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PHYTOCHEMICAL PROFILE, ANTIBACTERIAL AND ANTIDIABETIC EFFECTS OF CRUDE AQUEOUS LEAF EXTRACT OF *DATURA STRAMONIUM*

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ABSTRACT

The phytochemicals screening investigation for aqueous extract of *Datura stramonium* leaves revealed the presence of carbohydrates, tannins, steroidal glycosides, phenols and saponins. The antimicrobial potential of the extracts of leaves were evaluated against clinical bacterial isolates like *Bacillus, Escherichia coli, Staphylococcus aureus, Pseudomonas, Sarcina* and *Klebsiella*. The results showed the exhibition of antibacterial activity against *all isolates and revealed higher sensitivity* for *Sarcina* and *Staphylococcus aureus*. The present study also reveals that, the assay carried out on alpha-amylase enzyme showed the dose-dependent increase in inhibitory effect with IC_{50} 730 µg. The in vitro studies clearly indicate that the extract of *D. stramonium* leaves has antimicrobial activity and α-amylase enzyme inhibitory activity and hence may be considered as a potential candidate for the management of diabetes mellitus.

Keywords: α-Amylase, *Datura stramonium*, *In vitro* antidiabetic activity, antimicrobial activity.

INTRODUCTION

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. The intestinal digestive enzyme such as alpha-amylase plays a vital role in the carbohydrate digestion. Inhibition of this enzyme significantly decreases the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in management of blood glucose (Reddy *et al.,* 2010; Manikandan *et al.,* 2013). Drugs used in the management of diabetes mellitus (DM) are not without any side effects. This problem has led to the continuous search for new drugs for antidiabetic in the folklore/Ayurvedic medicine with the aim of getting less expensive and safer management options (Ajayi, *et al.,* 2012). A great number of medicinal plants used in the control of the Diabetes mellitus have been reported elsewhere. *Datura stramonium* is a bushy annual plant belonging to the family Solanaceae, also known as thorn apple, prickly burr, jimson weed, moon flower, devil's weed, devil's cucumber and devil's trumpet (Gachande and Khillare, 2013; Mandal and Shah, 2013). Most of the chemical constituents of the plant are found in the whole plant body, which has wide traditional application. The primary psychoactive chemicals in all plants in the genus *Datura stramonium* are the alkaloids, scopolamines, tannins, flavonoids, saponins and phenols which have antimicrobial, anti asthmatic, anti cancer, anti proliferative, free radical scavenging and hypoglycemic activity (Mandal and Shah, 2013). The aim of the present

study was to analyze the phytochemical compounds and to investigate the antibacterial and antidiabetic efficacy of crude aqueous leaves extract of *D. stramonium* by *in vitro* studies.

MATERIALS AND METHODS

Sample Collection

The *Datura stramonium* plants have been collected from Bangalore city northern outskirts in the June 2013. The plant leaves were grinded into powder form after separating them from plant, washing with tap water and drying. The sample was kept in cool dry place till further use. The powdered sample was used for extraction purpose.

Preparation of Aqueous Extracts

The powdered leaf extraction was carried out by decoction process with some modifications. The one part of dried powder sample and five parts of sterilized water were taken in boiling water flasks and boiled till one fourth of the extracts left behind after evaporation and this is allowed to stand for three days. After three days, the extracts were filtered through double layered muslin cloth followed by Whatman No.1 filter paper under aseptic conditions. The crude extract was collected in fresh sterilized bottles and stored in refrigerator for further use (Gachande and Khillare, 2013).

Phytochemicals Analysis

The Phytochemical analysis of aqueous extract was carried out for the presence of flavanoids, glycosides, alkaloids, tannins, saponins, Phenols, using standard qualitative method as described by standard phytochemical procedures (Evans WC and Evans, 2003).

Collection and Maintenance of Clinical Bacterial Strains

The predominant Bacterial strains present in Diabetic patients like *Bacillus, Escherichia coli, Staphylococcus aureus, Pseudomonas, Sarcina* and *Klebsiella* (Chandrakanth *et al.*, 2010; Banashankari *et al.*, 2012) were obtained from the microbiology laboratory of Sapthagiri Institute of Medical Sciences and Hospital, Bangalore, India and used them as test organisms. Bacterial strains were maintained on nutrient agar slant and sub-cultured on nutrient broth for incubation at 37 ^{o}C prior to each antimicrobial testing.

Antimicrobial Activity

Antibiotic discs chloramphenicol (10 mcg/disc), cloxacillin (30 mcg/disc), erythromycin (30 mcg/disc), ampicillin (30mcg/disc) were procured from Himedia, used along with plant extract to measure the antimicrobial activity by disc method. Antibacterial activity was checked by disc method. The discs were prepared using a Whatman filter paper and sterilized in an oven at 150 ^{o}C for 15 minutes before use. The 4-6 days old cultures of bacterial strains were inoculated on solidified nutrient agar in petri plates by spread plate method. Lab prepared discs containing aqueous leaf extracts and commercial antibiotic discs were carefully placed on the inoculated plates using a sterilized forceps. The plates were incubated at 37 ^{o}C for 24-48 hours and observed for zone of inhibition.

In Vitro Antidiabetic Study of A – AMYLASE

A total of 100-1000 µl of plant extracts and standard drug acarbose of 100-1000 µg/ml concentration were taken in 500 µl of 0.02 mM phosphate buffer (pH 6.9) to which 100 µl of α-amylase (27.5mg in 100 ml) added and to this 500 µl of 1% starch solution was added to each test tube. The reaction mixtures were incubated at 25^{0}C for 10 min. The reaction was stopped by adding 1 ml of 3, 5-dinitrosalicylic acid color reagent. The absorbance was measured at 540 nm. Control represents the 100% enzyme activity and was conducted in similar way by replacing extract (Narkhede *et al.*, 2011).

$$\% \text{ Reaction} = \frac{\text{(Maltose) test}}{\text{(Maltose) control}} \times 100$$

% Inhibition = 100- % reaction ± SD

Calculation of 50% Inhibitory Concentration (IC50)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract.

Percentage inhibition (I %) was calculated by

I % = (Ac-As)/Ac X 100, [10].

Where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULT

The phytochemical screening of aqueous extract of *D. stramonium* leaves revealed the presence of carbohydrates, tannins, steroidal glycosides, phenols and saponins (table 1). Any of these secondary metabolites, singly or in combination with others could be responsible for the antibacterial and anti-diabetic activity of the plant. Aqueous crude leaf extracts of *D. stramonium* and antibiotics showed the different levels of inhibition against bacterial strains tested. The leaf extract showed the positive results against all the bacterial isolates, out of which methicillin susceptible *Staphylococcus aureus* and *Sarcina* showed the highest sensitivity as compared to other isolates. The bacteria which are resistances to some of the antibiotics were also inhibited by leaf extract from *D. stramonium*. Results presented in table 2 summarize the antibacterial activities zone of inhibition in form of sensitivity. The in vitro antidiabetic study of *Datura stramonium* demonstrated (table 3) that there was a dose-dependent increase in percentage inhibitory activity against α-amylase and varied from 36% - 61% for the highest concentration with IC_{50} of 730 µg/ml which is compared with the standard drug acarbose having 50 % inhibitory concentration of 290 µg/ml.

DISCUSSION

International Diabetic Federation (IDF) has estimated that by 2025 every fifth diabetic subject in world will be an Indian. The factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. Plants shows antidiabetic activity with various mechanism, i.e.- alteration of glucose metabolism, insulin like effect/insulinotropic action, Improve glucose tolerance, reduction of absorption of glucose from intestine, enhancing insulin signal pathway, hypoglycemia through increase glucose uptake and glycogen synthesis, inhibiting for α-glucosidase and α-amylase, reduction of insulin resistance, reduction of oxidative stress and protecting against tissue damage, generation of beta cells in pancreas etc (Banshidhar and Yadav, 2013). α-amylase, an endoglucanase that catalyses hydrolysis of the internal α-1, 4 glucosidic linkages in the starch for the suppression of postprandial hyperglycemia. This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. acarbose-like drugs, that inhibit α-glucosidase present in the epithelium of small intestine, have been demonstrated to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion (Reddy *et al.,* 2010). Phytochemical screening helps to reveal the chemical constituents of the plant extract and also used to search for bioactive agents for starting products used in the synthesis of some useful drugs (Yakubu *et al.,* 2005). In the present study we screened the *Datura stramonium* for presence of various bioactive agents like carbohydrates, tannins, steroidal glycosides, phenols, saponins and we found in the leaves the presence of several of these bioactive agents (table 1). The antibacterial activity is highest for the *Sarcina* and Methicillin susceptible *Staphylococcus aureus* medium for *Bacillus* and Methicillin resistant *Staphylococcus aureus* and least for *Escherichia coli, Pseudomonas, Klebsiella* (table 2). Johnson *et al.,* have also reported that aqueous extract of *D. stramonium* showed the antibacterial against *Staphylococcus, E. coli* which is conformity to our findings. Gachande and Khillare (2013) studies showed antimicrobial effect of Datura sps stem extract against *Staphaureus, B. subtilis, S. typhi, E. coli. Datura stramonium* showed activity against *staphylococcus aureus & E. coli.* The aqueous extract displayed inhibitory zones against eight clinical bacterial isolates *Streptococcus b hemolytic, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus and Streptococcus dysenteriae* (Akharaiyi, 2011). The *in vitro* α-amylase inhibitory studies demonstrated that aqueous extract of *Datura stramonium* had α-

amylase inhibitory activity. The percentage inhibition at 200, 400, 500, 600, 800 and 1000 µg/ml concentrations showed a concentration dependant increase in percentage inhibition of α-amylase (Table 3). Thus the highest concentration of 1000 µg/ml tested showed maximum inhibition of nearly 61%. The percentage inhibition varied from 36-61 %. The 50 % inhibitory concentration, IC_{50} of aqueous extract was found to be 730µg/ml which is compared with standard drug acarbose having 50 % inhibitory concentration 290µg/ml (Table 3). Thus, data presented here indicate that aqueous extract of *Datura stramonium*. possesses significant *in vitro* antidiabetic activity. The mechanism by which *Datura stramonium* exerted action may be due to its action on carbohydrate binding regions of α-glucosidase enzyme, α- amylase, endoglucanases that catalyse hydrolysis of the internal α-1, 4 glucosidic linkages in starch and other related polysaccharides have also been targets for the suppression of postprandial hyperglycemia. This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. Seed powder of *D. metel* possed blood glucose lowering effect in normal glycemic and in alloxn induced hyper glycemic rats have been reported by (Yakubu *et al.,* 2005).

CONCLUSION

In this present study the *Datura stramonium* showed antibacterial effect against the predominant strains in diabetic patients and also the dose dependent inhibition against α-amylase enzyme. The presence of secondary metabolites, singly or in combination with others could be responsible for the antibacterial and anti-diabetic activity of the plant, so further isolation, purification and characterization of the compound which is responsible for inhibiting activity, is needed for the compound usage as antidiabetic drug.

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Table1: Phytochemical analysis of aqueous extract of *Datura stramonium* leaves

Sr. No.	Phytochemical Constituents	Aqueous leaf Extract
1	Carbohydrates	
	Molish Test	+
	Fehling Test	+
	Benedicts Test	+
	Starch	+
2	Tannins	+
3	Flavonoids	-
4	Phenols	+
5	Steroidal Glycosides	+
6	Picric	+
7	Saponins	+
8	Alkaloids	-

Table 2: Summary of the antibacterial activity by zone of inhibition in form of sensitivity

Micro organisms	Chloroamphenicol	Cloxacillin	Ampicillin	Erythromycin	Plant extract
Bacillus	+	-	+	-	++
Escherichia coli	-	-	+	+	+
Methicillin susceptible *Staphylococcus aureus*	+	+	+	-	+++
Pseudomonas	-	-	-	-	+
Sarcina	+	-	+	+	+++
Klebsiella	-	-	+	+	+
Methicillin resistance *Staphylococcus aureus*					++

Note: + = presence of inhibition zone and less sensitive; ++ = Moderate sensitive; +++ = highly sensitive; - = Absences of zone of inhibition/resistant.

Table 3: The inhibition of α-amylase at different concentration of *D. stramonium* aqueous leaf extract

Sample	Concentration (µg/ml)	% of inhibition (±SD)	IC_{50}
D. stramonium	200	36±0.02828	730 (µg/ml)
	400	38±0.00707	
	500	41±0.00707	
	600	45±0.000	
	800	53±0.00707	
	1000	61±0.01414	
Acarbose	200	44±0.00707	290 (µg/ml)
	400	56±0.00707	
	500	62±0.02121	
	600	65±0.00707	
	800	68±0.00707	
	1000	71±0.00707	

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