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Original Article

ANTIOXIDANT, ANTIHYPERGLYCEMIC AND ANTIGLYCATION PROPERTIES OF SOME *SWERTIA* SPECIES FROM WESTERN GHATS

PARTHRAJ KSHIRSAGAR[a], TANAJI MORE[b], AKALPITA ARVINDEKAR[b], NIKHIL GAIKWAD[a]*

[a]Department of Botany, Shivaji University, Kolhapur 416004, (MS) India, [b]Department of Biochemistry, Shivaji University, Kolhapur 416004, (MS) India.
Email: nbgaikwadsuk@gmail.com

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ABSTRACT

Objective: Oxidative stress and Advanced Glycation End-products have been associated with diabetic complications. Therefore, natural compounds or extracts that possess both antioxidant and anti-glycation activities might have great therapeutic potential for treating diabetic complications.

Methods: The main purpose of this study was to evaluate the total phenolics, total flavonoids, antioxidant, antihyperglycemic and anti-glycation properties of aqueous extracts of some *Swertia* species from Western Ghats.

Results: The present study revealed that the S. Minor showed the highest amount of total phenolics, total flavonoids and antioxidant activities as compared to other species under study. *Swertia angustifolia* var. *pulchella* showed prominent decrease in blood glucose level and the *S. lawii* distillate showed the highest reduction in fructosamine content with greater anti-glycation property.

Conclusion: All the *Swertia* species distillate analyzed in this study has exhibited potent hypoglycemic activity. Our study tends to support the traditional use of these medicinally important species and alternative source of diabetic medicines. Further study required to analyze the phytochemicals and their mechanism related to antioxidant, antidiabetic and antiglycation properties.

Keywords: *Swertia*, Antioxidant, Antihyperglycemic, Anti-glycation.

INTRODUCTION

Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, hyperlipidemia, hyper amino acidemia and hypo insulinaemia, it leads to decrease in both insulin secretion and insulin action [1]. It is the most common endocrine disorder world-wide; effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs including kidney, nerves, heart and gastrointestinal tract [2]. Diabetes is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including the superoxide radical, hydrogen peroxide and hydroxyl radicals or reduction of antioxidant defense system [3]. Oxidative stress is found to be increased in patients with diabetes mellitus [4]. Evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of Diabetes [5]. The plants having polyphenols, vitamins, carotenoids, flavonoids and terpenoids play an important role against free radicals [6]. There is huge demand for natural antioxidants in food industry for replacing the synthetic antioxidants.

Despite the advancement in the synthetic anti-diabetic drugs, diabetes is still remarkably not cured successfully. Similarly the herbal drugs have gained wider importance worldwide, mostly due to higher safety, less number of adverse effects and consistent blood glucose lowering capacity. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus. The use of herbal medicine for the victims of diabetes is encouraged in the developed countries, by the concern about the adverse effects and cost associated with chronic use of synthetic drug [7]. Advanced glycation end-products (AGEs) are generated in the diabetes as a result of chronic hyperglycemia and enhanced oxidative stress [8-10]. AGEs can accumulate at many sites of the body in diabetes, including the heart and large blood vessels. Because the abundance of AGEs has direct relevance to the pathogenesis of diabetic complications, a clear understanding of the factors contributing to AGE formation may help in ameliorating tissue damage. Advanced glycation end-products and oxidative stress have been implicated in the pathogenesis of diabetic complications. Both are known to interact with each other. Therefore, natural compounds or extracts that possess both antioxidant and antiglycation activities might have great therapeutic potential for treating diabetic complications. *Swertia* L. is ethnomedicinaly important genus of family Gentianaceae. *Swertia* plants are used widely as traditional medicines in the treatment of diabetes [11, 12]. The major class of compounds among the chemical constituents of this genus have been reported to show significantly hypoglycemic activities [13]. Recent review on species of *Swertia* showed the hypoglycemic activity such as *S. chirayita* [16], *S. japonica* [17] and *S. punicea* [18]. The *Swertia* species present in Western Ghats viz. *S. densifolia, S. lawii* and *S. minor* is used as adulterant [19, 20] and substitute [21] to *S. chirayita.*

There are several synthetic drugs currently available in market for treatment of diabetes. They suffer from several drawbacks such as beta cell burnout, weight gain and toxicity etc. The major problem with diabetes is that prolonged hyperglycemia leads to establishment of several complications such as nephropathy, neuropathy and retinopathy. This essentially arises from the non specific glycation of protein which brings out structural and functional changes in protein and initiates a cascade of reactions ultimately leading to tissue damage. Hence in the present study five species of *Swertia* found in the various parts of Western Ghats of India are studied for their total phenolics, total flavonoids, antioxidant, antihyperglycemic and also for glycation inhibition activity.

MATERIALS AND METHODS

Plant material

The plants of *Swertia* species were collected from different localities of Western Ghats (Table 1). The plant material was authenticated by Prof. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur and voucher specimens of the plants have been deposited in the herbarium of the Department of Botany, Shivaji University, Kolhapur.

Preparation of plant extract

The plants were cleaned and air-dried at room temperature and ground to a fine powder using a laboratory grinder, passed through a sieve to obtain uniform powder for the analysis.

Powdered materials were maintained at room temperature and protected from light until required for analysis. About 500 mg of the powder of *S. angustifolia var. pulchella* (D. Don) Burkill, *S. corymbosa* (Griseb.) Wight ex. C. B. Clarke, *S. densifolia* (Griseb.) Kashyapa, *S. lawii* Burkill and *S. minor* (Griseb.) Knobl was extracted with 50 ml distilled water at room temperature for 24 hrs. After that the extracts were filtered through filter paper and filtrate was used for further phenolics, flavonoids and antioxidant analysis. For the antidiabetic study the plant material (5 g) was soaked in 100 ml distilled water and distilled. The distillate was collected and used for further analysis.

Chemicals

All the chemicals and solvents used were of analytical grade. Folin-ciocalteu reagent, sodium carbonate, aluminium trichloride, ferric chloride, potassium acetate, ascorbic acid, 2,4,6- tripyridyl-s-triazine (TPTZ), 2,2-Diphenyl-1- picrylhydrazyl (DPPH), streptozotocin, bovine serum albumin and nitroblue tetrazolium (NBT) were obtained from Sigma chemical Co., USA.

Analysis methods

Determination of total phenol content (TPC)

Total phenolic content of the extracts was quantified using Folin-ciocalteu method [22] with some modification. The plant extracts with each solvent (0.125 ml) was mixed with 1.8 ml Folin-ciocalteu reagent (10 fold diluted reagent with distilled water) and kept for 5 min at 25 ^{0}C. before adding 1.2 ml of 15% sodium carbonate in the reaction and kept it for 90 min at room temperature.

The absorbance was measured at 765 nm on UV-Visible spectrophotometer (Shimadzu UV-190 Japan). The amount of total phenol was calculated as mg/g dry powder GAE (Gallic acid equivalents) from calibration curve of Gallic acid standard solution.

Table 1: It shows collection of *Swertia* species from Western Ghats.

S. No.	Name of the Species	Place of Collection	Latitude	Longitude	Voucher no.	Elevation
1	*Swertia angustifolia* var. *pulchella*	Pallakad	N 10^{0}33.312'	E076^0 42.527'	PRK-14	2940 ft
2	*Swertia corymbosa*	Bababhudangiri	N 13^{0}25.408'	E075^0 45.944'	PRK-13	1801 ft
3	*Swertia densifolia*	Kas	N 17^{0}42.579'	E073^0 54.220'	PRK-7	3641 ft
4	*Swertia lawii*	Panhala	N 16^{0}49.126'	E074^0 06.447'	PRK-11	2719 ft
5	*Swertia minor*	Panhala	N 16^{0}49.073'	E074^0 06.425'	PRK-5	2722 ft

Determination of total flavonoid content (TFC)

Total flavonoid content of all the plant extracts were quantified by using the aluminium chloride colorimetric method [23]. The extracts of each solvent (0.5 ml) was mixed with 1.5 ml methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The mixture was vortexed and the reaction was kept at the room temperature for 30 min and absorbance of reaction mixture was measured at 415 nm using UV-Visible spectrophotometer (Shimadzu UV-190 Japan). The content of the total flavonoids was expressed as mg/g dry powder Rutin equivalent (RE) according to the calibration curve obtained from rutin standard solution.

DPPH radical scavenging activity

The antioxidant activities of all the plant extracts were determined by using 2,2-Diphenyl-1- picrylhydrazyl (DPPH) assay [24]. The DPPH reagent was prepared by dissolving 2.4 mg of DPPH in 100 ml of chilled methanol. The different concentration of all plant extract were allowed to react with 3 ml of DPPH reagent. The reaction mixtures were allowed to interact properly and stand in the dark at room temperature for 30 min. The absorbance was measured at 517 nm on UV-Visible spectrophotometer. A control (without extract) also analyzed and the results were expressed as percent radical scavenging activity (% RSA) and calculated using following formula,

$$(\%\ RSA) = \frac{(A_{control} - A_{sample}) \times 100}{A_{control}}$$

Where, A= absorbance at 517 nm.

Ferric reducing antioxidant power (FRAP) activity

The FRAP assay was carried out according to described method [25] with some modifications. FRAP reagent formed by assimilation of the acetate buffer (300 mM- pH-3.6), 2, 4, 6- tripyridyl-s-triazine (TPTZ) in 40 mM HCL (10 mM), and $FeCl_3$ $6H_2O$ (20 mM) in 10:1:1 ratio former to use and heated to 37 ^{0}C in water bath for 10 min The plant extracts of various concentrations were allowed to react with 2.7 ml of the FRAP reagent and the final volume of the reaction was adjusted to 3 ml with distilled water, the reaction mixture was kept in dark for 30 min and the absorbance was recorded at 593 nm. The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

Experimental animals

Albino rats weighing between 150–200 g were used. The experimental protocol used was approved by Animal Ethics Committee (Registration No. 233/CPCSEA). The rats were fed with standard rat diet purchased from Amruth India. Animals were provided with food and water *ad libitum* and maintained 25–28 ^{0}C. Diabetes was induced by injecting 65 mg of Streptozotocin per Kg body weight intra-peritonealy to overnight fasted animals. The diabetic status of the animals was verified by checking their blood glucose on 14th day. Animals with blood glucose above 200 mg/dl were considered diabetic and included in the experimentation.

Oral glucose tolerance test (OGTT)

Glucose tolerance test was administered by feeding the rats with 3 mg glucose/g of body weight. The plant distillate 0.5 ml was given to diabetic rat with intragastric tubes. Blood was withdrawn from tail vein at defined time intervals and blood glucose measured using Accu-check glucometer purchased from Roche India, and results verified using appropriate controls. All the results presented are average values of experiments conducted on a set of three rats.

***In vitro* protein glycation**

Experiments were performed according to the Wu *et al.* method [26] with slight modification. Briefly, bovine serum albumin (BSA, 20 mg/ml) was mixed with glucose (300 mM) and 0.02% sodium azide in phosphate buffer (200 mM, pH 7.4). Distillate (0.5 ml) of each species was added to the final 5 ml reaction mixture, and then the mixture was incubated for 30 days at 37 ºC to obtain glycated sample. Aminoguanidine was used as a positive control.

Fructosamine assay

The procedure of NBT reductive assay followed the method discovered by Baker *et al.* [27] with slight modifications. The glycated sample (0.2 ml) and NBT reagent (0.3 mM, 0.8 ml) prepared in sodium carbonate buffer (100 mM, pH 10.35) were incubated at 37°C for 15 min, and the absorbance was read at 530 nm against a blank. The formation of fructosamine was measured in nM/mg protein.

RESULTS

The determined amount of TPC, TFC and antioxidant activity of *Swertia* species is shown in the table 2. All species showed superior but varying level of TPC, TFC and antioxidant activity.

The result revealed that the *S. minor* has the highest amount of TPC (24.84 mg GAE/g) and TFC (36.24 mg RE/g) as compare to other species of Western Ghats. All the species under study showed the antioxidant activity, similarly *S. minor* shows the strong DPPH radical scavenging activity (85.70 % RSA) and FRAP (2.75 mg

AEAC/g) activity among them. While the *S. corymbosa* showed the less amount of TPC (12.91 mg GAE/g), TFC (7.61 mg RE/g), DPPH radical scavenging activity (33.82 % RSA) and FRAP (1.07 mg AEAC/g) activity than other species under study. In all the species under study content of total phenolics, flavonoids and antioxidant activity pattern showed more or less similar like *S. minor* followed by *S. lawii* followed by *S. densifolia* followed by *S. angustifolia* var. *pulchella* followed by *S. corymbosa*.

The anti hyperglycemic activity of five *Swertia* species was analyzed by OGTT. The OGTT analysis of all species shows blood glucose lowering activity than the diabetic control (Fig 1a). Among the species studied *S. angustifolia* var. *pulchella* shows the very prominent activity for blood glucose reduction, which reduces blood glucose of diabetic animal up to normal level within 2 hour while the other species shows minimal blood glucose reduction. In the OGTT test, the glucose concentration reached at maximum state at first half hour in *S. densifolia*, *S. lawii* and *S. angustifolia* var. *pulchella* following the glucose overload and get decreased after wards, but *S. corymbosa* and *S. minor* showed the maximum level of glucose at one hour and the decreased at particular level. There was no any report on hypoglycemic activity of *Swertia* species under study except Selvameena [28] observed the significant hypoglycemic activity of *S. minor*.

The blood glucose lowering effect of *S. angustifolia* var. *pulchella* was also studied in normal healthy rat against control rat (Fig. 1b). It can be observed that *S. angustifolia* var. *pulchella* has the ability to lower blood glucose even in fasting condition. Insulin as well as secretagogues are have to lower blood glucose in this manner.

Table 2: It shows total phenolics, total flavonoids and antioxidant activities of different *Swertia* species.

Name of the Species	TPC (mg GAE/g)	TFC (mg RE/g)	DPPH (% RSA)	FRAP (mg AEAC/g)
S. minor	24.84±0.20	36.24±0.64	85.70±0.10	2.75±0.03
S. densifolia	15.85±0.15	08.30±0.14	48.65±0.03	1.03±0.02
S. lawii	19.24±0.20	10.33±0.07	60.05±2.10	1.84±0.02
S. corymbosa	12.91±0.06	07.61±0.13	33.82±0.51	1.07±0.01
S. angustifolia var. *pulchella*	13.32±0.15	07.59±0.01	47.23±0.05	1.54±0.00

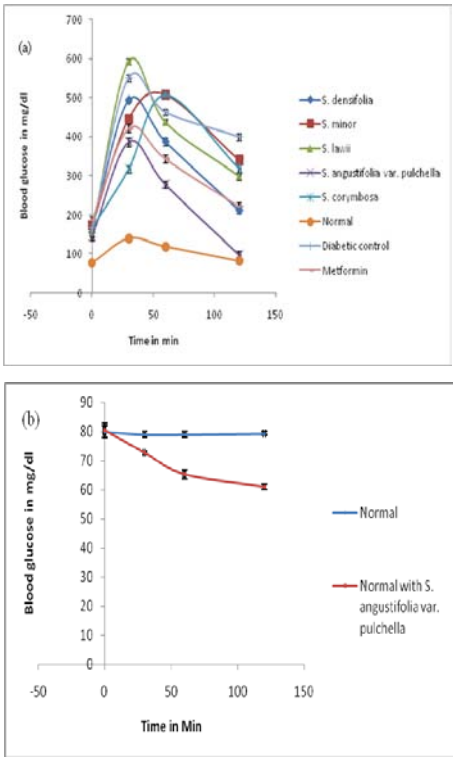

Fig. 1: a) OGTT of different *Swertia* species b) Reduction of blood glucose of normal rat treated with distillate of *S. angustifolia* var. *pulchella*.

Fructosamine is used as an indicator for short-term control of blood glucose level in diabetic patients [29]. The results demonstrated that the reduced level of fructosamine by *Swertia* species extracts (Fig. 2) associated with the decreased formation of AGEs. *S. lawii* showed the greater reduction in the fructosamine content than the other species under study followed by *S. minor*.

The species like *S. densifolia*, *S. angustifolia* var. *pulchella* and *S. corymbosa* does not show much reduction in fructosamine content than control. Reduction in fructosamine levels delays the progression of vascular complications [30].

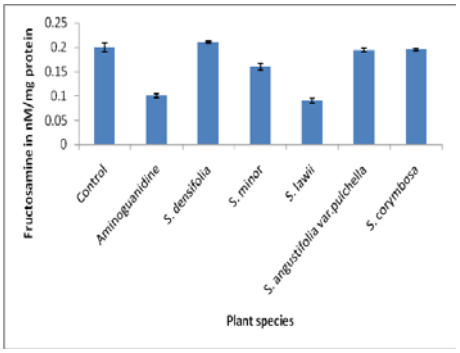

Fig. 2: Inhitibition of fructosamine formation by different *Swertia* species.

DISCUSSION

Researchers are interested in search of new drugs from medicinal plants for their biological activities like antioxidant and antidiabetic. The results of the present study showed that extract of all the five species namely *S. angustifolia* var. *pulchella, S. corymbosa, S. densifolia, S. lawii* and *S. minor* showed strong antioxidant activity. Many studies revealed that there is positive correlation between antioxidant activity potential and amount of phenolic compounds of the extracts [31, 32]. In general, plant flavonoids are highly effective free-radical scavengers and antioxidants [33].

The results of the present study also revealed that extract of all the five species produced a marked decrease in blood glucose levels in streptozotocin induced diabetic rats. It has been shown that the natural products like terpenoids, alkaloids, flavonoids, phenolics and some other categories have good antidiabetic potential [34]. But the presence of higher amount of phenolics and flavonoids in species under study does not showed the better antidiabetic potential, reason behind this may be other group of compounds or the specific compounds play an important role in the hypoglycaemic activity. In *S. japonica* bellidifolin [17] and *S. punicea* methyl swertianin and bellidifolin are xanthoids are reported to have hypoglycaemic activity [18].

Through all the *Swertia* species studied demonstrated good antidiabetic activity. *S. angustifolia* var. *pulchella* has demonstrated potent antihyperglycemic activity and *S. lawii* has shown the property of nonspecific glycation inhibition which is more potent

than the positive control aminoguanidine. It is essential to isolate and identify bioconstituents in these plants so as to generate new lead molecules for drug development in treatment of diabetes.

Abbreviations

(% RSA): percent radical scavenging activity

AEAC: ascorbic acid equivalent antioxidant capacity

DPPH: 2,2-Diphenyl-1- picrylhydrazyl

FRAP: ferric reducing antioxidant power

GAE: gallic acid equivalent

NBT: nitroblue tetrazolium

OGTT: oral glucose tolerance test

RE: rutin equivalent

TFC: total flavonoid content

TPC: total phenolic content

TPTZ: 2, 4, 6- tripyridyl-s-triazine

CONFLICT OF INTERESTS

Declared None

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