International Journal of Multidisciplinary Research and Growth Evaluation.

Phytochemical investigation and Antidiabetic activity screening of Bennincas Hispida (THUNB.)

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Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 01
January-February 2023
Received: 06-01-2023;
Accepted: 26-01-2023
Page No: 395-400
DOI: https://doi.org/10.54660/.IJMRGE.2023.4.1.395-400

Abstract
The crude extracts of *Benincasa hispida* i.e. ethanolic extract and aqueous extract were studied for the presence and detection of phytochemical such as of flavonoids, sterols, terpenoids, carbohydrates and phenolic compounds using standard procedures. On the basis of the results, the extracts were further used for in vivo evaluation of antidiabetic activity. The present study was designed to study the phytochemical screening and to investigate the antidiabetic potential of aqueous and ethanolic extract of dried leaves of *Benincasa hispida*. The antidiabetic potential was evaluated by Streptozotocin-Nicotinamide induced diabetic in rat model. The extracts showed significant potential in a dose dependant manner when compared with the Glibenclamide. The aqueous extract shows good glucose lowering ability than ethanoloc extract on day 15. Thus both the ME and AE may be useful as a natural antioxidants in the near future.

Keywords: *Benincasa hispida*; Streptozotocin, Nicotinamide; Type 2 diabetes; Flavonoid

Introduction

The number of diabetic patients is steadily increasing worldwide, and type 2 diabetes, especially among young people such as children and adolescents, is becoming a problem [1].

The causes of type 2 diabetes include environmental exposure and genetic factors [2]. Early diagnosis and management of risk factors are important because diabetes causes various serious complications. Blood sugar management is important for the prevention and management of type 2 diabetes [3], and agents with -glucosidase inhibitory activity are used as oral hypoglycemic drugs [4]. In the state of hyperglycemia, a sugar-derived substances called advanced glycation end-products (AGEs) are actively produced and accumulate in the blood and tissue [5, 6]. AGEs are a heterogeneous group of molecules formed by the Maillard reaction [7]. The reaction is a non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids [5]. Methylglyoxal (MGO) is one of the most reactive AGE precursors [8]. During aging and diabetes, increasing amounts of AGE-modified proteins can be detected. In other words, they are involved in the development of degenerative diseases such as diabetes [9]. Therefore, controlling the formation of AGEs is important for the prevention and treatment of diabetes and diabetic complications.

Agents that inhibit or reduce AGE formation include aminoguanidine, pyridoxamine, and OPB-9195. Aminoguanidine has been reported to be toxic during clinical evaluation [6]. Therefore, it is necessary to identify a safe anti-glycation agent. To find novel synthetic AGE inhibitors, scientists are focusing on researching anti-glycation compounds from natural products [7-10].

Benincasa hispida (Synonym: *Benincasa cerifera*) which usually known as (winter melon, ash gourd, ash guard, winter gourd, white pumpkin and wax gourd. white gourd, animal oil gourd, gourd melon and Chinese watermelon) belongs to the family Cucurbitaceae. it's common vegetable crop, notably among Asian countries both for biological and medicative purposes [11, 12]. All part of the plant is used medicinally. The plant grows annually. This plant could be a crawling with branched tendrils which

will climb over with the help of some support, cover fences or sprawl on the bottom. Stems are thick, hairy, grooved conspicuously and lined with sharp bristles. Leaves are spherical, kidney shaped with higher rough surface. These have beautiful flower of golden yellow color. Fruits contain varied white coloured embedded seeds [11-13]. However, the plant was used medicinally in various complains such as gastrointestinal problems, respiratory disease, heart diseases, diabetes mellitus and urinary diseases [13].

Fruits were historically used as a laxative, diuretic, tonic, aphrodisiac, cardiotonic, urinary calculi, blood disease, insanity, epilepsy, schizophrenia and other psychologic disorders, jaundice, dyspepsia, fever, and menstrual disorders [14-16]. Plants contain several phytochemicals like alkaloids, flavonoids, tannins, glycosides, phenolic compounds, amino acids, steroids, triterpenoids and saponins [17]. Polyphenols have good inhibitor activity, which helps to reduce the side effects of diseases like neurodegenerative diseases, cardiovascular, cancer, liver disease and infectious disease [18]. Tannins exhibit significant inhibitory effect on pancreatic lipase activity and fat absorption from the intestine [19]. The methanolic extract of the fruit is reportable to possess antiulcer [20], anti-inflammatory drug [21], antihistaminic, antidepressant drug [22] and bronchodilator [23] antihistaminic, analgesic and diuretic drug. The plant might have various kind of phytochemicals that are flavonoid, saponin, glycosides, tannins, steroids, beta-carotens, triterpenoids etc. There are also the presence of disaccharide like glucose, sucrose, maltose etc.

Materials and Methods

Collection of plant material

The leaves of *Benincasa hispida*, was selected for investigation and were procured from Sri Venkateswara University, Tirupathi, Andhra Pradesh, India. The plant material was taxonomically identified and authenticated by Dr. Madhavachetty, Head of Department, Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh. The chit sampling of the hedge plant partakes stood dumped in the Division of Pharmacognosy, of our institution.

Chemicals and instruments

Streptozotocin (Sigma Chemical Company, USA) nicotinamide (Ranbaxy Chemicals Ltd, Mumbai, India) was used to induce diabetes in mice and Glibenclamide (Hoechst Pharmaceuticals, Mumbai) was used as a standard hypoglycemic drug. Ethanol (BDH Ltd., England) and distilled water were used for extraction of the plant materials. ACCU CHEK Performa Glucometer (Roche Diagnostics India Pvt. Ltd., India) was used to measure the blood glucose level. For evaporating the solvents, BUCHI Rotavapour R-200, Switzerland and Lyophilizer (freeze dryer) (type: Heto power dry LL3000 Wag tech) was used. The following chemicals were used for phytochemical screening test: Chloroform and Ethyl acetate (ACS, Merck); Hydrochloric acid, Ferric sulphate, Lead acetate and Potassium ferrocyanide (BDH Ltd., England); Petroleum ether 60-80 °C (Labmerk Chemicals LTD India); Sulphuric acid (Farm Italia Carrloerba, Italy); Acetic anhydride and Methanol HPLC grade (Techno Pharmchem, Bahadurgarm, India); n-Hexane (Rathburn Chemicals Ltd., England); Acetonitrile (Sigma Aldrich, Germany) and Ferric chloride (FISHER Scientific Company, USA). All the chemicals were of analytical grades.

Experimental animals

Healthy male adult Wistar albino rats (150-300 gm body weight) were selected for study. The animals aged between 2 - 3 months. They were housed in polypropylene cages, maintained under standard conditions (12 h light; 12 h dark cycle; 25± 300C). They were fed with standard rat pellet diet (Pranav Agro Ltd., Vadodra, India and water *ad libitum*. Animal ethical norms were strictly followed during all experimental procedures.

Extraction of plant material

Extraction Method

The experiments were carried out using air-dried plant materials which were reduced to moderately coarse powder using mechanical grinder. The powdered was passed through sieve no. # 40 and stored in air-tight containers for further use. The ethanolic and aqueous extracts of dried powder drug were prepared as follows:

Preparation of ethanolic extract

The air-dried coarse powder of the roots was well packed in soxhlet apparatus and subjected for continuous hot extraction with 70% ethanol. The extract was filtered while hot and the filtrate was distilled under reduced pressure in order to remove solvent completely. The residue was dried and stored in desiccator, used for subsequent experiments.

Preparation of Aqueous extract

The air-dried coarse powder of the roots was refluxed with distilled water. The extract was filtered while hot and the resultant extract was distilled under reduced pressure in order to remove solvent completely. It was dried and stored in a desiccator, used for subsequent experiments.

Phytochemical screening

Preliminary phytochemical screening of the plant extracts was carried out using standard procedures [24], to check for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones.

Acute toxicity test [25]

The animals were divided into two groups and each group consisted of five mice. The defined or fixed dose level of aqueous and ethanolic extracts (2000 mg/kg) were given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death.

Hence in our studies we selected $1/4^{th}$ and $1/8^{th}$ of the highest dose.

Preparation of Doses

Doses equivalent to 500 mg and 250 mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v tween 80 solutions for the experiment.

Oral Glucose Tolerance Test [26]

It is the test for the diagnosis of diabetes. It can be made on the basis of individual's response to the oral glucose load, commonly referred to as oral glucose tolerance test (OGTT). The response of standard oral test dose of glucose was

determined. For this study normal rats were selected.

Procedure: Animals were divided into six groups and each group consisted of six rats. Overnight fasted rats were used for study.

Group I: Normal control rats administered saline (0.9% w/v);

Group II: Rats administered standard drug Glibenclamide (2.5 mg / kg) daily

Group III: Rats administered ethanolic extract (250 mg/kg);

Group IV: Rats administered ethanolic extract (500 mg/kg);

Group V: Rats administered aqueous extract (250 mg/kg);

Group VI: Rats administered aqueous extract (500 mg/kg).

Aqueous and ethanolic extracts were administered orally to all overnight fasted animals. After 30 min. extract administration, the glucose (4gm/kg) was administered orally to all groups. The blood samples were collected from the orbital plexus of each animal at 0 min, 30 min, 60 min and 120 min after glucose loading. The fasting blood glucose level was determined using a glucose oxidase-peroxidase reactive strips and a glucometer.

Induction of Diabetes

Diabetes was induced[27] by a single intraperitoneal injection of 60 mg/kg streptozotocin followed by nicotinamide 120 mg/kg, i.p, 15 min afterwards. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >126 mg/dl[93]. Only those rats that were found to have permanent NIDDM were used for the study.

The diabetic rats after confirmation of stable hyperglycemia, were divided into different groups of 6 rats each. That day was considered as the 0th day. Drug and doses were administered as mentioned.

Experimental design

Group I: Normal control rats administered saline (0.9% w/v);

Group II: Diabetic control rats administered saline (0.9% w/v);

Group III: Diabetic rats administered standard drug Glibenclamide (2.5 mg / kg) daily

Group IV: Diabetic rats administered ethanolic extract (250 mg/kg);

Group V: Diabetic rats administered ethanolic extract (500 mg/kg);

Group VI: Diabetic rats administered aqueous extract (250 mg/kg);

Group VII: Diabetic rats administered aqueous extract (500 mg/kg).

Blood samples were withdrawn from overnight fasted animals on day 5, 10 and day 15 following three hours after vehicle/extract/glibenclamide administration. Blood was withdrawn from the retro orbital plexus using heparinised haematocrits. The fasting blood sugar levels were determined by using glucose oxdiase peroxidase reactive strips.

Statistical analysis

Data are expressed as a mean ± standard deviation. Differences among treatment group means were assessed by two-way analysis of variance (ANOVA) and group means were considered to be significantly different at $P < 0.05$. Data were statistically evaluated using Statistical Package for the Social Sciences (SPSS) version 20.0 software. Bar and line charts were drawn using Excel 2007 software.

Result and Discussion

Phytochemical screening

The plants leaf extract of *Benincasa hispida* was studied for preliminary phytochemical and antidiabetic activities. Preliminary phytochemical studies revealed the presence of flavonoids, sterols, terpenoids, carbohydrates and phenolic compounds.

Acute toxicity test

The acute toxicity study showed that the administration of graded doses of both the aqueous and ethanol extracts of *Benincasa hispida* leaves did not generate any observable signs of toxicity up to the dose of 2000 mg/kg, This was confirmed by the absence of significant changes in behaviours such as alertness, motor activity, weight loss, sluggishness, paralysis, breathing, restlessness, diarrhoea, convulsions, and coma. In addition, no death was observed for two weeks and they were physically active. The result proves that the plant extracts had no observable adverse effect at the doses tested; implying that the medium lethal dose (LD_{50}) is greater than 2000 mg/kg body weight in mice.

Oral glucose tolerance test

The mean blood glucose levels of normal (negative control), diabetic rat untreated (positive control) and diabetic rat treated with *Benincasa hispida* leaves extracts that were subjected to glucose tolerance test after two weeks is presented in table no. 1. The animals in each group ($n = 5$) fasted 12–14 h and then the fasting mean blood glucose level was evaluated after oral administration of glucose (2 g/kg body weight) as a baseline. The mean blood glucose level in the normal control rat rise to a peak value after 60 min glucose load and decreased to near normal level after 120 min. In diabetic control rat, however, the value increased to a peak after 60 min of glucose load and remained high over the next 60 min, which is expected.

Table 1: Effect of the leaf extract of of *Benincasa hispida* on oral glucose tolerance test in rats

Sl. No.	Treatment	Blood glucose level (mg / dl)			
		0 min	30 min	60 min	120 min
1	Normal	91.42±0.92	132.33±1.12	117.29±1.11	111.03±1.17
2	Standard (Glibenclamide, 2.5mg/kg,)	94.01±0.73	110.33±0.56*	83.09±0.97*	79.39±0.55*
3	Ethanolic Extract (250mg/kg,)	95.01±1.32	123.33±1.48*	104.67±0.92*	92.01±0.37*
4	Ethanolic Extract (500mg/kg)	103.09±1.67	129.04±1.46	108.31±1.87*	94.83±2.11*
5	Aqueous Extract (250mg/kg)	98.33± 1.12	131.32± 1.28	113.33± 1.52	105.01±1.33
6	Aqueous Extract (500mg/kg)	104.33±1.11	132.67± 1.17	109.38±1.12	101.33±0.76

Normal Control- Vehicle 10 ml/kg, Reading are values ± S.E.M, n = Numbers of animals in each group * P < 0.05 v/s Normal control; One-way ANOVA followed by Dunnett test

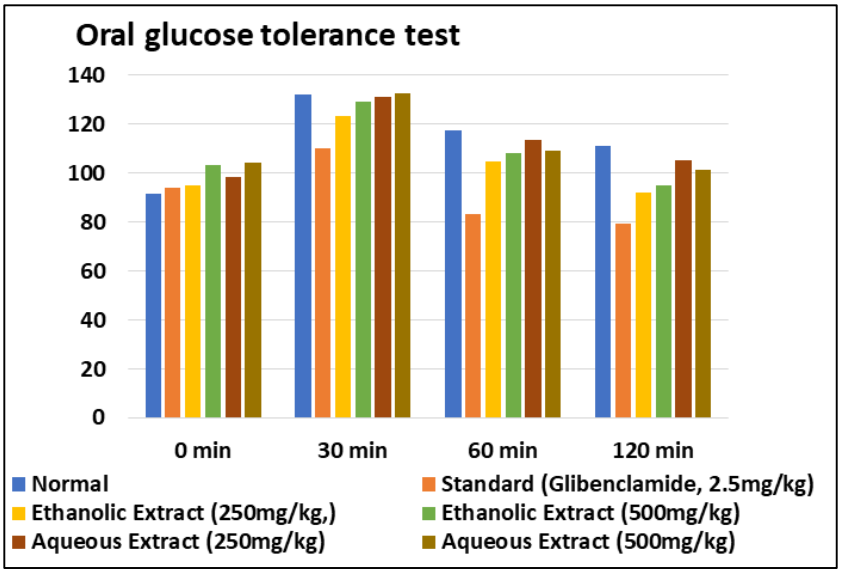

Fig 1: Effect of the leaf extract of of *Benincasa hispida* on oral glucose tolerance test in rats

The animals that were treated with the extracts showed a reduction in the mean blood glucose levels after 60 min of glucose loading. At 60 min, the blood glucose level reached the maximum in both the aqueous and ethanol extracts treated animals and then significant reduction was observed after 120 min of glucose administration. The aqueous extract at a dose of 500 mg/kg body weight showed a better reduction with 101.33±0.76 from the peak blood glucose level.

Effect of of *Benincasa hispida leaves* extract on blood glucose level

Streptozotocin-Nicotinamide induced diabetic rats were treated with aqueous and ethanol extracts of *Benincasa hispida* leaves, once a day orally, for 14 days. The effect of different doses of the extracts of *Benincasa hispida* leaves on fasting blood glucose level is presented in table no. 2. The present study was intended to examine the antidiabetic effects of the extracts of *Benincasa hispida* leaves. The dose of *Benincasa hispida* (250 and 500 mg/kg body weight) was selected.

Table 2: Effect of the leaf extract of of *Benincasa hispida* blood glucose levels in rats

Sl. No.	Treatment	Fasting blood glucose level (mg / dl)			
		Day 0	Day 5	Day 10	Day 15
1	Normal	97.14±1.53*	94.17 ± 1.25 *	91.83 ± 1.01*	88.67 ± 1.15 *
2	Diabetic control	181.67±1.12	189.11± 0.88	196.83± 1.08	199.8 ± 1.31
3	Standard (Glibenclamide, 2.5mg/kg,)	184.33±1.45	127.55±0.76*	116.51±1.01*	107.67±1.14*
4	Ethanolic Extract (250mg/kg,)	180.11±1.83	137.33±1.33*	125.83±1.34*	119.18±0.97*
5	Ethanolic Extract (500mg/kg)	179.67±0.65	135.65±1.50*	126.33±1.03*	116.52±1.08*
6	Aqueous Extract (250mg/kg)	175.12±1.06	140.51±0.96*	130.67±0.67*	122.09±1.79*
7	Aqueous Extract (500mg/kg)	177.52±1.78	138.8± 1.66*	128.08±2.56*	119.17± 1.9 *

Values expressed as mean ±S. E. M.; n = no. of animals in each group. * $p < 0.05$ significant Vs diabetic control. One-way ANOVA followed by Dunnett test

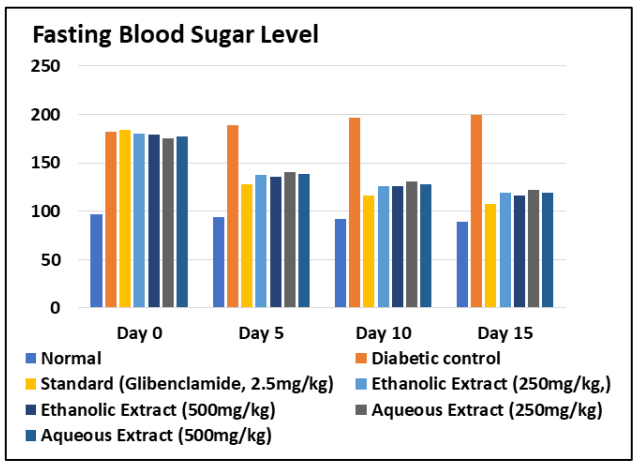

Fig 2

As expected in the diabetic control there was a 199.8 ± 1.31 increases in mean blood glucose level. The blood glucose level of diabetic rats was estimated before and after 0^{th}, 5^{th}, 10th and 15th days of treatment. Both the aqueous and ethanol *Benincasa hispida* leaves extract treatment groups show a statistically significant difference with normal and diabetic control mice with $p < 0.05$.

The average decrease in blood glucose levels (Table 1) showed a decrease in the blood glucose with a relative increasing dose administration of *Benincasa hispida* leaves extracts. However, the ethanol extract of *Benincasa hispida* leaves with dose of 500 mg/kg body weight had a greater decrease (116.52±1.08) than any of the extracts after 15 days of treatment administration. Glibenclamide (2.5 mg/kg body weight) treated diabetic rat showed a 107.67±1.14 reductions as positive control.

The extracts of *Benincasa hispida* leaves reduced elevated fasting blood glucose level. The mechanism of antidiabetic effects of the extracts of *Benincasa hispida* leaves might be due to presence of well-known antioxidant phytochemicals like flavonoids, polyphenols, and tannins, which acts as a free radical scavengers [28, 29]. The presumed mechanism of action of these antioxidants was because of an insulin mimetic effect on the peripheral tissues by either stimulation of regeneration process or release of pancreatic secretion of insulin from existing β-cells. On top of this mechanism; there are also other mechanisms that play a great role in the reduction of blood glucose levels as potential antidiabetic plants. Increasing the speed of the release of glucose from the circulation by accelerating filtration and renal excretion and increasing the release of glucose through enhanced metabolism or integrate into fat deposits, a process relating to the pancreas to produce insulin are among the others [28].

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over- production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues [30]. In the study, the difference observed between initial and final fasting blood glucose levels of the different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the 15 day experimental period. When extracts were administered to glucose loaded normal rats fasted for 18 hr., hypoglycemia was observed after 30 min. The decline in blood sugar level reached its maximum at 2nd hour. The investigations indicate the efficiency of the aqueous and the alcoholic extracts in the maintenance of blood glucose levels in normal and streptozotocin-nicotinamide induced diabetic rats. Fasting blood glucose levels in aqueous and alcoholic extracts treated group exhibited significant reduction of glucose levels as compared to the diabetic control group.

The present study has shown that the aqueous and the alcoholic extract of *Benincasa hispida* leaf are endowed with marked antidiabetic activity. The potent antidiabetic activity may be attributed to their protective effect on the tissue defence system against oxidative damage in STZ induced diabetes, as reported in earlier studies [31].

Conclusion

Medicinal plants have been in use for the control and management of life style and metabolic disorders since ancient times. Although synthetic drugs are highly efficient in controlling these, the use of plants, based on traditional knowledge still continues to be an effective and trustworthy medium for maintaining a normal and healthy life. The availability of modern techniques for extraction, isolation and characterization of phytochemicals has made it further feasible to carry out extensive study on medicinal plants and their active constituents. The present study provides a strong basis to augment the use of *Benincasa hispida* leaves in the current day systems of medicine in order to develop potent antidiabetic. The study also attributes a scientific base and support to the traditional claims of these plants.

References

1. Zimmet PZ, Magliano DJ, Herman WH, Shaw JE. Diabetes: A 21st century challenge. Lancet Diabetes Endocrinol. 2014; 2:56-64. [CrossRef]
2. Hussain A, Claussen B, Ramachandran A, Williams R. Prevention of type 2 diabetes: A review. Diabetes Res. Clin. Pract. 2007, 76:317-326. [CrossRef]
3. Russell WR, Baka A, Björck I, Delzenne N, Gao D, Griffiths HR, *et al*. Impact of Diet Composition on Blood Glucose Regulation. Crit. Rev. Food. Sci. Nutr. 2016; 56:541-590. [CrossRef]
4. Seçil Ö; Suna T; Burcu O; Figen Z. Inhibition of-Glucosidase by Aqueous Extracts of Some Potent Antidiabetic Medicinal Herbs. Prep. Biochem. Biotechnol. 2005; 35:29-36.
5. Peppa M, Uribarri J, Vlassara H. Glucose, Advanced Glycation End Products, and Diabetes Complications: What Is New and What Works. Clin. Diabetes. 2003; 21:186-187. [CrossRef]
6. Rhee SY; Kim YS. The role of advanced glycation end products in diabetic vascular complications. Diabetes Metab. 2018; 42:188-195. [CrossRef]
7. Chuyen NV. Toxicity of the AGEs generated from the Maillard reaction: On the relationship of food-AGEs and biological-AGEs. Mol. Nutr. Food. Res. 2006; 50:1140-1149. [PubMed]
8. Kilhovd BK, Giardino I, Torjesen PA, Birkeland KI, Berg TJ, Thornalley PJ, *et al*. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. Metabolism. 2003; 52:163-167. [CrossRef] [PubMed]
9. Nass N, Bartling B, Santos AN, Scheubel RJ, Börgermann J, Silber RE, *et al*. Advanced glycation end products, diabetes and ageing. Z. Gerontol. Geriat. 2007; 40:349-356. [CrossRef]
10. Velichkova S, Foubert K, Pieters L. Natural Products as a Source of Inspiration for Novel Inhibitors of Advanced Glycation Endproducts (AGEs) Formation. Planta Med. 2021; 87:780-801. [PubMed]
11. Nimbal SK, Venkatrao N, Ladde S, Pujar B. Anxiolytic evaluation of *Benincasa hispida* (Thumb) Cogn. fruit extracts. International Journal of Pharmacy and Pharmaceutical Science Research. 2011; 1(3):93-97.
12. Zaini NAM, Anwar F, Abdul Hamid A, Saari N Kundur [*Benincasa hispida* (Thub.) Cogn.]: A potential source for valuable nutrients and functional foods. Food Res Int. 2011; 44:2368-2376.
13. Joshi S. *Benincasa hispida*. In: Joshi S, editor. Medicinal Plants, 2000, 152.
14. Blatter E, Caius JF, Mhaskar KS (eds.). Indian Medicinal Plants, 2nd vol, Bishen Singh Mahendra Palsingh, 1975, 1126-1128.
15. Sharma PV, Medhyadi Varg. In The Dravya Gun-Vijnana Vegetable Drugs. Sharma PV (ed.) Chaukhambha Bharti Academy, Varanasi. 2005, 14.
16. Jayasree T, Kishore K, Vinay M, Vasavi P, Chandrasekhar N, Manohar VS, *et al*. Evaluation of the Diuretic effect of the chloroform extract of the *Benincasa hispida* rind (Pericarp) Extract in Guinea-pigs. Journal of Clinical and Diagnostic Research. 2011; 5(3):578-582.
17. Iqbal Hussain, Riaz Ullah, Rooh Ullah, Muhammad Khurram, Naseem Ullah, Abdul Baseer, *et al*. Phytochemical analysis of selected medicinal plants. African journal of Biotechnology. 2011; 10(38):7487-7492.
18. Ames BM, Shigena MK, Hagen TM. Oxidants, antioxidant and the degenerative diseases of aging proceeding of the National Academy of sciences. 1993; 90:7915-7922.

19. FL XN Zhang, W Wang, DM Xing, WD Xie, H Su, LJ Du. Evidence of anti-obesity effects of pomegranate leaf extract in high fat diet induced obese mice. International journal of obesity. 2007; 31:1023-1029.
20. Grover JK, Adiga G, Vats V, Rathi SS, Extracts of *Benincasa hispida* prevent development of experimental ulcers. J Ethnopharmacol. 2001; 78:159-64.
21. Chandrababu S, Umamaheshwari S. Studies on the anti-inflammatory activity of fruit rind extract of *Benincasa hispida* Cogn. Indian Drugs. 2002; 39:651-3.
22. Anilkumar D, Ramu P. Effect of methanolic extract of *Benincasa hispida* against histamine and acetyl choline induced bronchospasm in guinea pigs. Indian J Pharmacol. 2002; 34:365-361.
23. Rashmi SP, Yogendra P, Pranay W, Ankita W. *Benincasa hispida*-brihatphala A-review cited on, 2018.
24. Trease GE, Evans WC. Text book of Pharmacognosy. 13. London: Bailiere-Tindall Ltd, 1989, 343-383.
25. OECD Guidelines for testing of chemicals, revised draft 420, Documents on acute oral toxicity and acute toxicity class method, Revised, 2001.
26. Bakirel T, Bakirel U. *In vivo* assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. Journal of Ethnopharmacology. 2000; 116:64-73.
27. Pellegrino M, Christopher B, Michelle M, Gerard R. Development of a new model of type II diabetes in adult rats administered with streptozotocin and nicotinamide. Diabetes. 1998; 47:224-30.
28. Muhtadi Primarianti AU, Sujono TA. Antidiabetic Activity of Durian (*Durio zibethinus* Murr.) and Rambutan (*Nephelium lappaceum* L.) Fruit Peels in Alloxan Diabetic Rats. Procedia Food Science. 2015; 3:255-261.
29. Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. Food Chem Toxicol. 2008; 46:2376-2383. doi: 10.1016/j.fct.2008.03.020.
30. F Jahan, M Hossain, A Mamun, *et al*. An evaluation of antinociceptive effect of methanol extracts of Desmodium gangeticum (L.) Dc. Stems and Benincasa hispida (Thunb.) Cogn. Leaves on acetic acid-induced gastric pain in mice," Advances in Natural and Applied Science. 2010; 4:365-369.
31. Griesmacher A, Kinder HM, Andert S. Enhanced serum levels of thiobarbituric acid reactive substances in diabetes mellitus. American Journal of Medicine. 1995; 16:331-335.