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ANTI-INFLAMMATORY ACTIVITY AND ANTIDIABETIC ACTIVITY FROM FLOWER EXTRACT OF SENNA AURICULATA(L.) Roxb. AN IN VITRO STUDY

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ABSTRACT: The present study investigated the *in-vitro* anti-inflammatory and anti-diabetic activities of flower extract (aqueous, acetone and chloroform) of *Senna auriculata*. Phytochemical analysisrevealed that the plant contains bioactive compounds such as alkaloids, flavonoids, phenols, fats, saponins and steroids. Anti-inflammatory activity was evaluated using albumin denaturation. Acetone extract showed the highest inhibition rate of 73.84% at 100 µg/ml and aspirin was used aspositive control. Anti-diabetic activity was evaluated using inhibition of alpha amylase enzyme. Acetone extract showed the highest inhibition rate of 73.24% at 100 µg/ml and acarbose was used as positive control.From the results, it is concluded that alkaloids and flavonoids present in the acetone extract of *Senna auriculata* flower may be responsible for the activity.
Key Words: *Senna auriculata*, Phytochemicals, Antiinflammatory, Antidiabetes, Albumin denaturation, Alpha Amylase Enzyme.

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INTRODUCTION

Medicinal plants are the "backbone" of traditional medicines. [1]The World Health Organization (WHO) reported that 4 billion people (80% of the world's population) use herbal medicines for some aspect of primary healthcare. [2-4] These medicinal plants are considered as a rich resource of ingredients which can be used in synthesis and drug development. *Senna auriculata*(L.) Roxbis a native plant found in India and belongs to family Fabaceae. Its synonym is *Cassia auriculata* L and commonly known by other names as avaramsenna, matara tea, styptic weed or tanner's cassia. [5]In Tamil, it is called as Avaram tree. [6] It is distributed throughout hot deciduous forests of India. It is available in dry regions of Madhya Pradesh, Tamilnadu, Rajasthan and other parts of India.[6] The plant possessed antipyretic,[7] hepatoprotective, anti-peroxidative, anti-hyperglycemic[8]and microbicide activity.[9] It is used in the traditional system of medicine for female antifertility, leprosy, worm infestation, diarrhea and disease of pitta.[10]The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation.[11]The seeds of *Cassia auriculata* find their application in purulent ophthalmic *i.e*. inflammation of the eye or conjunctiva. They should be finely powdered and blown into the affected eyes. [12]

Inflammation is a local response of living mammalian tissues to the injury. [13,14] It is a body defense reaction in order to eliminate or limit the spread of injurious agents. [15] There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury and pain. Even though most of the synthetic anti-inflammatory drugs are available in the market, due to their well-known side effects, toxic effects and production cost, presently people are searching for natural anti-inflammatory drugs without any adverse effects. [16] A systematic study of anti-inflammatory effects of Indian medicinal plants began in 1956 and a number of plants have been screened for their anti-inflammatory effects. Subsequently, various workers from different laboratories in India have made significant contributions. [17].

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin. There are two general types of diabetes mellitus: Type I diabetes is also called insulin dependent diabetes mellitus (IDDM) andcaused by lack of insulin secretion. Type II diabetes, also called non-insulin dependent diabetes mellitus (NIDDM) and is caused by decreased sensitivity of target tissues to the metabolic effect of insulin. This reduced sensitivity to insulin is often referred to as insulin resistance.[18]

In this study the flower extracts of *Senna auriculata*(L.) Roxb were studied to determine the anti-inflammatory and anti-diabetic activities by using *in-vitro* assays.

MATERIALS AND METHODS

Collection of plant samples

Senna auriculata plant was collected from Siruvani regions of Coimbatore. The collected plant materials were fresh and young flower samples. The flower samples were washed thoroughly with distilled water and then shade dried. Flower samples of the plant were taken and made into fine powder. The plant was authenticated by Botanical Survey of India, Southern Regional Center, TNAU Campus, Coimbatore -03. (BSI/SRC/5/23/2018/TECH/3220)

Preparation of plant extract

About 20 gramsflower sample of *Senna auriculata* were taken in a 250 ml Erlenmeyer flask containing 100 ml of sterile water, acetone and chloroform solvents and kept in a shaker for 8hours and filtered. The filtered mixture was dried with the help of rotary evaporator. The extract was collected and stored at 4° C.

Phytochemical screening [19]

Alkaloids Test

2ml of extract was mixed with 2-3 drops of Wagner's reagent. The formation of reddish brown precipitate indicated the presence of alkaloids.

Flavonoids Test

2ml of extract was mixed with 1ml of 10% lead acetate solution. The formation of yellow precipitate indicated the presence of flavonoids.

Phenol Test

2ml of extract was mixed with 2ml of 3% Ferric chloride solution. The formation of bluish green colour indicates the presence of Phenol.

Protein Test

2ml of extract was mixed with 2ml of water and 0.5 ml of conc. nitric acid. The formation of yellow color indicated the presence of protein.

Saponins Test

2ml of extract was shaken vigorously with 2ml of distilled water in a test tube. The formation of stable foam indicated the presence of saponins.

Steroids Test

2ml of extract was dissolved in 2ml of chloroform and 2ml of conc. sulphuric acid was added. Appearance of red color in upper layer and yellow with green fluorescence indicate the presence of Steroids.

Terpenoids Test

2ml of extract was dissolved in 2ml of chloroform and 2ml of acetic anhydride was added following addition of 2ml of conc. Sulphuric acid was added. The formation of greyish colour indicates the presence of terpenoids.

Glycoside Test

2ml of extract was mixed with 0.4ml glacial acetic acid containing trace amount of ferric chloride and 0.5ml of conc. Sulphuric acid. The formation of persistent blue colour indicated the presence of glycoside.

Anti-inflammatory activity by protein denaturation [20]

About 1% of bovine serum albumin was dissolved in phosphate buffer and pH was adjusted to 6.9. The sample was taken at different concentrations (20, 40, 60, 80 and 100). Along with the sample 0.5ml of distilled water was added with 1 ml of 1% bovine serum albumin. The tubes were incubated in dark for 20 minutes. Then the tubes were incubated in water bath for 5-10 minutes at 57°C. After that 2.5ml of phosphate buffer saline was added to stop the reaction. The turbidity was absorbed at 660nm in UV-Spectrophotometer. Aspirin was used as a control.

Percentage of inhibition = (Absorbance of control – Absorbance of test sample)/ (Absorbance of control) ×100

Anti-diabetic activity by alpha amylase inhibition assay [21]

The extract was dissolved in di methyl sulfoxide solution and different concentrations of the sample were taken in test tubes (20, 40, 60, 80 and 100). Then the alpha amylase was prepared by dissolving it in sodium phosphate buffer and the pH was adjusted to 6.9. After pre-incubation, 500µl of 1% starch solution was added to each tube. The reaction mixtures were then incubated at 25ºC for 10 minutes. The reaction was stopped with 1ml of di nitro salicylic acid reagent and incubated in boiling water bath for 5 minutes. The content was cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540 nm. Control was maintained without the inhibitor. Positive control was done with acarbose.

Percentage of inhibition = (Absorbance of control – Absorbance of test sample)/ (Absorbance of control) ×100

RESULTS AND DISCUSSION

In the present study, phytochemicals present in the aqueous, acetone and chloroform extracts of *Senna auriculata* were analyzed by standard phytochemical screening methods. The phytochemical analysis revealed that the plant contains bioactive compounds such as alkaloids, flavonoids and proteins in all the extracts. Phenols and saponins were present in aqueous and acetone extract. Steroids were present in acetone and chloroform extracts. Terpenoids and glycosides were absent in all the extracts (Table 1).

Flavonoids and steroids were present in ethanol, methanol and acetone extracts whereas saponin was found to be present in ethanol, methanol and aqueous extracts of *Cassia auriculata*. Presence of phytochemicals saponin has been reported.[22-23] The flower has been reported to contain flavonoids, proanthocyanidins and β-sitosterol.[24-25]

Table-1: Phytochemical analysis of *Senna auriculata*(L.) Roxb.

S.No	Phytochemicals	Aqueous extract	Acetone extract	Chloroform extract
1.	Alkaloids	+	+	+
2.	Flavonoids	+	+	+
3.	Phenols	+	+	-
4.	Proteins	+	+	+
5.	Saponins	+	+	-
6.	Steroids	-	+	+
7.	Terpenoids	-	-	-
8.	Glycosides	-	-	-

'+' indicates Presence '-' indicates Absence

Anti-inflammatory activity by protein denaturation

The anti-inflammatory activity of the aqueous, acetone and chloroform flower extracts of *Senna auriculata* were determined using bovine serum albumin denaturation. Aqueous extract of *Senna auriculata* showed the highest inhibition rate of 62.18% at 100 µg/ml concentrations andthe lowest inhibition rate of 29.21% at 20 µg/ml concentrations. In acetone extraction, the highest inhibition rate of 73.84% was achieved at 100 µg/ml concentration andthe lowest inhibition rate of 34.16% at 20 µg/ml concentration. (Table 2, Figure 1) In chloroform extraction, samples showed the highest inhibition rate of 56.82% at 100 µg/ml concentrations andthe lowest inhibition rate of26.93% at 20 µg/ml concentrations. Aspirin was taken as positive control and sterile distilled water was taken as negative control.The results showed that there was a dosedependent increase in percentage inhibitory activity against the protein.

In this investigation the mechanism of the anti-inflammatory activity, ability of extracts to inhibit protein denaturation was studied. A variety of disorders produced during inflammatory is due to the release of lysosomal enzymes. The activity of these enzymes is said to be related to inflammation. The drugs act either by inhibiting their enzymes or by stabilizing the lysosomal membrane. The presence of anti-inflammatory activity may be due to the presence of higher secondary metabolites present in the extracts. These observations produce a scientific basis for the use of this medicinal plant in traditional medicine for the treatment of inflammatory diseases. Flavonoids inhibited biosynthesis of prostaglandins, which act as secondary messengers and are involved in various immunologic responses. [26-28] Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory disorders.

Denaturation of proteins is a well-documented cause of inflammation. [29] As part of the investigation on the mechanism of the anti-inflammation activity, ability of different solvent plant extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation.

Ability of *Senna auriculata* extract to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity. Saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects. [30-31] The therapeutic applications of flavonoids on inflammation have previously been reported by Abesundara *et al*. (2004). [32] Bandawane *et al.* (2012)[33] reported that methanolic extract of *Cassia auriculata* leaves showed potent anti-inflammatory activity compared to aqueous, hydroalcoholic and ethyl acetate extracts in wistar rats using carrageenan induced rat paw edema.

Antidiabetic activity by alpha amylase inhibition

The antidiabetic activity of the flower extract of *Senna auriculata*in different solvents (aqueous, acetone, chloroform) was determined using inhibition of alpha amylase enzyme. Aqueous extract of *Senna auriculata* showed the highest inhibition rate of 66.24 % at 100 µg/ml concentration and the lowest inhibition rate of 33.27 % at 20 µg/ml concentrations. (Table 3, Figure 2) In acetone extraction, *Senna auriculata* showed the highest inhibition rate of 73.24 % at 100 µg/ml concentration and the lowest inhibition rate was 36.73 at 20 µg/ml concentration. In chloroform extraction, samples showed the highest inhibition rate of 57.83 % at 100 µg/ml concentration and the lowest inhibition rate of 31.82 %at 20 µg/ml concentration. Acarbose was taken as positive control and sterile distilled water was taken as negative control.The result of the present experiment showed that there was a dose dependent increase in percentage inhibitory activity against the amylase enzyme.

Table-2: Anti-inflammatory activity of *Senna auriculata* flower extracts by protein denaturation

S.No	Conc. of the sample (µg/ml)	Percentage of inhibition		
		Aqueous extract	Acetone extract	Chloroform extract
1.	20	**29.21**	34.16	31.93
2.	40	39.39	46.28	37.43
3.	60	45.94	55.32	42.94
4.	80	53.27	64.74	49.22
5.	100	62.18	**73.84**	56.82

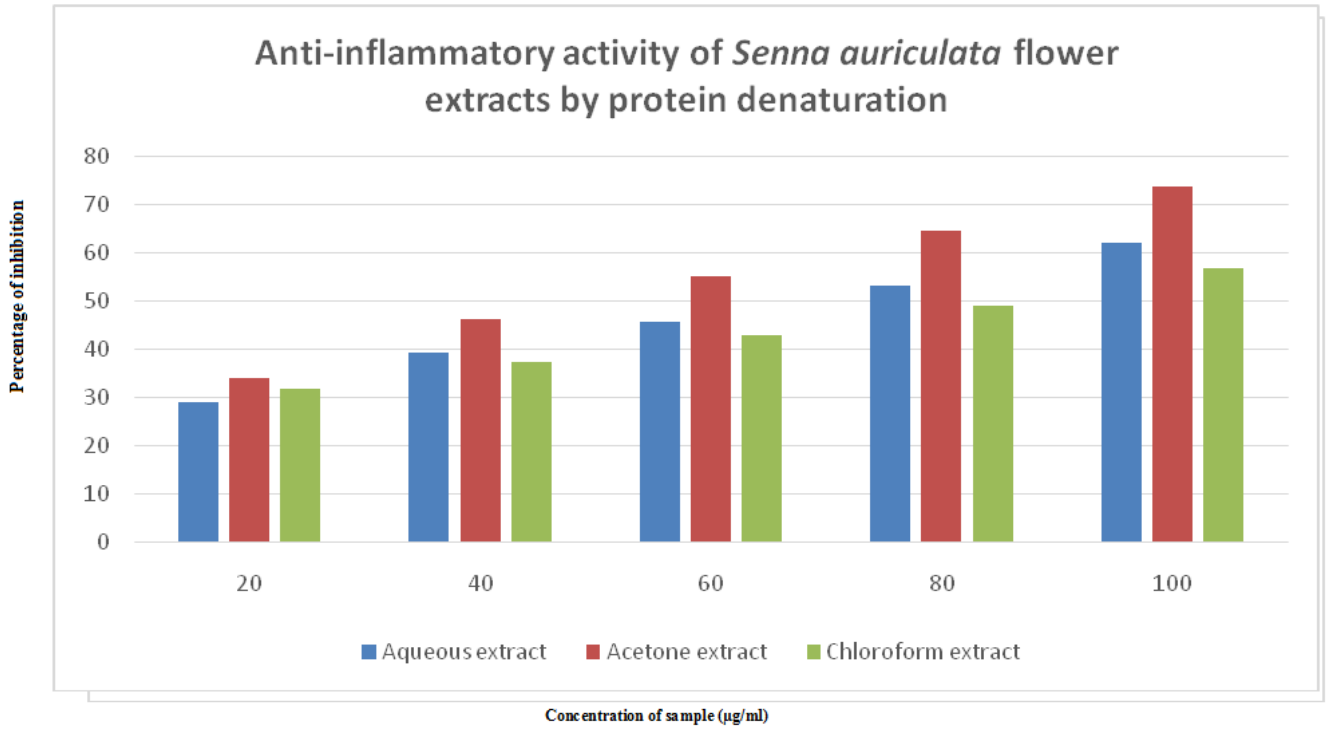

Figure-1: Anti-inflammatory activity of *Senna auriculata* flower extracts by protein denaturation

Table-3: Anti-diabetic activity of *Senna auriculata* flower extracts by alpha amylase inhibitory assay

S.No	Conc of the sample (µg/ml)	Percentage of inhibition (%)		
		Aqueous extract	Acetone extract	Chloroform extract
1.	20	33.27	36.73	**31.82**
2.	40	41.82	46.27	39.48
3.	60	49.32	54.84	44.27
4.	80	57.34	62.63	51.31
5.	100	66.24	**73.24**	57.83

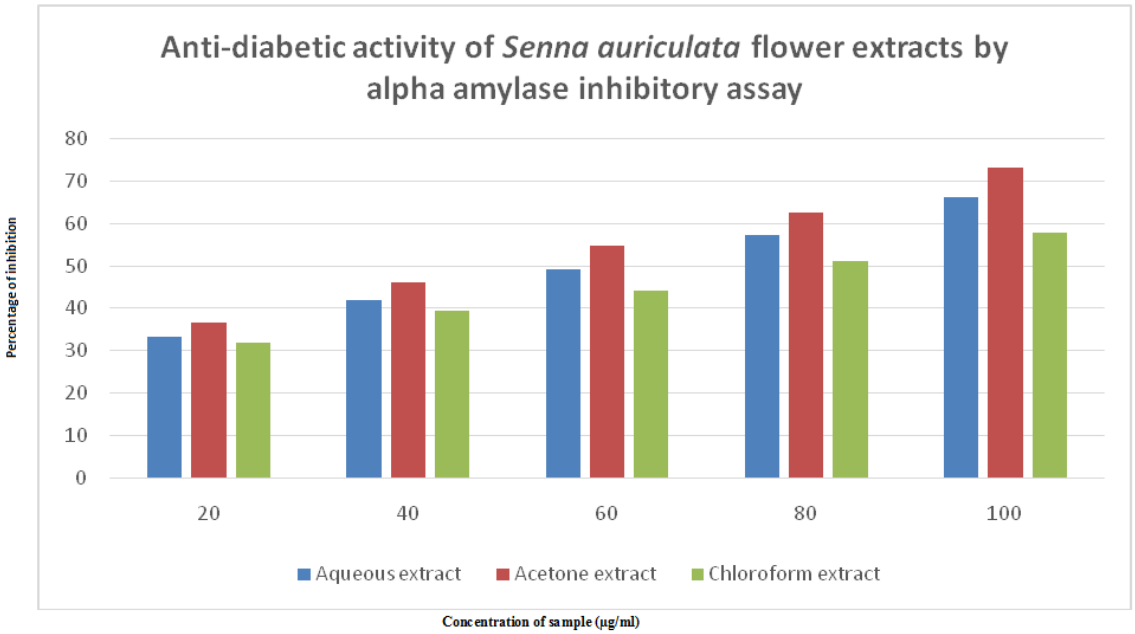

Figure-2: Anti-diabetic activity of *Senna auriculata* flower extracts by alpha amylase inhibition

In vitro study is on the principle of inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes. [34] *Senna auriculata* is the not only effective anti-diabetic agent but also helpful to reduce associated complications. [35]Pari and Latha, (2008)[36] reported that *Cassia auriculata* flower extract (CFEt) has hypoglycemic action. The n-butanol fraction of *Cassia auriculata* exhibited significant reduction ($p<0.001$) in blood glucose levels and was also found effective in restoring the blood lipids and proteins to normal level. [37]

The antidiabetic effect of extract is potentially due to presence of phytochemicals like flavonoids, terpenoids, glycosides, steroids, saponin and phenols. [35].

CONCLUSION

Medicinal plant species constitute important alternatives to conventional medicine in a large number of developing countries, especially within poor communities that inhabit rural areas and lack access to health services. *Senna auriculata* flowers might be a potential alternative agent for anti-inflammatory and antidiabetic activity. Hence it is anticipated that *Senna auriculata* flower would be a useful pharmaceutical material to treat diseases. This investigation may focus research field to develop clinical studies which might be of great scientific contribution for the society.The importance of medicinal plants in traditional health care practices clues to new areas of research and in biodiversity conservation.

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