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Antiglycation and Insecticidal Potential of *Heliotropium strigosum* Willd.

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ABSTRACT

Heliotropium strigosum Willd. (Chitiphal) is a medicinally important herb belongs to the Boraginaceae family. Traditionally, this plant was used in the medication therapy of various ailments in different populations of the world. The main objective of the current research was to probe the antiglycation and insecticidal potential of this plant. In the present study, the dichloromethane and methanol crude extracts of whole plant of *H. strigosum* were screened to explore the antiglycation and insecticidal potential of this plant by using bovine serum albumin (BSA)-methylglyoxal assay and contact toxicity method respectively. The results showed that the methanolic extract showed weak antiglycation activity at the concentration of 2mg/ml with the inhibition of 52.93% and IC_{50} of 1.8 ± 0.15 while the dichloromethane extract found to be inactive and showed 47.30% inhibition at the same concentration. On the other hand, the dichloromethane crude extract demonstrated moderate insecticidal activity against *Rhizopertha dominica* with 40% inhibition and low activity with 20% inhibition against *Sitophilus oryzae* respectively while the methanol extract exhibited no significant activity against all the tested insects. The presence of various groups of secondary metabolites such as saponins, flavonoids and tannins were also confirmed by different phytochemical tests. On viewing the above contributions, the researchers come to know that from the isolation and purification of valuable phytoconstituents of this plant by using advanced scientific methodologies must be helpful in the preparation of therapeutic agents of desired interest in the world of drug discovery.

Key words: *Heliotropium strigosum* (Willd), Antiglycation activity, Insecticidal potential, Secondary metabolites, Boraginaceae family.

INTRODUCTION

Among the group of endocrine disorders, diabetes mellitus is one of the commonly known chronic ailments which is being characterized by the severity of numerous micro-vascular and macro-vascular complexities of peripheral

nerves, skin, eyes, kidneys and blood vessels along with the occurrence of long term hyperglycemia [1]. The manifestation of diabetes mellitus is usually accompanying with the process of protein glycation (non-enzymatic condensation reaction between the reducing sugars and amino groups of proteins, nucleic acids and lipo-proteins) and sometimes oxidative stress [2]. The formation of advanced glycation end products (AGEs) considered to be the chief complementary factor causing the pathogenesis of different damaging complications of diabetes [3]. Glycation process and formation of AGEs displayed the propensity for the generation of free radicals along with several reactive oxygen species (ROS) that might causes the autooxidation of glycated proteins and reducing sugars [4]. The injurious effects on various body tissues during diabetes might be due to the formation of these free radicals. In the field of drug discovery, advanced scientific approaches are used for the identification and isolation of bioactive constituents from different plant species which demonstrated inhibitory effects against AGEs and showed significant physiological management of glycation process. Thus, therapeutic targeting of glycation process and AGEs found to be very beneficial in the control of different pathogenic complications of diabetes [5].

Medicinal plants are the rich source of naturally occurring phytoconstituents that exhibited substantial role in the planning and development of unthreatened environmental methodologies that are used to protect the harmful outcomes of insect predation and infestation [6]. Pharmacological mechanisms of various extracts of different plant species and their biologically active compounds are observed on insects to explore the pernicious consequences of different classes of insects by probing several systemic methods which involved mortality and morbidity, toxicology, growth inhibitory effect, antifeedant potential, unwanted behavioral alterations related to the process of reproduction followed by diminishing the fertility and fecundity rate [7]. World widely, annual reduction of total harvesting yields up to 10-30% should be reported due to the damaging effects of insects, deterioration of micro-organisms and some other harmful factors [8]. On viewing different scientific investigations related to the existing interactions between various plant and insect species [9], researchers give serious attentiveness upon the discovery and development of newer and safer insect control agents (insecticides) in the world of drug discovery [10].

Heliotropium is one of the complex and largest genus of family Boraginaceae. In tropical and temperate regions, it was represented by 270-275 species while in Pakistan 23 species of this genus are present [11]. In the folk medicinal history, species of genus *Heliotropium* attained the noticeable pharmacological importance. In Malaysia, the paste of whole plant material of *H. indicum* was considered to be effective against putrefaction, pyoderma and ringworm infection [12]. In Tanzania, the juice of leaves of *H. dasycarpum* was applied externally on cuts to stop bleeding and to prevent infection. In Mauritius, the decoction of whole plant of *H. amplexicaule* was used in the therapeutic management of cough and fever [13]. *H. strigosum* exhibited significant therapeutic potential as prescribed in traditional folklore history of medicines. The variety of traditional medicinal uses of *H. strigosum* made it distinguishable among other species of genus *Heliotropium*. For the curing of snake bites, gum boils, eye sores and nettles of stings, the juice of this whole plant is administered. This juice has also used as diuretic and demonstrated some laxative effect [14]. The powder and decoction of whole plant material of *H. strigosum* was used in the medication therapy of rheumatic arthritis, jaundice and also used as blood purifier [15].

Keeping in view, the increased complications of diabetes in the recent years and the development of newer and safer insecticidal agents, this artifact is arranged to examine the antiglycation and insecticidal potential of dichloromethane (DCM) and methanolic extracts of *H. strigosum*. In antiglycation study, we also discuss particularly the co-existing relationship between phenolic compounds (flavonoids and tannins), antioxidants and antiglycation potency in the lights of previously cited literature.

MATERIALS AND METHODS

The current study was performed in the natural product chemistry laboratory, Faculty of Pharmacy, Bahauddin Zakariya University, new campus Multan and International Centre for Chemical and Biological Sciences, H.E.J Research Institute of Chemistry, University of Karachi, Karachi, Pakistan, from August 2014 to August 2015.

a. Collection and identification of plant material

The plant *Heliotropium strigosum* was collected in September 2014 from the surrounding areas of railway ground district Khanewal (Pakistan) and identified by Dr. Muhammad Zafarullah, Assistant professor of Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. A voucher no. "Stewart 591" was assigned to the specimen and preserved in the University herbarium.

b. Preparation of plant extracts

To achieve the purpose of maximum extraction, the whole plant material of *H. strigosum* was dried under shade by putting it on an old newspapers for 20-25 days. When the plant material was completely dried, make the coarse powder of it by crushing in the grinding mill. The extraction of this powdered plant material was accomplished by the process of simple maceration. About 600g of measured powdered material was put into the extraction bottle and a known volume (3×1.5) of dichloromethane was added into the bottle. This mixture was continuously shaken after every 15 minutes for 3 to 4 hours to attain effective extraction and then make it homogenized by the method of ultrasonication. After 24 hours, this mixture was filtered off. This procedure was carried out thrice with dichloromethane in the same manner. After the third collection, the marc was macerated with methanol similarly as that of dichloromethane. To make the extracts of both dichloromethane and methanol concentrated, both of them were employed separately to rotary evaporator under the reduced pressure. After this, both of these extracts were separately collected in the sample bottles and assigned the code names as HSWPD and HSWPM respectively. The extraction of powdered whole plant material yielded 5.15g and 24.50g of crude dichloromethane and methanolic extracts respectively, approximately 0.85% and 4.08% of the total dry weight.

c. Detection of secondary metabolites

Preliminary phytochemical screening of whole plant material of *H. strigosum* was performed for the identification of various groups of secondary metabolites such as alkaloids, glycosides, saponins, flavonoids and tannins. Alkaloids were detected by performing phytochemical tests with Dragendorff's reagent, Hager's reagent, Mayer's reagent and Wagner's reagent. Borntrager's test and Modified Borntrager's test was carried out for the identification of free and bound anthraquinone glycosides. For the detection of cardiac glycosides, Keller-Kiliani test was executed. Similarly, for saponin glycosides, Froth test; for flavonoids, Lead acetate solution test and for tannins, Ferric chloride test and Catechin test was performed [16,17].

d. Antiglycation activity

The dichloromethane and methanolic extracts of whole plant of *H. strigosum* were examined for significant antiglycation activity by using bovine serum albumin (BSA)-methylglyoxal assay. The middle stages of protein glycation process was evaluated by means of this assay.

Bovine serum albumin (BSA)-methylglyoxal assay

In this method, 100mM of phosphate buffer containing sodium azide (3mM) which is used as antimicrobial agent was taken. Prepared the 10mg/ml solution of bovine albumin serum in the phosphate buffer and its pH was adjusted up to 7.4. In the same buffer, prepared the 14mM solution of methylglyoxal. Prepared the solutions of tested crude extracts and the drug which is used as standard inhibitor (Rutin) in dimethyl sulfoxide at the concentration of 1mM. Added 20 μ l of standard inhibitor solution, 50 μ l of methylglyoxal, 50 μ l of bovine serum albumin and 80 μ l of phosphate buffer into every well of 96 well plate. 20 μ l of dimethyl sulfoxide was served as control. The control did not contain any testing sample. The accumulative volume of this reaction mixture was 200 μ l respectively. Incubated this reaction mixture at the temperature of 37°C for almost 9-10 days. When the incubation was completed, recorded the specific development in florescence of each sample by measuring the excitation at 330nm and emission at 420nm respectively by using the microplate reader [18]. The percent inhibition of AGEs formation by tested samples against the control was calculated by the formula given as under.

$$\% \text{ inhibition of AGEs formation} = [1 - (\text{Florescence of the tested group} / \text{Florescence of the control group})] \times 100.$$

e. Insecticidal activity

The methanolic and dichloromethane extracts of whole plant of *H. strigosum* were tested for prominent insecticidal activity by using contact toxicity method against different species of insects particularly *Tribolium castaneum*, *Callosbruchus analis*, *Sitophilus oryzae* and *Rhizophthera dominica*.

Contact toxicity method

In this method, prepared the sample solution by dissolving 200mg of each crude extract in 3ml of ethyl alcohol. Cutting of filter paper was done in such a way that it was easily adjusted into the petri-plate according to its size. The sample was completely loaded upon the filter paper by means of micro-pipette. To evaporate the solvent completely, left these petri-plates for twenty four hours. Very next day, took the healthy insects of same age group and placed ten insects of every species in each petri-plate (both tested sample and control) by using an uncontaminated brush. All these plates were incubated for 24 hours at the temperature of 27°C and maintained the

relative humidity of growth chamber up to 50%. Number of survivors of every species were counted. Permethrin was used as standard insecticidal drug while Permethrin, volatile solvent (acetone) and tested insects were served as positive and negative control respectively [19,20]. The results were expressed in terms of either percentage inhibition or percentage mortality. The percentage (%) mortality can be calculated by the formula described below. Percentage (%) mortality = $[100 - (\text{No. of insects in test} / \text{No. of insects alive in control}) \times 100]$

RESULTS

a. Phytochemical tests for the detection of secondary metabolites

Various phytochemical tests were carried out for the identification of different groups of secondary metabolites in the tested powdered plant material. The consequences of this phytochemical screening exposed the presence of tannins, saponins and flavonoids while alkaloids and glycosides were found to be absent in the tested plant material (Table 1).

Table 1: Results of phytochemical screening of whole plant of *H. strigosum*

Class of secondary metabolites	Name of test	Detection
Alkaloids	Dragendorff's reagent	–
	Wagner's reagent	–
	Mayer's reagent	–
	Hager's reagent	–
Anthraquinone glycosides	Borntrager test (Free anthraquinones)	–
	Modified Borntrager test (Bound anthraquinones)	–
Cardiac glycosides	Keller-Kiliani test	–
Saponins	Froth test	+
Tannins	Ferric chloride test	+
	Catechin test	+
Flavonoids	Lead acetate solution test	+

Note: (–) = Absent and (+) = Present

b. Antiglycation activity

The methanol and dichloromethane extracts of whole plant of *H. strigosum* were studied for the significant antiglycation activity. The results revealed that the methanol extract showed weak antiglycation activity at the concentration of 2mg/ml with the inhibition of 52.93% and IC_{50} of 1.8 ± 0.15 while the dichloromethane extract found to be inactive and showed the inhibition of 47.30% at the same concentration when compared with the standard drug Rutin which displayed 95% inhibition with IC_{50} of 0.20 ± 0.01 (Table 2).

Table 2: Results of antiglycation activity of dichloromethane and methanol extracts of whole plant of *H. strigosum*

Name of sample	Concentration used (mg/ml)	Percent (%) inhibition	$IC_{50} \pm SEM$ (mg/ml)
Dichloromethane extract	2	47	>2
Methanol extract	2	53	1.8 ± 0.13
Rutin (Standard drug)	2	95	0.20 ± 0.01

Positive control= Rutin (standard drug); Negative control= DMSO (Dimethyl sulfoxide).

Data is expressed as mean \pm SEM of three independent readings.

Table 3: Results of insecticidal activity of dichloromethane and methanol extracts of whole plant of *H. strigosum* by contact toxicity method

Name of Insects used	Percent (%) mortality		Percent (%) mortality of tested samples	
	+ve control	-ve control	Dichloromethane extract	Methanol extract
<i>Tribolium castaneum</i>	100	0	NA	NA
<i>Callosobruchus analis</i>	100	0	NT	NT
<i>Sitophilus oryzae</i>	100	0	20	NA
<i>Rhopipertha dominica</i>	100	0	40	NA

+ve control= Permethrin (standard insecticide) and the tested insects; -ve control= Volatile solvent (acetone) and the tested insects; NA= not active; NT= not tested. Concentration of tested sample used= $1.019 \mu\text{g}/\text{cm}^2$; Concentration of standard drug used= $239.5 \mu\text{g}/\text{cm}^2$.

c. Insecticidal activity

The dichloromethane and methanolic crude extracts of whole plant of *H. strigosum* were tested for prominent insecticidal activity. The results showed that dichloromethane crude extract demonstrated moderate insecticidal activity against *R. dominica* with the percent inhibition of 40% and low insecticidal activity with the inhibition of 20% against *S. oryzae* respectively. While the methanol extract exhibited no significant activity against all the tested insects and found to be inactive (Table 3).

DISCUSSION

Diabetes mellitus (DM) is linked with various acute and chronic complications. World-widely, about 1-2% people of different populations are affected by diabetes. Moreover, more than 100 million patients suffering from diabetes are present all around the world which expected to be double in the upcoming 10-20 years [21]. Hyperglycemia is the hall mark of diabetes in which blood sugar level is increased. Long term hyperglycemia stimulates the synthesis of advance glycation end products (AGEs). With the formation of Schiff bases between the amino group of proteins and carbonyl group of reducing sugars, glycation cascade start, which gradually rearranges to moderately stable product known as Amadori adducts. AGEs trigger the critical intracellular signaling pathways and impart a crucial role in the evolution or worsening of different degenerative disorders such as diabetes, Alzheimer's syndrome, ailments of connective tissues mainly rheumatoid arthritis, chronic renal dysfunction and atherosclerosis. The causative effects of AGEs also lead towards the development of destructive micro-vascular and cardiovascular complications during diabetes mellitus [18,22]. In the present study, the methanolic extract of whole plant of *H. strigosum* showed weak antiglycation activity while dichloromethane extract exhibited no significant activity and found to be inactive. The particular mechanism involved in exposure of antiglycation principle of plant extracts was still unknown. But researchers explored the specific relationship between antioxidative potential and inhibition capacities of glycation potency and also focused upon the significant role of phenolic compounds (flavonoids, tannins) behind this correlation. Different scientific reports on numerous plant species mainly *Thymus vulgaris*, *Petroselinum crispum*, *Murraya koenigii*, *Curcuma longa*, *Allium cepa*, *Allium fistulosum*, *Coriandrum sativum* [23], *Aframomum melegueta* and *Zingiber officinale* [5] have clearly elucidate the correlated behavior of phenolic compounds, antioxidant properties and antiglycation activity. Koko et al. [24] narrated the antioxidant and antiglycation potential of ethanolic extracts of twenty Sudanese medicinal plants. Among these extracts, *Acacia nilotica* (bark) displayed significant antioxidant and antiglycation activity with 75% and 78% inhibition respectively while the rest of the plant extracts were less active and only few of them showed more than 50% inhibition capacity. Green tea (a very beneficial and popular drink throughout the world) which is obtained from the leaves of *Camellia sinensis* contains large quantities of tannins and flavonoids which are responsible for its antioxidant and antiglycation effects. Recent studies on commercially available dosage form of *Allium sativum* (garlic) which is known as aged garlic extract (AGE) highlighted that some of the products within AGE obtained after Maillard reaction possessed both antioxidative and antiglycation capacity but more advance studies are required to investigate the dual features of AGE [1]. Garcinol (benzophenone derivative) which is isolated from the ethanolic extract of fruit rind of *Garcinia indica* has shown *in vitro* antioxidation and aniglycation properties along with its metal chelating role [25]. Rutin, a potent antioxidative agent which is also found in the water soluble fraction of Tomato established strong inhibitory action against AGEs formation and revealed prominent antiglycation activity [26]. On viewing the previous studies, to some extent it was cleared that the acute toxicity induced by non-enzymatic protein glycation and AGEs was accompanying with the enlarged production of free radicals and other reactive oxygen species during diabetes. Thus, in the recent years the major area of interest is the discovery of such pharmaceutical agents which demonstrate the synergistic properties of both antioxidant and antiglycation potential rather than targeting each separately [23]. In our study, the consequences of phytochemical tests confirm the presence of phenolic compounds particularly flavonoids and tannins. Hussain et al. [14] demonstrated the significant antioxidative behavior of *H. strigosum*. Quercetin, a flavonoid (flavonol) reported in many plant species, was also isolated from *H. strigosum* [27]. Morimitsu et al. [28] explored antiglycation potential of Quercetin. Thus, from the above contributions, the researchers come to know that further investigations are required for the isolation and purification of different groups of phenolic compounds mainly flavonoids and tannins from *H. strigosum* that might be used as lead in the synthesis of newer phytomedicines which served as strong antioxidants and simultaneously prevent the formation of AGEs and retard the protein glycation process.

More than one million species of insects are present that attain intrinsic places among different ecosystems of the world. Mainly insects have vital role in the transmittance of detrimental pests and microbes that are involved in causing several ailments in humans such as malaria, Lyme and Chagas diseases and dengue fever. The damaging

effects of various insect species namely termites, locusts and gypsy moths on household articles, farming crops and seedling plants and trees can also be reported. Certain diseases and threatening outcomes caused by insects lead towards the massive economical loss which is immeasurable in different populations of the world. Herbal medicines are considered to be the suitable substitute of synthetic and commercial insecticides which possess simple, safer and feasible methods of insect control. Moreover, the practice of chemical insecticides available in markets is strictly prohibited recently because these agents are very expensive, non-biodegradable in nature, fetch harmful effects on environment and increase insecticidal resistance [29]. In our study, the dichloromethane crude extract demonstrated moderate and low insecticidal activity against *R. dominica* and *S. oryzae* respectively while methanol extract exhibited no significant activity against all the tested insects and found to be inactive. The insecticidal potential of this plant is extremely compatible with the previously reported developments in the world of insecticides. The methanolic extracts of *Rumex nepalensis*, *R. hastatus* and *R. dentatus* showed significant and moderate insecticidal activity against *C. analis* and *R. dominica* respectively while exhibited no mortality against *T. castaneum*. Some other medicinal plants such as *Polygonum persicaria*, *P. plebejum* and *Rheum australe* also displayed insecticidal potential in the consistent manner [30]. The methanolic extract of the leaves of *Peucedanum Beluchistanicum* [31] and dichloromethane extract of aerial parts of *Salvia cabulica* [20] revealed prominent insecticidal activity against *T. castaneum* while least activity was shown against *C. analis*. The insecticidal potential of essential oil of *Cinnamomum cassia* and its major bioactive constituent trans-cinnamaldehyde was also examined against the booklice, *Liposcelis bostrychophila* [32]. Recent scientific reports on the insecticidal behavior of *Sapindus mukorossi* (ethanolic extract) [33], *Blumea lacera* (aqueous extract) [34] and *Xanthium strumarium* (aqueous extract) [35] were also under contemplation. At the present times, some of the commonly used insecticidal agents obtained from botanical sources are pyrethrum, plant essential oils (mixtures of total phenolic contents, sesquiterpenes and monoterpenes), neem and rotenone (isoflavonoid). These are seem to be the valuable alternatives of commercially available chemical insecticides [36]. Keeping in view the above mentioned scientific descriptions, the dichloromethane extract of *H. strigosum* exhibited moderate insecticidal potential. This gives the scientists a new approach to pure and isolate such compounds from *H. strigosum* that should accomplish this plant a perceptible role in the field of insect controlling medicinal agents.

CONCLUSION

The dichloromethane and methanolic screening of *H. strigosum* was done for the first time to investigate its antiglycation and insecticidal potential. The methanolic extract of whole plant of *H. strigosum* showed weak antiglycation activity while dichloromethane extract exhibited no significant activity and found to be inactive. However, the dichloromethane crude extract demonstrated moderate and low insecticidal activity against *R. dominica* and *S. oryzae* while methanol extract exhibited no significant activity against all the tested insects and found to be inactive. In conclusion, scientists and pharmacologists give serious attention towards the screening of this plant by using some other scientific bioassay methodologies which might serve as a source for the identification, purification and isolation of beneficial bioactive constituents that seems to be helpful in the synthesis of new therapeutic agents of desired interest. So, in future *Heliotropium strigosum* will be used globally as a source of safer phytomedicines.

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