



Original Research Article

Evaluation of Hypoglycemic Property of Gurmar (*Gymnema Sylvestre*) Leaves Methanolic Extract (GSLME) in Streptozocin (STZ) induced Diabetic Albino Rats

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Abstract

Background: *Diabetes is almost everywhere a most common growing health issue in the world and is now emerging as an epidemic world over. currently, so much of importance is being paid to the natural products study as potential antidiabetics. The study of plants which possess Antidiabetic activity may give a new idea in treatment approaches of Diabetes mellitus(DM).*

Objective: *The present research study was aimed to evaluate the Hypoglycemic action of *Gymnema Sylvestre* (*G. sylvestre*) plant leaf methanolic extract (GSLME) in streptozocin - induced diabetic albino rat model.*

Methods: *Albino rats with a weight of 150- 200 grams were weighed & were grouped into 6 equal groups taking 6 rats in each. Group A served as control (normal), Group B as diabetic control, received streptozocin (60mg/kg.b.w.) after overnight fasting. Group C ,D & E was received streptozocin+ GSLME suspension at 100 ,200 & 300 mg/kgdoses orally respectively, Group E was given streptozocin + standard drug (Glibenclamide 5mg/kg.b.w.) suspension for 30 days orally & the GSLME effect on blood sugar(BS) was measured with glucometer at regular intervals. At the end of this research study samples of blood were collected from all rats for biochemical estimation of BS.*

Results: *This present research study has revealed that GSLME has Hypoglycemic effect against streptozocin induced diabetic albino rats on i.p. streptozocin injection at 60mg/kg.b.w. & confirms that on i.p. streptozocin injection causes a significant raise in fasting blood sugar (FBS) in untreated albino*

rats when compared to control group. Diabetic rats treatment with GSLME for 30 days caused a dose dependent fall in FBS levels. Diabetic rats treated with Glibenclamide also showed a significant ($P < 0.00$) fall in FBS levels after 30 days of treatment.

Conclusion: It is concluded that GSLME has shown significant Hypoglycemic action at 100, 200 & 300 mg/kg. b.w. doses in alloxan monohydrate induced diabetic albino rats.

Keywords: GSLME, Glibenclamide, Hypoglycemic, albino rats, BS.

Introduction

Manymore countries has been used most of the medicinal plants to treat and control diabetes mellitus (DM). The hypoglycemic action of these plants is being studied.^[1] Diabetes is a group of disorder of metabolism which is characterized by hyperglycemia which is resulting from the defective insulin action or insulin secretion or even both. Broad research work on diabetes was resulted the synthesis of most of synthetic oral antidiabetic drugs like thiazolidinedione's, biguanides & sulphonyl ureas which are still being in use to treat DM by most of the Physicians (Diabetician/ Diabetologists). But all of these synthetic oral antidiabetic drugs have their own side effects which are associated with their use. On the other side, all of these traditional plants of medicine & their various biological constituents have been used effectively by the different communities since long periods for the diabetes treatment.

Plants have been used abundantly as drug sources for the DM treatment in most of the developing countries where the price of the conventional medicines is a big burden to the population². However, despite these psychosocial implications, as well as the big financial burden associated with DM management, existing treatment options are almost costly & have limited, palliative effects. DM (Diabetes mellitus) is defined as a disorder of metabolism that is characterized by an elevated levels of blood sugar (BS) concentration due to a relative or absolute insulin lack leading to hyperglycemia. DM is usually associated with abnormalities of carbohydrates, protein & fat metabolism^{3,4,5}. The anti-diabetic bioactive compound (bioactive molecules) in the medicinal plants of China are flavonoids, terpenoids, polysaccharides, sterols, and alkaloids⁶. In many countries,

much more care has been paid for the natural antidiabetic drugs discovery from the various medicinal plants⁷. Though at present different types of oral antidiabetic drugs are available along with insulin for the DM treatment, there is an raised importance/value of natural medicinal products with antidiabetic action by most of the patients for diabetes treatment. Orally Insulin cannot be used and continuous usage of the synthetic antidiabetic drugs causes side effects & toxicity^{8,9}. Therefore, it is most important to isolate the correct bioactive molecules from the traditional anti-diabetic plants.

As per as DM management concerned which may include diet, exercise, lifestyle modifications, long term insulin therapy use or oral hypoglycaemic agents. The search for the plants of medicine with hypoglycaemic action is a compartment/section/zone/sector which draws research workers attention. There are roughly a 45 globally reviewed medicinal plants & their products which have been used in the Indian system of traditional medicine. Diabetes mellitus management without any side effect is yet a big task/ challenge/test/problem to the medical community where the routine synthetic oral hypoglycemics only the biggest option for the Diabetes management. There is an continuous search for alternative drugs for Diabetes treatment. Even though effectively herbal medicines have been used from a lengthy period in diseases treatment in Asian communities & even in throughout the world because of their selective action. Therefore, it is so much important to look for more herbal medicines for the DM treatment. From the ancient times, most of the herbal medicinal plant products have been used in DM treatment. So, for the diabetes treatment & for the DM management many

more traditional plants were used. The active components/ constituents/ compounds of these medicinal plants play a key role in the DM management especially in the countries developing yet.

Gurmar (*G. sylvestre*) is a climbing plant & woody plant, native plant of India. The *G. sylvestre* plant leaves Chewing also destroys the ability to differentiate “sweet” taste, which gave it its name, as “sugar destroyer or “gurmar.” Diabetes mellitus (DM) is the most commonest metabolic disorder with both micro & macro vascular complications that results in significant morbidity & mortality. Even WHO has also suggested that the plants with its antidiabetic potential evaluation as effective therapeutic agents, especially in the areas in which harmless newer drugs are usually lacking¹⁰. Although, numerous traditional plants of herbal medicine are reported to have antidiabetic & hypoglycemic properties.

Gymnema sylvestre plant has some important constituents include 2 resins (one is alcohol soluble), 6% gymnemic acids, stigmasterol, quercitol, saponins & the derivatives of amino acid choline, betaine & trimethylamine.¹¹ *G. sylvestre* is a diuretic, stomachic, astringent, refrigerant, and tonic.¹¹ Gurmar has been found to augment /raised urine output & reduce the hyperglycemia in both human & animal studies. Two animal studies on beryllium nitrate & streptozotocin --diabetic rats found that the *G. sylvestre* extracts to double the insulin-secreting beta cells number in pancreas & to return BS to almost normal.^{12,13} *Gymnema sylvestre* increases the enzymes that are responsible for the uptake of glucose & utilization,¹⁴ and inhibits glucose peripheral utilization by somatotropin & corticotrophin.¹⁵

In India *Gymnema sylvestre* leaves, called “Gurmar”, and are most popular for their sweet taste suppressing activity & are used for the diabetes mellitus treatment for over 2000 year, hence the name “Gurmar” meaning 'sugar destroying' to it. *Gymnema* is used in food additives against obesity^[16,17,18,19]. *G. sylvestre* is

presently being used in all of the natural ingredients for diabetes with other plant-based medication. The main objective of the present research study thus strongly focuses on the hypoglycemic action of methanolic extract of leaves of *Gurmar* streptozocin (STZ) induced diabetic albino rats model. Even though, reports of literature indicate that the *G. sylvestre* plant possess hypoglycaemic activity, the plant has not yet been subjected to its scientific investigation. This plant leaves are used in this present research study to check the full effectiveness of the test drug compound in the treatment of Streptozocin (STZ) induced diabetes, on BS levels in experimental albino rat model.

Materials and Methods

Chemicals

Streptozocin ((Sigma Aldrich, United State of America.), Tab Glibenclamide 5mg, glucometer, methanol, 0.5% Tween-80.

Animals

Both sexes of the animals (Albino rats) with a weight between 150gm – 250 gm were used in this experiment. Albino rats were kept in polypropylene cages & were grouped in a group of 6 in every group & were housed at a controlled room temperature of $25 \pm 2^\circ\text{C}$, and a relative humidity of 55% & 12 hrs. light: dark cycle. The rats were fed with the supplied standard food pellet diet & water ad libitum during this experiment. Prior to this present experiment the rats were almost fasted for an exact time period of 12 hrs with water ad libitum given & were weighed. All the study protocols of this research study were cleared and approved by CPCSEA (Committee for the Purpose of Control & Supervision of Experiments on Animals) & were cleared by Institutional Animal Ethics Committee (IAEC) clearance at Mamata Medical College (MMC), Khammam, Telangana state.

Plant Material and Preparation of Test Extract

Gymnema sylvestre plant leaves were collected from local regions near Khammam city at Bhadrachalam in the month of October – November and authenticated by Assistant Professor & Head, Department of Botany, Govt. SRBGNR PG College, Khammam. The collected plant leaves were washed thoroughly with distilled water & shade dried, powdered & stored in air tight containers. The dried *Gymnema* leaves were pulverized & were passed through a 40 mesh sieve. The finely coarsed powder was then extracted with 95% v/v methyl alcohol (methanol) at 600 – 750⁰ C for 48 hours. The collected extract was then filtered, concentrated & dried under the reduced pressure by a rotating evaporator (yield 6.3%) and the residue was kept in desiccators to remove moisture. The methanolic extract suspension was prepared by using Tween-80 0.5% in saline.

Experimental Induction of Diabetes

Full night the Albino rats were fasted & blood was withdrawn from tail vein of the rats of all groups on 10th, 20th, 30st day of the given test drug GSLME from the each rats group using glucometer and FBS values were analyzed and were observed. Serum of collected blood was actually used for biochemical parameter estimation like BS. Almost all of the rats were fasted upto 18 hrs. before Diabetes induction. Intraperitoneal (i.p.) streptozocin (STZ) single dose (60 mg/kg, b.w.) injection was dissolved in 0.1 M sodium citrate buffer of pH 4.5 was used for DM (Diabetes mellitus) induction in rats after full night fasting. After the intraperitoneal streptozocin injection the albino rats had free access to standard food pellets and water (after the 48 hrs of streptozocin administration). The Albino rats were actually allowed to drink a solution of 5% glucose full night to overcome the attack of hypoglycemic shock. The diabetes mellitus development in rats was confirmed after 48hrs of the streptozocin injection. The rats were stabilized for a week & rats showing BS level >200 mg/dL were

actually selected for the study. FBS levels were monitored in blood samples with a glucometer before administration of the drugs.

Experimental Study Design

36 Albino rats were completely divided into 6 groups (A, B, C, D, E & F), 6 animals in each group. Group A was served as normal control or non diabetic received only 10 mL/kg/day of distilled water orally for 30 days on routine diet. Group B was served as diabetic control received single dose of streptozocin (60 mg/kg, b.w., i.p.) dissolved in 0.1 M sodium citrate buffer of pH 4.5 was used for type 2 diabetes (Diabetes mellitus) induction in rats after full night fasting. Whereas the Group C, D & E (Experimental Groups) were treated as Diabetic animals and were received streptozocin (60 mg/kg, b.w., i.p.) with GSLME suspension at a doses of 100, 200 & 300 mg/kg b.w. distilled water /day respectively for 30 consecutive days. Group E was received streptozocin (60 mg/kg, b.w., i.p.) + suspension of Glibenclamide (5mg/kg b.w.) orally for 30 consecutive days. After the 30 minutes of treatment, each group rats were given glucose (5gm/kg) in distilled water orally. Blood samples were collected on experimental days like on day 0 (initial) day, 10th, 20th, 30st days of the given test drug GSLME from each rats group with a glucometer and the BS levels were analyzed and blood sugar (FBS) values were observed.

Statistical analysis

Results of biochemical estimation like BS are reported as mean \pm SD of 6 animals in every group (n=6). The data were actually subjected to ANOVA (one-way analysis of variance) and for the multiple comparisons followed by Dunnett's test was applied for determining statistical significance of difference in blood serum glucose (BS). P values of less than 0.05 (p<0.05) were considered statistically significant.

Results

The present research study investigation has declared/confirmed that the GSLME has Hypoglycemic activity against streptozocin (STZ) induced diabetic albino rats on intraperitoneal streptozocin injection at 60mg/kg body weight dose. This study also confirms that on intraperitoneal streptozocin injection at a 60mg/kg b.w. dose causes significant rise in BS level in untreated albino

rats (diabetic control) groups when compared to control group was shown in (Table 1). Treatment of diabetic rats with GSLME for 30 days caused dose a dependent fall in blood sugar levels(FBS) in diabetic albino rats. treated Diabetic rats with Glibenclamide were also showed a significant ($P < 0.00$) decrease inBS levels after the 30 days of treatment as shown in (Table 1)

Figure -1: Gurmar leaves



Figure-2: Effect of Gurmar leaves methanolic extract (GSLME) on FBS levels in streptozocin (STZ) induced diabetic albino rats.

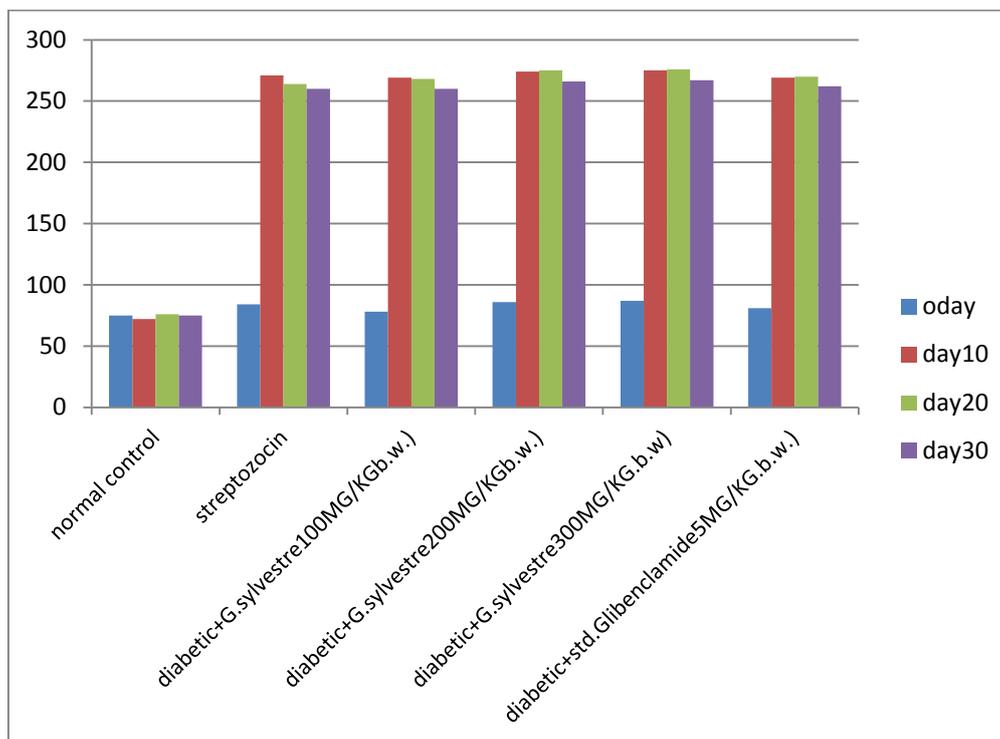


Table 1: Effect of Gurmar leaves methanolic extract (GSLME) on FBS levels in streptozocin (STZ) induced diabetic albino rats.

Treatment Groups	Fasting blood serum glucose (mg/dl)			
	0 Day	Day10	Day 20	Day 30
Duration of treatment	0 Day	Day10	Day 20	Day 30
Normal control group (given only (10ml/kg distill water orally)	75.1±2.1	72.8±1.6	76.8±2.4	75.8±2.1
Diabetic control (given streptozocin (60 mg/kg, b.w., i.p.) dissolved in 0.1 M sodium citrate buffer in a single i.p. dose	84.8±6.9	271.8±5.1	264.3±4.3	260.7±3.9
Diabetic+Gurmar leaves methanolic extract (GSLME) (100mg/kg/ b.w. /day) orally	78.5±5.6	269.8±6.3*	268.8±6.4**	260.5±2.5**
Diabetic+Gurmarleaves methanolic extract (GSLME) (200mg/kg/ b.w. /day) orally	86.5±5.6	274.2±5.3*	275.5±5.8**	266.3±3.2**
Diabetic+Gurmarleaves methanolic extract (GSLME) (300mg/kg/ b.w. /day) orally	87.8±5.4	275±5.3*	276.3±5.6**	267.3±3.2**
Diabetic+standard drug Glibenclamide(5mg/kg b.w.day) orally	81.8±5.4	269.2±5.3*	270.3±5.6**	262.7±3.2**

All values are expressed in Mean±SD. Analyzed by ANOVA (one wayanalysis of variance) followed by Dunnet's test for multiple comparison tests. * = p<0.05 when compared to normal control group (significant p value). ** = p<0.00 when compared to diabetic control group(Highly significant p value).

Discussion

Streptozocin ((STZ) (2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose] is a diabetic inducer, which is produced from streptomyces a chromogens. Rakieten et al at first described the diabetogenic property of STZ²⁰. Streptozocin (STZ), a glucose analogue, was originally isolated from cultures of streptomyces chromogens by Herr et al.(1960).The glucose transporter 2(GLUT2), which mediates uptake of glucose into β – cells STZ causes DNA fragmentation through the free alky lating radicals formation leading to reduction in cellular nucleotides levels & related compounds, particularly NAD⁺, which causes a rapid necrosis of β – cells. Streptozocin (STZ), is now widely used for experimental diabetes induction in the various laboratory animals²¹. STZ produces nuclear DNA damage through the more reactive carbonium ions production which ultimately cause cell death²². Streptozocin is a beta -cytotoxin, which induces chemical diabetes in most of the species of animal including rat by selective damage of the insulin-secreting β -cells of the pancreas. The exact purpose of selecting or choosing of streptozocin as a diabetes-inducer was known to producetype -2 diabetes irreversibly with streptozocin single dose i.p. administration by relative necrotic action on the β -cells of pancreas leading to deficiency of insulin^[23].The main constituents of streptozocin were saponins,

flavonoids & tannins²⁴. The Flavonoids are considered as active principles in most of the medicinal plants²⁵ & natural products with positive effect for human health²⁶.

Treatment of diabetic rats with Gurmar leaves methanolic extract at 100mg/kg, 200 mg/kg& 300 mg/kgb.w. doses for 30 days caused a significant fall in FBS levels in a dose dependent manner when compared to diabetic control group. Most of the studies of G.sylvestre on diabetes revealed that there is a remarkable improvement in the condition of damaged β -cells in histological study of the pancreas with G. sylvestre leaves methanolic extract treated diabetic albino rats. However, the G. sylvestre leaves methanolic extract did not restore the disturbed biochemical parameter like BS levels to normal value in diabetic rats. Hence, the extract can be used in combination with other established anti-diabetic drugs or herbal formulations for more effective outcomes.

Conclusion

The present study, methanolic Gurmar leaf extract of doses of 100mg/kg, 200mg/kg & 300 mg/kg b.w. have potentlyhypoglycemic activity. The exact mechanism of the antidiabetic property of Gurmar methanolic extract is unknown.

However, further studies are required to identify the probable mechanism of action to establish its antidiabetic property.

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Declarations

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee.

References

1. Tenpe C.R. and Yeole P.G. "Comparative evaluation of antidiabetic activity of some marketed polyherbal formulation in alloxan induced diabetic rats". *Journal of Pharma Tech Research*, 2009; 1(1): 43-49.
2. Oliveira ACP, Endringer DC, Amorim LAS, Brandao MGL, Coelho MM. *J* 2005. *Ethnopharmacol*; 102:465.
3. Dinesh, k.B., Analava, M., Manjunatha, M., 2011. Azadirachtolide: an anti-diabetic and hypolipidemic effects from *Azadirachta indica* leaves. *Pharmacognosy Communication*1, 78–84.
4. Shah, J.G., Patel, M.S., Patel, K.V., Gandhi, T.R., 2008. Evaluation of anti-diabetic and antioxidant activity of *Centrathurum anthelmintica* in STZ-induced diabetes in rats. *The International Internet Journal of Pharmacology*, 6:1–10.
5. Sharma, V.K., Kumar, S., Patel, H.J., Hugar, S., 2010. Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rat. *International Journal of Pharmaceutical Sciences Review and Research*, 1: 18–22.
6. Li, W.L., Zheng, H.C., Bukuru, J., De Kimpe, N., 2004. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *Journal of Ethnopharmacology*, 92:1–21.
7. Ugochukwu, N.H., Babady, N.E., 2003. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Sciences* 73:1925–1938.
8. Yamamoto, Y., Nakajima, M., Yamazaki, H., Yokoi, T., 2001. Cytotoxicity and apoptosis produced by troglitazone in human hepatoma cells. *Life Sciences*, 70: 471–482.
9. Tolman, K.G., Chandramouli, J., 2003. Hepatotoxicity of the thiazolidinediones. *Clinics in Liver Disease*, 7: 369–379.
10. WHO expert committee on Diabetes mellitus. Technical reports series World Health Org, Geneva; 1980.
11. Kapoor LD. *Handbook of Ayurvedic Medicinal Plants*. Boca Raton, FL: CRC Press, Inc; 1990:200-201.
12. Prakash AO, Mather S, Mather R. Effect of feeding *Gymnema sylvestre* leaves on blood glucose in berylliumnitrate treated rats. *J Ethnopharmacol* 1986;18:143-146.
13. Shanmugasundaram ER, Gopinath KL, Shanmugasundaram KR, Rojendran VM. Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given *Gymnema sylvestre* leaf extracts. *J Ethnopharmacol* 1990;30:265-279.
14. Shanmugasundaram KR, Panneerselvam C, Samudram P, Shanmugasundaram ER. Enzyme changes and glucose utilisation in diabetic rabbits: the effect of *Gymnema sylvestre*, R.Br. *J Ethnopharmacol* 1983;7:205-234.
15. Gupta SS, Variyar MC. Experimental studies on pituitary diabetes IV. Effect of *Gymnema sylvestre* and *Coccinia indica* against the hyperglycemia response of somatotropin and corticotrophin

- hormones. *Indian J Med Res* 1964;52:200-207.
16. Kapoor LD. CRC Handbook of Ayurvedic Medicinal Plants; CRC, Boca Raton. 1990; 200–201.
17. Dixit RS., Pandey HC. Plant used as folk-medicine in Jhansi and Lalitpur sections of Bundelkhand, Uttar Pradesh. *Int J Crude Drug Res.* 1984; 22: 47–51.
18. Gupta SS., Seth CB. Experimental studies on pituitary diabetes II: Comparison of blood sugar level in normal and anterior pituitary extract induced hyperglycemic rats treated with a few Ayurvedic remedies. *Indian J Med Res.* 1962; 50: 708-714.
19. Reddy MB., Reddy KR., Reddy MN. A survey of plant crude drugs of Anantapur district, Andhra Pradesh, India. *Int J Crude Drug Res.* 1989; 3: 145–155.
20. SK Gupta. Antidiabetic Agents. In : Drug Screening Methods. 2nd Edition. New Delhi: Jaypee Brothers Medical Publishers; 2009. P 308.
21. M.N. GHOSH some common evaluation techniques In: fundamentals of experimental pharmacology 5th Edition. kolkata: Hilton & company; 2011. P 160-61.
22. SK. Kulkarni Practical Pharmacology and Clinical Pharmacy. 1st Edition. New Delhi: Vallabh Publications; 2007. P 205-06.
23. Kumar NJ, Loganathan P. Hypoglycemic effect of *Spinaciaoleracea* in alloxan induced diabetic rat. *Global J Bioech Biochem* 2010; 5(2): 87-91.
24. Jouad H, Lacaille-Dubois MA, Lyoussi B, Eddouks M (2001c) Effects of the flavonoids extracted from *Spergularia purpurea* Pers. On arterial blood pressure and renal function in normal and hypertensive rats. *J Ethnopharmacol*, 76: 159–63.
25. Wollenweber E (1988) Occurrence of flavonoid aglycones in medicinal plants. In: Cody V, Middleton EJR, Harborne JB, Beretz A (Eds.). *Plant flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties.* Progress in Clinical and Biological Research, vol. 280, Alan R. Liss, New York, 45–55.
26. Das NP, Ramanathan L (1992) Studies on flavonoids and related compounds as anti-oxidants in food. In: OngASH, Packer L (Eds.) *Lipid-soluble anti-oxidants: Biochemistry and Clinical Applications*, Birkhauser, Basel, 295–306.