolation and structural elucidation the current studies are extended for determination of antidiabetic efficacy. Plant compounds continue to serve as viable source of naturally derived drugs for the world population are in extensive clinical use. Antioxidants are defined as substances that can delay or prevent the oxidation of lipids or other molecules by oxidizing chain reaction initiation or propagation and by many other mechanisms to prevent from disease (Zheng and Wang, 2001 and Rajendra Prasad Gujjeti and Estari Mamidala, 2014). Plants with feasible antimicrobial activity should be assayed against appropriate microbial strains to confirm the possessed activity and to find out the parameters associated with it. The efficacies

of these plant extracts on bacteria have been studied by numerous researchers present around world. Especially, relatively a large number of studies have been conducted on ethnomedicinal plants made of increased interest in isolation of large number of traditional natural products (Taylor, 1996).

Plant-based medicaments have been become basis and alternative for many modern pharmaceuticals we use today. Ethno-pharmacological information based phytochemical research is generally considered as effective approach for the discovery of new anti-infective agents from higher plants. Knowledge related to the chemical constituents of plant is desirable, not only for the discovery of therapeutic agents, and also to economic materials such as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants is also valuable for further discovering the actual value of folkloric remedies (Mojab et al., 2003 and D. Krishna Gopal Rao, 2013 and Sateesh Poojari et al, 2014). Medicinal plants have been provided a source of inspiration for novel drug molecules and made large contributions to human health. Their role is two-fold in the development of new drugs: (1) they have been become base for the development of a medicine (a natural blue print for the development of new drugs) or; (2) a phytomedicine to be used for treatment of various diseases. Among the best examples of phytochemicals tannins have been found take important place in the prevention of several diseases. Theses form an irreversible complex with proline a rich protein resulting in the inhibition of cell protein synthesis.

These protein reactions of tannins are known to play important in the treatment of inflamed or ulcerated tissues. Herbs that constitute tannins as their main components are astringent in nature and are utilized for the treatment of intestinal disorders such as diarrhea and dysentery. The biological properties of tannins have been reviewed and also reported that, tannins exhibit anticancer properties and can be used for cancer prevention. The other classes of phytochemicals that attribute medicinal properties are alkaloids. These alkaloids are also widely studied for their potential utility in the elimination and reduction of human cancer cell lines. Alkaloids are the largest groups of phytochemicals found in plants with amazing effects on human beings. Alkaloids have led to the development of powerful pain killer medications. Saponin inhibitory effects on inflamed cells have also been revealed. Steroidal extracts from some medicinal plants are also investigated and reported to exhibit antibacterial activities and antiviral properties. Flavonoids offer a wide range of biological properties such as antimicrobial, antioxidant properties, antidiabetic, anti-inflammatory, anti-angionic, anti-allergic, cytostatic and analgesic (Igbinosa et al., 2009; Janovska et al., 2003; Devdatta Gopal Lad, 2014, Devdatta Gopal Lad, 2014, D. Krishna Gopal, 2013).

4. Conclusion:

In the present study we have been hypothesized the *in vitro* antidiabetic efficacy of *P. angulata* fruit methanol extract. Our findings, directly indicate that the isolated compound Physagulin-F possess anti-diabetic activity resulted in inhibition of α-amylase and α-glucosidase at significant levels. This study supports the ethnobotanical usage of P. *angulata* in the treatment of diabetes and its associated complications. However, further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

Competing interests

The authors have declared that no competing interests exist.

References

[1]. Ahmed, A. B. A., Rao, A. S. and Rao, M. V., In vitro callus and in vivo leaf extract of Gymnema sylvestre stimulate β-cells regeneration and anti-diabetic activity in Wistar rats. Phytomedicine, 2010, 17, 1033–1039.
[2]. D. Krishna Gopal Rao (2013). Hypoglycemic activity of extracts from Elytraria acaulis L. Leaves in alloxaninduced diabetic rats. Biolife. 1(1):11-16.
[3]. Devdatta Gopal Lad. (2014). Comparative histopathological remedial study of polyherbal formulation
[4]. Diabcure and Glibenclamide on Streptozotocin induced diabetic rats. Biolife. 2(4), 1376-1379.
[5]. Esampally Sucharitha, Mamidala Estari (2013). Evaluation of antidiabetic activity of medicinal plant extracts used by tribal communities in rural areas of Warangal district, Andhra Pradesh, India. Biology and Medicine, 5:20–25.
[6]. G. Krishna and M.A Singara Charya V. Pranitha. 2014. Evaluation of antibacterial and anifungal activity of fruiting body extracts of Trametes versicolar. 2(4). 1181-1184
[7]. Grover, J. K. and Vats, V., Shifting paradigm from conventional to alternate medicine. An introduction on traditional Indian medicine. Asia Pac. Biotech News, 2001, 5, 28–32.
[8]. Harborne JB (1 998). Phytochemical methods. A guide to modern techniques of plant analysis.3rd ed., Chapman and Hall Int ed., New York.
[9]. Igbinosa, O.O., Igbinosa, E.O., Aiyegoro, O.A (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha cuccas (Linn). Afr. J. Phar & Pharmacol, 3: 58-62.
[10]. Islam, M. S., Choi, H. and Loots, D. T. L., Effects of dietary onion Allium cepa L. in a high-fat diet streptozotocininduced diabetes rodent model. Ann Nutr. Metab., 2008, 53, 6–12.
[11]. Janovska, D., Kubikova, K., Kokoska, L (2003). Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. Czech. J. Food Sci 21: 107-110.
[12]. Januário AH, Filho ER, Pietro RC, Kashima S, Sato DN, França SC (2002). Antimycobacterial physalins from Physalis angulata L. (Solanaceae). Phytother Res 16: 445-448.
[13]. Joshi, S. R. and Parikh, R. M., India – diabetes capital of the world: now heading towards hypertension. J. Assoc. Physicians India, 2007, 55, 323–324.
[14]. Kazushi Shingu, Yahara, Shoji, Nohara, Toshihiro, Okabe, Hikaru (1992). Three new withanolides, physagulins A, B and C from Physalis angulata L.Chemical & Pharmaceutical Bulletin (tokyo). 40(8): 2088-2091.
[15]. Lin YS, Chiang HC, Kan WS, Hone E, Shih SJ, Won MH (1992). Immunomodulatory activity of various fractions derived from Physalis angulata L. extract. Am J Chin Med, 20: 233-243.

[16]. Mohan, V., Sandeep, S., Deepa, R., Shah, B. and Varghese, C., Epidemiology of type-2diabetes: Indian scenario. Indian J. Med. Res., 2007, 125, 217–230.
[17]. Mojab, F., Kamalinejad, N., Ghaderi, Vahidipour, H (2003). Phytochemical screening of some Iraninan plants. Iran.J.pharma. sci, 2: 77-82.
[18]. Noor, A., Gunasekaran, S., Manickam, A. S. and Vijayalakshmi, M. A., Antidiabetic activity of Aloe vera and histology of organs in streptozotocin-induced diabetic rats. Curr. Sci., 2008, 94, 1070– 1076.
[19]. Rajendra Chary and Estari Mamidala. (2013). Phytochemical and Chromatographic studies in the stem bark extract of Ficus recemosa (L). The Ecoscan, 2013. Special Issue.IV, 27-31.
[20]. Rajendra Prasad Gujjeti and Estari Mamidala (2013). Phytochemical Screening and Thin Layer Chromatographic Studies of Aerva lanata root extract. International Journal of Innovative Research in Science, Engineering and Technology. 2(10), 5725-5730.
[21]. Rajendra Prasad Gujjeti and Estari Mamidala. (2013). Phytochemical Analysis and TLC Profile of Madhuca indica Inner Bark Plant Extract. International Journal of Scientific and Engineering Research. 10, 1507-1510.
[22]. Rajendra Prasad Gujjeti and Estari Mamidala. Anti-HIV Activity and Cytotoxic Effects of Aerva lanata Root Extracts American Journal of Phytomedicine and Clinical Therapeutics. 2014. 2(7) 894-900.
[23]. Raju Porika, Sateesh Poojari, Venkanna Lunavath and Estari Mamidala. 2014. Preliminary Phytochemical Investigation and TLC Analysis of P. Angulata Fruit
[24]. Extract. Journal of Pharmacy and Biological Sciences. Volume 9, Issue 2 .11-14.
[25]. Sateesh Poojari, Raju Porika and Estari Mamidala Phytochemical Analysis And In Vitro Antidiabetic Activities Of Physalis angulata Fruit Extracts.. National Journal of Integrated Research in Medicine (NJIRM) 2014; Vol. 5(2).34-38.
[26]. Sultana N, MA Hassan, Momtaz Begum, Mahbuba Sultana. Physalis angulata L. (solanaceae) - A new Angiospermic Record for Bangladesh. Bangladesh J Bot.2008;37(2):195–198.
[27]. Taylor, R.S.L., Manandar, N.R. Hudson, J.B (1996).Antiviral activities of Nepalese medicinal plants. J. Ethanopharmacol, 52:157-163.
[28]. Valiathan, M. S., Healing plants. Curr. Sci., 1998, 75, 1122–1126.
[29]. Van Valkenburg JLCH, N Bunyapraphatsara, editors. Medicinal and Poisonous Plants 2.Bogor, Indonesia: Prosea Foundation; 2002. Plant Resources of Southeast Asia No.12 (2) p. 782.
[30]. Vinatha Naini and Estari Mamidala. 2013. An ethnobotanical study of plants used for the treatment of diabetes in the Warangal district, Andhra Pradesh, India. Biolife. 1(1):24-28.
[31]. Zheng, W and Wang, S.Y (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem, 49: 5165-5170.