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Research Article
Exploring α-Amylase Inhibitory Activity of Cow's Urine Extract of *Alternanthera sessilis*

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Abstract

Background and Objective: The current α-amylase and α-glucosidase inhibitors in clinical use such as acarbose, miglitol and voglibose are associated with undesirable side effects such as hypoglycemia, flatulence and diarrhoea due to the inhibitory effect of gastrointestinal digestion which limits their use in the treatment of diabetes when compared to the active principles of plant origin. This study explores the antidiabetic enhancing property of *Alternanthera sessilis* with cow's urine as compared to aqueous extract. **Materials and Methods:** The aqueous and cow's urine extract of *Alternanthera sessilis* was prepared by maceration and both extracts were subjected for *in vitro* α-amylase inhibitory activity. The obtained data were compared and analysed by MS office excel. **Results:** Cow urine has enhanced the time of hydrolysis of starch by α-amylase, which in turn exhibited an inhibitory effect on the α-amylase enzyme. **Conclusion:** From the research work it was revealed that cow's urine extract shows promising α-amylase inhibitory activity compared to aqueous extract. The cow's urine distillate as a solvent for the extraction and compare the α-amylase inhibitory activity of cow's urine extract with aqueous extract and analyze the bio-enhancing effect on bio-active principles of aqueous extract.

Key words: α-amylase, *Alternanthera sessilis*, starch iodine assay, cow's urine, maceration, hypoglycemia, antidiabetic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The global population of people facing major and increasing health problems is diabetes[1]. This is continued to be a global health challenge and economic burden, which is due to increased consumption of carbohydrates and the modern lifestyle. Type-II diabetes cases account for the major portion of diabetes worldwide along with many diabetes-associated complications. The strategy to manage these associated risk factors is by controlling the postprandial hyperglycemia[2]. One of the therapeutic targets currently used in the management of type-II diabetes is the inhibition of α-amylase and α-glucosidase enzymes to decrease the reabsorption of glucose in the intestine[3]. The α-amylase is a major secretory product of the pancreas and salivary gland responsible for the initial hydrolysis of complex carbohydrates to a mixture of oligosaccharides in the intestinal mucosa and the oral cavity to a smaller extent. These sugars are further digested into monosaccharides by the action of α-glucosidase.

The current α-amylase and α-glucosidase inhibitors in clinical use such as acarbose, miglitol and voglibose are associated with undesirable side effects such as hypoglycemia, flatulence and diarrhoea due to the inhibitory effect of gastrointestinal digestion which limits their use in the treatment of diabetes[4]. The above complications have made the researchers think of an alternative method to inhibit α-amylase and α-glucosidase especially biofriendly principles mainly from plant origin.

Cow's urine a divine medicine is believed to have therapeutic value alone[5] or with many herbal drugs for the treatment of various infections and disorders of human beings. The distillate of cow's urine is also reported as an efficient bioactivity enhancer and availability facilitator for bioactive molecules (antibiotic, antifungal and anti-carrier drugs) and has been used along with herbs to treat various ailments by traditional healers[6,7].

For this comparative study, a well-known traditional plant *Alternanthera sessilis* was used, as a vegetable in the Southern Region of India and also as a household remedy to get relief from many ailements[8-11]. The current study planned to explore cow's urine distillate as a solvent for the extraction and compare the α-amylase inhibitory activity of cow's urine extract with aqueous extract and analyze the bio-enhancing effect on bio-active principles of aqueous extract.

MATERIALS AND METHODS

Study area: The research work has been carried out at Bapuji Pharmacy College, S. S. Layout, Davangere-577 004, Karnataka, India during the period of October, 2021 to March, 2022.

Materials: The α-amylase, starch, iodine and potassium iodide were purchased from Sigma-Aldrich, USA and monosodium dihydrogen phosphate and disodium mono hydrogen phosphate was obtained from E. Merck (India) and used without further purification.

Plant material: The leaves of *Alternanthera sessilis* were collected from the local areas of Davangere. It was identified and authenticated by Dr. Haleshi, Assistant Professor, Department of Botany, Davangere University, Davangere. Leaves were dried under shade, coarsely powdered and stored in an airtight container.

Preparation of extracts: The aqueous extract has been prepared by taking 50 g of the shade dried leaves of *Alternanthera sessilis* and macerated in 500 mL of purified water for 72 hrs with occasional shaking. Similarly, the cow's urine extract has been prepared with 500 mL of cow's urine, both were filtered, concentrated in a water bath and stored in a desiccator.

Preparation of reagents

Iodine solution: Iodine 0.254 and 4.00 g of potassium iodide were weighed and dissolved in distilled water and the volume was made to 1 L in a volumetric flask. The above solution was protected from light by keeping it in a dark place to avoid photochemical decomposition.

Starch solution (freshly prepared): Starch soluble 1 g is weighed and dissolved with the aid of a small quantity of boiling distilled water, cooled and quantitatively transferred to 100 mL volumetric flask and made up to 100 mL with distilled water.

Buffer: Monosodium dihydrogen phosphate 0.2651 g and disodium-monohydrogen phosphate 0.825 g were transferred to a 100 mL volumetric flask, dissolved with a small quantity of distilled water and a pinch of sodium chloride was added (to enhance the solubility) and then the volume was made up to 100 mL with distilled water.

α-amylase solution: The α-amylase solution was prepared by dissolving 400 mg with 10 mL buffer in a volumetric flask.

***In vitro* procedure:** The *in vitro* α-amylase inhibitory activity[12] was carried out in triplicate in phosphate buffer solution (pH 8.0 at 37°C) using the method of starch iodine colour assay. The values depicted in the table are the average of triplicate runs. A freshly prepared stock solution of α-amylase

in 10 mL of phosphate buffer was stored under refrigeration. The working plant extract was prepared using a buffer to get a 1 mg mL^{-1} concentration.

The earlier prepared mixture of 0.3 mL of α-amylase and 0.3 mL of plant extract was dissolved in 0.6 mL of phosphate buffer, mixed well and incubated at 37±1°C for 15 min. The 0.4 mL of the above solution was transferred to a test tube containing 3 mL of starch solution and 2 mL of buffer.

To a series of test tubes containing 10 mL of iodine solution, the 0.1 mL of the above solution was added at 0 and 10 sec. The absorbance was measured at 620 nm on each addition at 37±1°C till the absorbance obtained was not significant. The absorbance of aqueous extract was measured similarly.

Percentage inhibition was calculated using the following equation[13].

$$\text{Inhibiton (\%)} = \frac{(A_o - A_t)\,\text{Aq. extract} - (A_o - A_t)\,\text{cow's urine extract}}{(A_o - A_t)\,\text{Aq. extract}} \times 100$$

where, A_o is the absorbance at 0 sec and A_t is the absorbance at 't' seconds.

Statistical analysis: Possible statistical analysis was done using MS office excel.

RESULTS

The parameter measured was absorbance by using a double beam UV-Visible Spectrophotometer. The absorbance is dimensionless (that is No unit). The units of time and wavelength were mentioned as second and nm, respectively.

The absorbance value of cow's urine extract was 0.232, whereas, of aqueous extract was 0.128 at 30 sec. It was found that the cow's urine extract of the plant has delayed the hydrolysis of the starch. The maximum percentage cumulative inhibition of 66.24 was observed at 30 sec. The comparative absorbance and results of aqueous and cow's urine extract were summarized in Table 1.

DISCUSSION

According to the ancient manuscripts from the times Vedic, cow urine is considered a Sanjeevani a medicine capable of curing any kind of alignment. Cow urine is trusted to have beneficial value and is used in various drug formulations. As cow urine consists of 95% water, 2.5% urea, minerals 24 types of salts, hormones and 2.5% enzymes it is considered to be non-toxic. Other than that cow urine also contains iron, calcium, phosphorous, carbonic acid, potash, nitrogen, ammonia, manganese, sulfur, phosphates, urea, uric acid, amino acids, cytokine and phenolic acids and so on[14-16].

There is the various disease which was proven to be cured by cow urine are gastrointestinal tract disorders, urological disorder, migraine, constipation, cancer, heart disease, gynaecological disorder and so on.

In reality, if cow urine is taken consistently even without having ailments, it boosts our immune response by eliminating various toxic substances from our body and keeps us healthy[17].

Various herbal extracts are known to exhibit antidiabetic activities and are used in ayurveda for the treatment of diabetes. Herbal extracts have been used directly or indirectly for the preparation of numerous modern medicines. The *Alternanthera sessilis* of leaves are known to reveal the phytochemicals such as alkaloids, flavonoids, amino acids, carbohydrates, phenols, steroids, terpenoids, saponins and glycosides.

The present study was conducted to validate the medicinal properties of cow urine scientifically. In this regard, it was focused that cow urine has not been used as a solvent for the extraction of phytoconstituents from plant origin, compared to aqueous extract of the same plant through various literature surveys. In the current study, *in vitro* inhibitory effect of α-amylase activity on hydrolysis of starch was compared among the aqueous extract with that of cow urine extract. It was observed that both extracts were capable of inhibiting the α-amylase and thus preventing the digestion of starch to some extent. The percentage inhibition of the

Table 1: Absorbance and percentage of inhibition data of aqueous extract and cow's urine extract (time 0-60 sec)

	Absorbance at 620 nm			
Time in sec ('t')	Absorbance of aqueous extract	Absorbance of cow urine extract	Increase inhibition (%)	Cumulative inhibition (%)
0	0.560	0.562	~0.00	~0.00
10	0.422	0.452	20.29	20.29
20	0.278	0.343	22.34	42.63
30	0.128	0.232	23.61	66.24
40	0.103	0.178	15.97	82.21
50	0.079	0.133	10.81	93.02
60	0.055	0.079	4.35	97.37

extracts was measured by the absorbance values, which in turn directly related to the intensity of colour formation due to the formation of coloured complexes between starch and iodine. The high absorbance value depicts that the intensity of the coloured complex formed between starch and iodine is high, which in turn reflects the hydrolysis of starch was retarded.

It is clear from Table 1 that at 0 sec, the rate of absorbance and percentage inhibition of aqueous extract and cow urine extract of *Alternanthera sessilis* leaves were almost the same and there was no inhibitory activity of α-amylase.

At regular time intervals of 10, 20, 30, 40, 50 and 60 sec the absorbance value of aqueous extract and cow urine extract were compared and it exhibits an increase in percentage inhibition up to 30 sec for both the extracts. Whereas percentage cumulative inhibition keeps on increasing up to 60 sec. However, at 30 sec it represented the maximum percentage of inhibitions of cow's urine extract as compared to aqueous extract.

The above results of cow's urine extract were stimulated to compare the similar work carried out by another researcher. In this connection, Manalo *et al.*[18] have prepared the methanol extract of *Alternanthera sessilis* leaf and fractionated it with three solvents of different polarities: Water, ethyl acetate and hexane. These fractions were screened for mammalian α-amylase and α-glucosidase inhibitory activities *in vitro*. All fractions displayed inhibitory activities in porcine α-amylase and intestinal rat α-glucosidase, with the highest activity observed in the ethyl acetate fraction at 0.52 ± 0.072 mg mL^{-1} and for glucosidase at 2.82 ± 0.21 mg mL^{-1}. Further, Chai *et al.*[19] have carried out similar α-glucosidase inhibitory activity for a methanolic extract of *Alternanthera sessilis.* As a result, the inhibitory activity exhibited at as high as 6 mg mL^{-1} concentration whereas, cow's urine extract in our study exhibited the same activity at the concentration of 1 mg mL^{-1}. Similarly Hossain *et al.*[9] have shown that the methanolic extract of *Alternanthera sessilis* reduced the blood sugar level by 22.9, 30.7, 45.4 and 46.1% at a dose of 50, 100, 200 and 400 mg kg^{-1} b.wt., respectively. Whereas, the cow's urine extract of the same plant in our study reduced the blood glucose level by a percentage cumulative inhibition of 66.24% at 30 sec. This indicates cow urine extract showed more prominent activity.

CONCLUSION

Ayurveda narrates regarding cow urine (Gomutra) as an effective medicinal secretion of animal origin with countless therapeutic values. It is the safest method of therapy and the most effective natural remedy granted by nature to mankind. On analyzing a comparative study of cow's urine extract of *Alternanthera sessilis* leaves with that of aqueous extract, the study revealed that cow's urine extract exhibited promising α-amylase inhibitory activity as compared to aqueous extract. The results of this study could be used as a first approximation to synergize the biological activity of plant sources for other diseases with cow urine as a solvent. The promising results of cow urine *Alternanthera sessilis* leaves extract may be due to the synergistic activity of cow urine along with leaves extract. The chemical constituents of the cow may have enhanced the biological activity of leaf extract.

SIGNIFICANCE STATEMENT

It's clear from the above findings that cow urine extract might have amplified α-amylase inhibitory activity compared to aqueous extract of the same plant. Henceforth, cow urine can be considered as a solvent to enhance the biological activity of the active principles of the plant. Cow urine formulation would reduce the burden on the existing use of chemotherapy, as extensive research is going on all over the world to prove cow urine formulation to be a potential medicine as it is the safest method of treatment which is gifted by nature. And it has also been mentioned in ayurveda that gomutra is capable of removing the entire imbalance in the body, thus maintaining general health. More well-planned studies in an animal model are required and later in human subjects to fully assess its potential as an effective inhibitory agent as most of the studies quoted are *in vitro*. Subsequent evolution of novel drugs can involve cow urine in therapy as an antidote which could open the doors for curing a wide variety of disorders as cow urine is eco-friendly, easily available in abundance and economically cheap.

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