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EVALUATION OF QUALITATIVE PHYTOCHEMICAL CONSTITUENTS AND *IN VITRO* ANTI-DIABETIC ACTIVITY OF *Cardiospermum halicacabum* L.

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ABSTRACT

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates due to absolute or relative deficiency of insulin. In recent years, there is a sharp increase in the incidence and prevalence of diabetes mellitus which demands an effective and immediate approach to cure it. One of the anti-diabetic therapeutic approaches is to reduce the post prandial glucose level in blood by the inhibition of the intestinal digestive enzymes, such as alpha-amylase and alpha-glucosidase. The objective of the present work was to analyze the phytochemicals qualitatively and to evaluate the anti-diabetic activity of different polar and non-polar extract of *Cardiospermum halicacabum* L. *in vitro* conditions. Different concentrations of extracts were used for the evaluation of alpha-amylase and alpha-glucosidase inhibition activity. The assay results suggest that all the extract of *C. halicacabum* exhibit dose-dependent increase in inhibitory activity on α-amylase and α-glucosidase enzymes due to the presence of phytoconstituents. This infers that *C. halicacabum* can be used as a potent herbal drug for the treatment of diabetic patients.

Keywords: ***Cardiospermum halicacabum*. L, Phytochemicals, Anti-diabetic, α-amylase, α-glucosidase.**

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INTRODUCTION

During the last decades a global trend to focus on green medicines due to minimum side effects and cost effectiveness. Medicinal plants play an appreciable role in the development of modern herbal medicines as many diseases like cancer, liver diseases and arthritis finds no complete cure in allopathy [1]. Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [2]. Type 2 diabetes is complicated by several factors inherent to the disease process, such as insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin mediated glucose uptake, and utilization [3].The chronic hyperglycaemia of diabetes is associated with the long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [4]. It is a leading non-communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world [5]. According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% [6]. Currently available therapies for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigations [7]. Plant- based products have been used as a natural drug from ancient times. These alternative phytomedicines are cost effective and without any side effects. Plant foods have reported to have good enzyme inhibitors in type 2 diabetes [8,9]. Biological actions of the plant products used as alternative medicines to treat diabetes are in relevance to their chemical composition. Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which help to reduce blood glucose levels [10]. Mostly, these phytoconstituents act on pancreas, elevate insulin secretion or action and thus trigger hypoglycemia. Another mode of antihyperglycemic action offered by these bioactive compounds is through inhibition of enzymes engaged in carbohydrate digestion [11,12]. The intestinal enzymes like α-amylase and α-glucosidase are found to be very important in carbohydrate digestion and glucose absorption. The suppression of the activity of such digestive enzymes would delay the degradation of starch and oligo saccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation [13]. *Cardiospermum halicacabum* L. is a highly important medicinal plant belongs to the family Sapindaceae. This herbaceous plant is extensively dispersed in tropical and subtropical areas of the world [14]. This plant is used in the Indian traditional medicine of Ayurveda and folk medicine for the treatment of rheumatism, lumbago, earache, and fever [15]. It is also a diuretic, stomachic and rubefacient [16]. Studies conducted on the plants antibacterial activity [17,18], antipyretic [17,19], analgesic [17], anti-parasitic [20], anti-diarrheal [21], antioxidant [22], suppression of TNF production [23] and anticancer [24] revealed its therapeutic potential. Various pharmacological actions of *C. halicacabum* have also been investigated in animal models for anti-inflammatory [25], analgesic and vasodepressant [26], antipyretic [27] and anti-ulcer activities [24]. The present investigation is directed to the exploration of the anti-diabetic activity based on the study of the various extracts of *C. halicacabum*. To better understand the biological activities of *C. halicacabum*, we determined the phytochemicals present in the various extracts of leaf and stem and to determine the potential of different extracts as anti-diabetic agents, we investigated the effect of extracts on inhibition activity of alpha amylase and alpha glucosidase enzyme.

MATERIALS AND METHODS

Collection of Plant Material and authentication

The fresh plant material was collected from local market of Tambaram, Chennai, India. The plant was botanically identified as *Cardiospermum halicacabum* L. and authenticated by Dr. Arvind, National Institute of Siddha, Medicinal Plants of Research Unit, Tambaram, Chennai.

Extraction of Plant material

The leaves and stem part of *C. halicacabum* were thoroughly washed under running tap water, dried in oven (50° C) for 4-5 days. Then the dried materials were homogenized to fine powder and kept in sterile plastic air-tight container for further use. For extraction, two polar solvents such as aqueous, methanol and two non-polar solvents, heptane, benzaldehyde used separately for leaves and stem. About 100 g of plant powder was mixed with 300 ml of each solvent. The extraction was carried out by continuous percolation method using Soxhlet apparatus for 36 h accompanying with occasional shaking and stirring. The extract was underwent a coarse filtration by muslin cloth followed by a filtration through Whatmann filter paper. Each extract was concentrated by distilling off the solvent and evaporated to dryness under vacuum. The crude extracts were used for the phytochemical analysis. Different concentration (50 - 1000 µg/ml) of plant extract was used for anti-diabetic analysis extracts by dissolving dried extract in 1 % carboxy methyl cellulose (CMC).

Qualitative phytochemical screening

Phytochemical screening was done to investigate the plant material in terms of its active constituents. In order to establish the profiles of the extracts for their nature of chemical composition and identification of various phytoconstituents, different qualitative chemical tests were performed [28,29].

Evaluation of in vitro anti-diabetic activity

Inhibition of alpha amylase enzyme assay

A total of 500 µl of test samples and standard drug (50- 1000 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic (DNS) acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Acarbose was used as a standard drug for assay. The control samples represent 100 % enzyme activity and were prepared without any plant extract [30]. Each test was performed three times and the mean absorption was used to calculate the percentage α-amylase inhibition.

% Inhibition was calculated according to the formula

$$\% \text{ Inhibition} = \frac{\text{A540 Control} - \text{A540 Sample}}{\text{A540 Control}} \times 100$$

Inhibition of alpha glucosidase enzyme assay

The α-glucosidase inhibitory effect of *C. halicacabum* was determined according to the Standard method using different concentration (50- 1000 µg/ml) of leaves and stem extracts [31]. 10 µl of α-glucosidase enzyme solution and varying concentrations of the extract is incubated together for 10 minutes, at 37°C, and the volume was made up to 210 µL with maleate buffer, pH 6.0. The enzyme reaction is started by adding 200 µl of 2 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) solution and further incubated at 37°C for 30 minutes. Enzymatic reaction was stopped by adding 2 ml 0.2 M sodium carbonate solution. After the addition of 1.0 ml of 0.1 M disodium hydrogenphosphate solution, the absorption of

liberated p-nitrophenol is read at 400 nm. Each test was performed three times and the mean absorption was used to calculate the percentage α-glucosidase inhibition. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing the plant extracts prepared with different solvents.

percentage Inhibition was calculated according to the formula

$$\% \text{ Inhibition} = \frac{\text{A400 Control} - \text{A400 Sample}}{\text{A400 Control}} \times 100$$

Statistical Analysis

All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences. Values were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Diabetes is considered as one of the five leading causes of increasing premature death in worldwide, which demand immediate and alternative remedies. Natural drugs are being used against various diseases in the world since the past history. The discovery, development and the use of modern medicines have a deep routed connection with the age old practice of folk and traditional medicinal background of the natives [32]. Antihyperglycemic activities of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/or to stimulate insulin secretion [33]. In this study, *Cardiospermum halicacabum* leaf and stem extract are used for the analysis of phytochemicals and the assessment anti-diabetic using different solvents.

Qualitative Phytochemical screening

Two polar solvents, namely, aqueous and methanol and two non-polar solvents, such as heptane and benzaldehyde were used for extraction of different phytoconstituents from *Cardiospermum halicacabum.* Here leaf and stem part of the plants were used and all the tests were performed separately. The leaf (Table 1) and stem (Table 2) extracts of *C. halicacabum* confirms the presence of various secondary metabolites such as terpenoids, alkaloid, saponins, phenol, coumarin, tannin. Any of these secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic activity of the plant.

Table 1
Qualitative phytochemical analysis of Cardiospermum halicacabum leaf extract

Phytoconstituents	Aqueous	Methanol	Heptane	Benzaldehyde
Terpenoids	-	+	+	-
Phenol	+	+	+	-
Flavonoids	+	+	+	+
Coumarin	+	+	-	-
Tannins	+	+	+	-
Amino acids	-	-	-	-
Alkaloids	+	+	+	+
Cardiac glycosides	-	-	+	-
Saponins	+	+	-	-
Carbonyl	-	-	-	-

(+) Presence of Constituents, (-) Absence of Constituents

Table 2
Qualitative phytochemical analysis of Cardiospermum halicacabum stems extract

Phytoconstituents	Aqueous	Methanol	Heptane	Benzaldehyde
Terpenoids	-	+	+	+
Phenol	+	+	+	-
Flavonoids	+	+	+	+
Coumarin	+	-	-	-
Tannins	+	+	+	-
Amino acids	-	-	-	-
Alkaloids	+	+	+	+
Cardiac glycosides	+	+	+	-
Saponins	-	-	-	-
Carbonyl	-	+	+	-

(+) Presence of Constituents, (-) Absence of Constituents

Evaluation of in vitro α-amylase inhibitory activity

Different concentrations of stem and leaf extract of *C. halicacabum* were used to evaluate *in vitro* α-amylase inhibitory activity. All the extracts showed significant anti-diabetic activity. The percentage inhibition of alpha amylase is dose dependent, as dose increases, inhibition increases by all the extracts. Among the all four leaf extracts, the activity of polar solvent i.e. aqueous extracts was found to be better of 99.75 %, even than standard drug acarbose (Figure 1). Similarly in case of stem extract, polar solvent, methanolic extract showed maximum anti-diabetic activity of 97 % (Figure 2). For both the cases, the highest concentration 1000 mg/ml showed maximum inhibition activity.

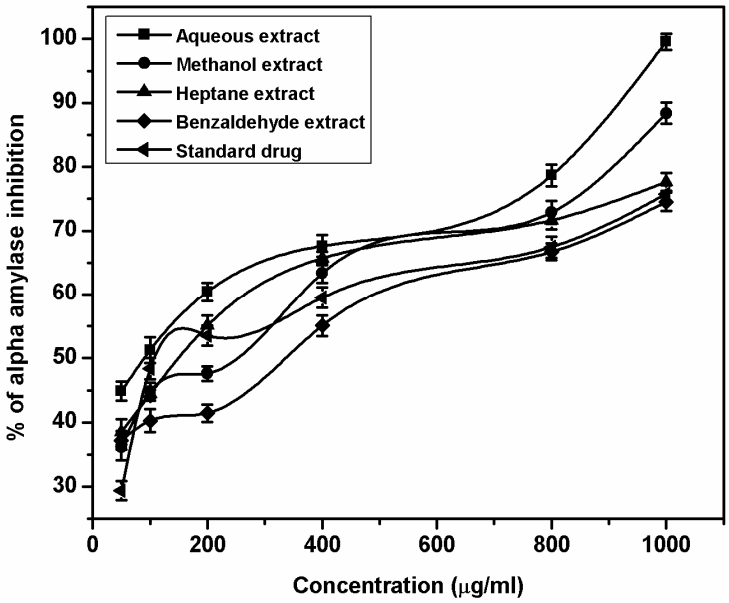

Figure 1
In vitro alpha amylase inhibition of leaf extract of C. halicacabum

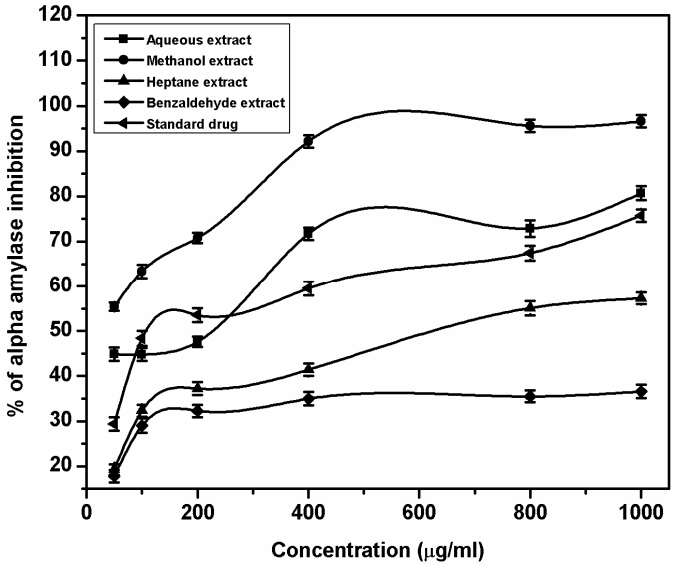

Figure 2
In vitro alpha amylase inhibition of stem extract of C. halicacabum

Evaluation of in vitro α-glucosidase inhibitory activity

The *in vitro* α-glucosidase inhibitory action was demonstrated using different solvents extract of *C. halicacabum* leaf and stem. The percentage inhibition at 50, 100, 200, 400, 800 and 1000 µg/ml concentration showed a concentration dependent increase in percentage inhibition for leaf (Figure 3) and stem (Figure 4). Thus the highest concentration of 1000 µg/ml tested showed maximum α-glucosidase inhibition of nearly 99 % by leaf extract and 96 % by stem extract. The stem and leaf of *C. halicacabum* heptane extract revealed significant a significant inhibitory action of alpha-glucosidase enzyme. The results revealed that heptane extract produced effective α-glucosidase inhibition activity (86 %) even at low concentration (50 µg/ml). In addition, the plant extract showed better anti-diabetic activity than the standard drug.

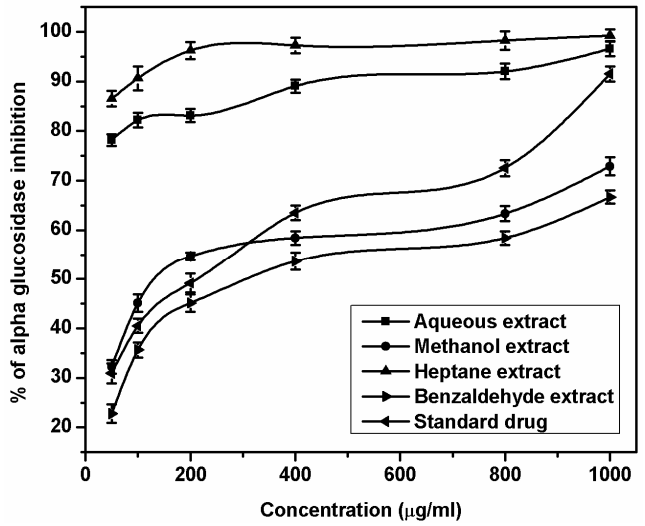

Figure 3
In vitro alpha glucosidase inhibition of leaf extract of C. halicacabum

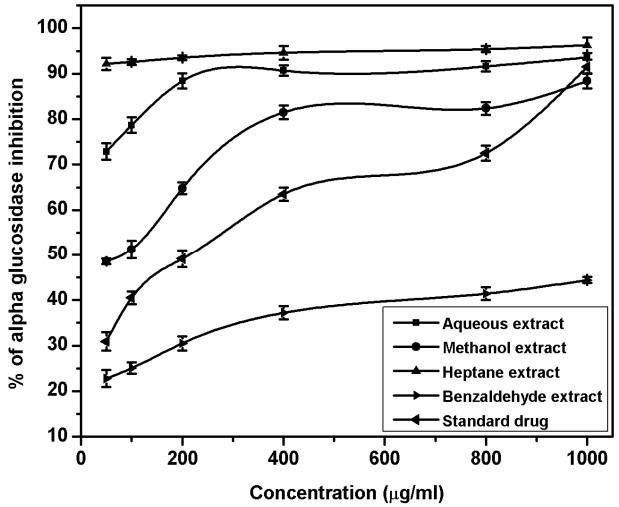

Figure 4
In vitro alpha glucosidase inhibition of stem extract of C. halicacabum

CONCLUSION

The present observation provides evidence that the ability of various extracts of *Cardiospermum halicacabum* efficiently inhibits both alpha amylase and alpha glucosidase enzymes *in vitro* in a dose dependent manner. In particular, aqueous, methanol and heptane leaves and stem extracts of *C. halicacabum* represent maximum anti-diabetic activity. This effect may be due to the presence of various phytoconstituents present in the leaves which could act independently in enhancing the inhibition activity of intestinal carbohydrate-digestive enzymes. Thus, further comprehensive chemical and pharmacological investigation should be carried out to isolate, purify and characterize the active compound and appropriate elucidation of its mechanism of action. However the results of this study provide the information about the significant anti-diabetic properties of *C. halicacabum* by *in vitro* methods, these effects need to be confirmed by employing different *in vivo* models and clinical trials for their effective utilization as therapeutic agents.

REFERENCES

1. Nair SS, Kavrekar V, Mishra A, Evaluation of *In Vitro* Anti diabetic Activity of Selected Plant Extracts, International Journal of Pharmaceutical Science Invention, 2(4): 12–19, (2013).
2. Rajiv Gandhi G, Sasikumar P, Antidiabetic effect of *Merremia emarginata Burm.* F. in streptozotocin induced diabetic rats, Asian Pacific Journal of Tropical Biomedicine, 2: 281–286 (2012).
3. Gruenwald J, Freder J, Armbruester N, Cinnamon and health, Critical Reviews in Food Science and Nutrition, 50(9): 822–834, (2010).
4. American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care, 30: S42–S47, (2007).
5. Zimmet PZ, Diabetes epidemiology as a tool to trigger diabetic research and care, Diabetologia, 42: 499–518, (1999).
6. Abdalla M, Abdelatif, Mariam Y, Ibhrahim, Mahmoud S, Antidiabetic Effects of Fenugreek (*Trigonella foenum-graecum*) Seeds in the Domestic Rabbit (*Oryctolagus cuniculus*), Research Journal of Medicinal Plant, 6: 449–455, (2012).

7. Balamurugan K, Nishanthini A, Mohan VR, Antidiabetic and antihyperlipidaemic activity of ethanol extract of *Melastoma malabathricum* Linn. leaf in alloxan induced diabetic rats, Asian Pacific Journal of Tropical Biomedicine, 4: S442–S448, (2014).
8. Kwon Y-I, Apostolidis E, Kim Y-C, Shetty K, Health Benefits of Traditional Corn, Beans, and Pumpkin: *In Vitro* Studies for Hyperglycemia and Hypertension Management, Journal of Medicinal Food, 10(2): 266–275, (2007).
9. Cheplick S, Kwon Y, Bhowmik P, Shetty K, Phenolic-linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension, Bioresource Technology, 101(1): 404–413, (2010).
10. Jung M, Park M, Chul HL, Kang Y, Seok-Kang E, Ki-Kim S, Antidiabetic agents from medicinal plants, Current Medicinal Chemistry, 13: 1–16, (2006).
11. Kumar P, Singh VK, Singh DK, Kinetics of enzyme inhibition by active molluscicidal agents ferulic acid, umbelliferone, eugenol and limonene in the nervous tissue of snail *Lymnaea acuminate,* Phytotherapy Research, 23(2): 172–177, (2009).
12. Mahesar H, Bhutto MA, Khand AA, Narejo NT, Garlic used as an alternative medicine to control diabetic mellitus in alloxan-induced male rabbits, Pakistan Journal of Physiology, 6(1): 39–41, (2010).
13. Davis SN, Granner DK, Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas, In: Brunton LL, Lazo JS, Parker KL,Ed., Goodman and Gilman's: The pharmacological basis of therapeutics, 11th ed. McGraw-Hill Medical Publication Division, New York, 1706-1707, (2001).
14. Raza SA, Hussain S, Riaz H, Mahmood S, Review of beneficial and remedial aspects of *Cardiospermum halicacabum* L., African Journal of Pharmacy and Pharmacology, 7(48): 3026–3033, (2013).
15. Nadkarni KM, Ed. Indian Materia Medica, 3rd Ed, Vol 1, Popular Book Depot,Bombay, 857–858,, (1976).
16. Joshi SK, Sharma BD, Bhatia CR, Singh RV, Thakur RS, The Wealth of India Raw Materials, CSIR Publication, New Delhi, (1992).
17. Dhar LM, Dhar MM, Dharwan NB, Mehrotra NB, Ray C, Screening of Indian plants for biological activity, Indian Journal of Experimental Biology, 6: 232–247, (1968).
18. Raman N, Radha A, Ravi M, Antibacterial activity of *Cardiospermum halicacabum* against human and plant pathogens, Indian Drug, 35: 29–31, (1998).
19. Guitton E, Montastruc JL, Lapeyre-Mestre M, Influence of HCV or HBV coinfection on adverse drug reactions to antiretroviral drugs in HIV patients, European Journal of Clinical Pharmacology, 62: 243–249, (2006).
20. Boonmars T, Khunkitti W, Sithithaworn P, *In vitro* antiparasitic activity of extracts of *Cardiospermum halicacabum* against third-stage larvae of *Strongyloides stercoralis,* Parasitology Research, 97: 417–419, (2005).
21. Venkat Rao N, Chandra Prakash K, Shanta Kumar SM, Pharmacological investigation of *Cardiospermum halicacabum* L. in different animal models of diarrhea, Indian Journal of Pharmocology, 38: 346–349, (2006).
22. Kumaran A, Karunakaran RJ, Antioxidant activities of the methanol extract of. *Cardiospermum halicacabum,* Pharmaceutical Biology, 44: 146–151, (2006).
23. Venkatesh Babu KC, Krishnakumari S, *Cardiospermum halicacabum* suppresses the production of TNF-alpha and nitric oxide by human peripheral blood mononuclear cells, African Journal of Biomedical Research, 9: 95–99, (2006).
24. Sheeba MS, Asha VV, Effect of *Cardiospermum halicacabum* on ethanol induced gastric ulcers in rats, Journal of Ethnopharmacology, 106: 105–110, (2006).

25. Sadique J, Chandra T, Thenmoshi V, Elango V, Biochemical modes of action of *Cassia occidentalis* and *Cardiospermum halicacabum*in inflammation, Journal of Ethnopharmacology, 19: 201–212, (1987).
26. Gopalakrishnan C, Dhannanjayan R, Kameshwaran L, Studies on the pharmacological actions of. *Cardiospermum halicacabum,* Indian Journal of Physiology and Pharmacology, 20: 203–208, (1976).
27. Asha VV, Pushpagadan P, Antipyretic activity of *Cardiospermum halicacabum*, Indian Journal of Experimental Biology, 37: 411–414, (1999).
28. Mary Helen PA, Prinitha, Jaya Sree S, Madoen Abisha SM, Jacob A, Phytochemical characterization and Antimicrobial activity of oil and solvent extracts of *Curcuma longa,* Research Journal of Pharmaceutical and Biological Sciences, 3(3): 49–52,(2012).
29. Patil SG, Honrao BK, Phytochemical studies in the genus *Cayratia Juss. Vitaceae* and their significance, Journal of Economic and Taxonomic Botany, 24(3): 688–694, (2000).
30. Thalapaneni NR, Chidambaram KA, Ellappan T, Sabapati ML, Mandal SC, Inhibition of carbohydrate digestive enzyme by *Talinum portulacifolium* (Forssk) leaf extract, Journal of Complementary and Integrative Medicine, 5(1):1–10, (2008).
31. Dewi RT, Iskandar YM, Hanafi M, Kardono LBS, Angelina M, Dewijanti ID, Banjarnahor SD, Inhibitory effect of koji *Aspergillus terreus* on α-glucosidase activity and postprandial hyperglycemia, Pakistan Journal of Biological Sciences, 10(8): 3131–3135, (2007).
32. Das MP, Kumar SS, Preliminary Phytochemical Analysis of *Illicium verum* and *Wedelia chinensis*, International Journal of PharmTech Research, 5(2): 324–329, (2013).
33. Gray AM, Abdel-Wahab YHA, Flatt PR, Insulin-like and insulin-releasing actions of the traditional antidiabetic plant *Sambucus nigra* (elder), Journal of Nutrition, 130: 15–20, (2000).