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Preliminary phytochemical and anti-diabetic activity of *Cassia sophera* Linn

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Abstract
The medicinal plant *Cassia sophera* was analysed for screening of phytochemical and anti-diabetic activity. The objective of the present study was to investigate anti-diabetic activity of *Cassia sophera* bark, using Streptozocin induced diabetic rats as model. The biochemical study was carried out and the results shown that ethanolic extract of dose 400 mg/Kg has more effective against Streptozocin induced diabetic rats. We conclude from the present study that the ethanolic extract of bark of *Cassia sophera* may be beneficial in the management of diabetes.

Keywords: *Cassia sophera* Linn. Poonaverie, Blood glucose, Anti-diabetic.

Introduction
Cassia sophera Linn. (Caesalpiniaceae) known as Poonaverie in Tamil, is an evergreen herb found in India and most tropical countries. It is common in waste lands, on roadsides and in the forests. The plant is used in the treatment of diabetes, hepatoprotective, asthma, psoriasis and in ringworm infections [1-4].
Diabetes is known as "diabetes mellitus" - where diabetes comes from the Greek word for siphon, which describes the excessive thirst and urination of this condition, and mellitus is the Latin word for honey, because diabetic urine is filled with sugar and is sweet. Diabetes is the common term for several metabolic disorders in which the body no longer produces insulin or uses the insulin it produces ineffectively. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. The cause of diabetes continues to be a mystery, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles [5-7].

Materials and Methods
The fresh bark of *Cassia sophera* was collected from Kerala state and the plant specimen was authenticated by Dr. V. Chelladurai, Research Officer Botany, Survey of Medicinal Plants Unit, Tirunelveli.

Preparation of Extracts
The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of *Cassia sophera* (500g) was first extracted with petroleum ether (60 – 80 °C) in Soxhlet apparatus and then with solvents of increasing polarity like chloroform, ethyl acetate and ethanol at 60 – 70 °C. Each extracts was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and preceded for further detailed phytochemical and pharmacological screening.

Phytochemical studies [8-10]
Phytochemical evaluation is used to determine the nature of phyto constituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. Therefore a complete investigation is required to characterize the phyto constituents qualitatively and quantitatively.

Preliminary phytochemical screening
The chemical tests for various phyto constituents in the dried powder and exracts of bark of *Cassia sophera* Linn. were carried out as described below.

Detection of Alkaloids
Dragendorff's reagent
The substance was dissolved in 5ml of distilled water, to this 5 ml of 2 M HCl was added until an acid reaction occurs, then 1ml of Dragendorff's reagent was added and examined for an immediate formation of an orange red precipitate.

Mayer's reagent
The substance was mixed with little amount of dil. HCl and Mayer's reagent and examined for the formation of white precipitate.

Wagner's reagent
The test solution was mixed with Wagner's reagent and examined for the formation of reddish brown precipitate.

Detection of Glycosides
Borntrager's test
The powdered material was boiled with 1ml of sulphuric acid in a test tube for five minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dil. Ammonia. A rose pink to red colour is produced in the ammoniacal layer.

Modified Borntrager's test
The test material was boiled with 2ml of dil. Sulphuric acid. This was treated with 2ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dil. Ammonia. A rose pink to red colour is produced in the ammoniacal layer.

Detection of Steroids and Triterpenoids
Libermann Burchards test
The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added from the sides of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

Salkowski test
The extracts was treated with few drops of Conc. Sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Detection of Flavonoids
Shinoda test
To the solution of extract, few piece of magnesium turnings and conc. HCl was added dropwise, pink to crimson red, occasionally green to blue colour appear after few minutes indicates the presence of flavonoids.

Alkaline reagent test
To the test solution, few drops of sodium hydroxide solution was added. Intense yellow colour is formed which turns to colorless on addition of few drops of dilute acid indicate the presence of flavonoids.

Detection of Carbohydrates
Molisch's test
To the test solution few drops of alcoholic α- naphthol solution and few drops of con. sulphuric acid were added through the sides of the test tube, purple to violet colour ring appears at junction.

Fehling's test
The test solution was mixed with Fehling's A and B and heated and examined for the appearance of red coloration for the presence of reducing sugar.

Detection of Phenols
Ferric chloride test
A small quantity of substance were dissolved with 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for the appearance of blue or green colour.

Detection of Proteins
Biuret test
The sample was treated with 5-8 drops of 10%w/w copper sulphate solution, violet colour is formed.

Detection of Tannins
Lead acetate test
The test solution was mixed with basic lead acetate solution and examined for the formation of a white precipitate.

Ferric chloride test
A few drops of 5% aqueous ferric chloride solution was added to 2 ml of aqueous extract of the drug and examined for the appearance of bluish black colour.

Detection of Saponins
A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3 minutes. Development of any honey comb like froth was examined.

Detection of Gum and Mucilage
Small quantities of test substances was dissolved in 5 to 10ml of acetic anhydride by means of heat, cooled and add 0.05ml of conc. Sulphuric acid; it is examined for the formation of bright purplish red colour.

Detection of Fixed oils and Fats
Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.

Quantitative Estimation of Phytoconstituents
Glycosides Estimation [11]
Materials
Tincture
Baljet reagent (freshly prepared)

Preparation of tincture
A 10% extract in 70% alcohol is prepared by shaking for 2 hrs then filtered.

Spectrophotometric Determination
In the assay of tincture, 10ml of the 10% tincture (+0.8g powdered bark) are diluted during purification to 200ml, from which 10 ml (=0.04g powdered bark) are used in the assay, after being treated with 10 ml Baljet's reagent, and diluted with 20ml of water(to a total of 40ml). measure the absorbance of the tincture at 495 nm.

Fluorescence Analysis [12]

Fluorescence analysis was carried out in day light and in UV light. The bark powder and extracts were treated with different solvents and the fluorescence was observed in day light and in near and far UV light.

***In vitro* Glucose Diffusion Inhibition Study** [13]

A simple model system was used to evaluate the effects of plant extracts on glucose movements *in vitro*. The model was adapted from a method described by Edwards *et al*., which involved the use of a sealed dialysis tube into which 15ml of a solution of glucose and sodium chloride (0.15 M) was introduced and the appearance of glucose in the external solution was measured. The model used in the present experiment consisted of a dialysis tube into which 1 ml of 50 g/l plant extract in 1% CMC and 2ml of 0.15 M sodium chloride containing 0.22 M D- glucose was added. The dialysis tube was sealed at each end and placed in a 50ml centrifuge tube containing 45 ml of 0.15 M sodium chloride. The tubes were placed on an orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored at set time intervals.

***In vivo* Anti-Diabetic Activity** [14, 15]

Healthy Wistar albino rats weighing about 150-230g were kept fasting overnight but allowed for access to water. The rats were injected intra peritoneally with Streptozocin dissolved in citrate buffer of pH 4.5 at a dose of 555 mg/Kg body weight. After 48 hours, rats with blood glucose level 250 mg/dl were selected for the study.

Treatment

The animals were randomly divided into 5 groups of six animals each, after the induction of diabetes.

Group 1: Non diabetic control rats received 1ml of 1% CMC orally once daily for 4 weeks.

Group 2: Diabetic control rats received 1ml of 1% CMC orally once daily for 4 weeks.

Group 3: Diabetic control rats given Ethanolic bark extracts (200 mg/Kg) of *Cassia sophera* Linn. Made fine suspension with 1 ml of 1% CMC once daily for 4 weeks.

Group 4: Diabetic control rats given Ethanolic bark extracts (400 mg/Kg) of *Cassia sophera* Linn. Made fine suspension with 1 ml of 1% CMC once daily for 4 weeks.

Group 5: Diabetic control rats given Glibenclamide 3 mg/Kg body weight made fine suspension with 1ml of 1% CMC once daily for 4 weeks.

Biochemical analysis

Blood samples were collected from the retro orbital plexus of the rats at the end of 0 hr, 3 hr, 5 hr, 7 hr, 24 hr (Acute study) and 1st, 2nd, 3rd and 4th week (chronic study), samples were analysed for blood glucose content by glucometer.

Results

Table 1: Percentage yield of Successive solvent extracts of the bark of *Cassia sophera* Linn.

S. No	Extracts	Methods of Extraction	Physical Nature	Colour	Yield (%w/w)
1.	Petroleum ether	Soxhlet extraction	Semisolid	Green	4.5
2.	Chloroform		Semisolid	Green	3.8
3.	Ethyl acetate		Semisolid	Brownish Green	6.2
4.	Ethanol		Solid	Brownish Green	9.6

Table 2: Qualitative phytochemical analysis of bark of *Cassia sophera* Linn.

S. No	Chemical Constituents	Powder Drug	Petroleum Ether	Chloroform	Ethyl acetate	Ethanol
1.	Carbohydrate	-	-	-	-	-
2.	Alkaloids	-	-	-	-	-
3.	Steroids	+	+	-	-	-
4.	Glycosides	+	-	-	+	+
5.	Saponins	-	-	-	-	-
6.	Flavonoids	-	-	-	-	-
7.	Tannins	+	-	-	-	+
8.	Phenolic compounds	-	-	-	-	-
9.	Proteins	-	-	-	-	-
10.	Amino acids	-	-	-	-	-
11.	Gums and Mucilage	+	-	-	-	+
12.	Terpenoids	-	-	-	-	-
13.	Resins	-	-	-	-	-
14.	Chlorogenic acid	-	-	-	-	-
15.	Fats and Oils	+	+	-	-	-

Table 3: Fluorescence Analysis of powdered bark of *Cassia sophera* Linn.

S. No	Treatment	Day light	Short UV (254nm)	Long UV (366nm)
1.	Powder	Yellowish Green	Pale Green	Pale Green
2.	Powder + Water	Pale Green	Pale Green	Pale Green
3.	Powder+ 1N HCl	Pale Yellow	Pale Yellow	Pale Yellow
4.	Powder+ 1N H_2SO_4	Pale Yellow	Pale Yellow	Pale Yellow
5.	Powder+ HNO_3	Red	Red	Red
6.	Powder+ Acetic acid	Green	Pale Green	Pale Green
7.	Powder+ 1N NaOH	Pale Yellow	Pale Yellow	Pale Yellow
8.	Powder+ Picric acid	Yellow	Yellow	Yellow
9.	Powder+ 1N KOH	Pale Yellow	Pale Yellow	Pale Yellow

10.	Powder+ Acetone	Yellowish Green	Pale Green	Pale Green
11.	Powder+ Ammonia	Yellowish Brown	Brown	Brown
12.	Powder+ Iodine	Brown	Brown	Brown
13.	Powder+ $FeCl_3$	Green	Green	Green
14.	Powder+ Ethanol	Pale Yellow	Pale Yellow	Pale Yellow

Table 4: Fluorescence analysis of various extracts

S. No	Extracts	Day light	Short UV (254nm)	Long UV (366nm)
1.	Petroleum Ether	Dark Green	Green	Green
2.	Chloroform	Green	Green	Green
3.	Ethyl acetate	Brown	Brown	Brown
4.	Ethanol	Greenish Brown	Greenish Brown	Greenish Brown

Table 5: Quantitative estimation of phytoconstituents of *Cassia sophera* Linn.

S. No	Phytoconstituents	Quantity (%w/w)
1.	Glycosides	11.2

Table 6: *In vitro* anti-diabetic activity

Extract	1 hour	3 hours	5 hours	24 hours	27 hours
Control (Absence of extract)	172.3±0.3	204.73±0.8	268.93±0.2	291.03±2.5	319.43±1.9
Petroleum Ether (50g/l)	157.65±1.3	189.95±2.2	251.25±1.3	280.65±2.3	302.35±0.3
Chloroform (50g/l)	132.48±1.5	163.04±1.4	232.77±0.8	253.47±0.8	283.13±2.9
Ethyl acetate (50g/l)	105.76±3.2	134.19±2.9	204.49±3.3	218.99±2.4	248.55±2.0
Ethanol (50g/l)	90.45±2.2	99.23±2.5	177.12±1.9	201.77±3.4	214.22±0.6

Values are expressed as mean ±SD (n=3)

In vivo anti-diabetic activity

Table 7: Effects of ethanolic extracts of the bark of *Cassia sophera* Linn. on blood glucose level in streptozocin induced diabetic rats (mg/dl) – Acute study

Group	0 Hr	1 Hr	3Hr	5 Hr	7 Hr	24 Hr
Normal	83±1.3	93±1.6	102±1.2	112±1.8	99±1.1	101±1.9
Diabetic control	372±0.4	374±1.1	385±0.2	390±1.5	407±1.8	431±2.1
Diabetic+ Glibenclamide (3mg/Kg)	342±1.2	315±1.4	288±1.7	246±2.3	225±1.2	198±1.8
Diabetic+ Ethanolic Extract (200mg)	352±2.1	348±2.2	332±2.1	276±1.3	252±1.6	232±1.6
Diabetic+ Ethanolic Extract (400mg)	334±1.4	329±0.8	297±1.3	262±2.3	233±2.2	210±2.3

One way Anova values are expressed as mean ±SD n=6

*$p<0.05$ compared to diabetic control

Table 8: Chronic study

Group	Initial	1st week	2nd week	3 rd week	4th week
Normal	90±1.4	96±1.3	100±1.2	99±1.2	93±1.7
Diabetic control	264±1.4	278±1.6	282±0.8	290±1.1	307±1.9
Diabetic+ Glibenclamide (3mg/Kg)	250±0.9	248±0.5	200±1.3	182±1.3	148±1.2
Diabetic+ Ethanolic Extract (200mg)	278±1.3	274±2.7	216±1.9	209±2.3	197±1.5
Diabetic+ Ethanolic Extract (400mg)	270±1.2	261±2.3	206±1.5	191±2.5	173±1.6

Discussion

In the present study the preliminary phytochemical screening was carried out on *Cassia sophera* indicates the presence of steroids in petroleum ether and ethanol, glycosides in ethanol and tannins in ethanolic extract. The research has been carried out to evaluate the potential of various extracts to additionally retard the diffusion and movement of glucose in the intestinal tract. *In vivo* study showed that the administration of ethanolic bark extracts of *Cassia sophera* Linn. at the dose of 200mg/Kg and 400mg/Kg produced a significant reduction in blood glucose level. However, the ethanolic extract at the dose of 400mg/Kg was found to be more effective. It may be due to the presence of phytochemical constituents present in the plant. The results of the study strongly suggest that *Cassia sophera* Linn. Is useful in the treatment of diabetes.

Conclusion

From the overall study it is concluded that the ethanolic extract of *Cassia sophera* Linn. Is found to have anti-diabetic effect in Streptozocin induced diabetes in Wistar albino rats. Therefore, this treatment can safely be considered to be an alternative antihyperglycemic drug for diabetic patients. Further studies are warranted to isolate and characterise the anti-diabetic principles from the bark of *Cassia sophera*.

References

1. Nadkarni KM. Indian Materia Medica. 2009, 1:285-286.
2. Khare CP. Indian Medicinal Plants, 2007, 129-130.
3. Tagboto S, Townson S. Antiparasitic properties of medicinal plants and other naturally occurring products. Adv Parasitol 2005; 50:199-295.
4. Gupta RK. Medicinal and Aromatic plants, 2010, 116-117.

5. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hypoglycemic crises in adult patients with diabetes. Diabetes care 2009, 32(7):1335.
6. Guyton and Hall. Text book of Medical Physiology. 11th ed. Pennysylvania: Elsevier, 2006, 848-851, 972-976.
7. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. 6thed. Elsevier, 403-408.
8. Harbone JB. Phytochemical methods. A guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall, 1973, 04-34.
9. Tyler VE, Brady Lr, Robber Je. Pharmacognosy. 9th ed. Philadelphia USA: Lea and Febriorer, 1998, 78.
10. Khandelwal KR. Practical Pharmacognosy. Pune: Nirali Prakashan, 1998, 20-42.
11. Bahoran T, Neergheen VS. Aruoma OI. Phytochemivcal constituents of *Cassia fistula*. African Journal of Biotechnology. 4, 1530-1540.
12. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drug under ultraviolet radiation. Journal of American Pharmaceutical system science. 1949, 28, 324-333.
13. Gallagher AM, Flatt PR, Duffy G, Abdul-Wahab YHA. The effects of traditional anti-diabetic plants on *in-vitro* glucose diffusion. Nutritional Research. 2003, 715-717.
14. Sakthi PS, Vadivu R, Jayshree N. *In vitro* and *In vivo* anti-diabetic activity of leaves of *Ravenala madagascariensis*. On alloxan induced diabetic rats. Journal of Pharmaceutical Science and Technology. 2010, 312-317.
15. Ramakrishna D, Vidyasagar G, Pavan Kumar K, Madhusudhana I, Gupta VSS. Evaluation of antidiabetic activity of *Triumfetta piosaroth* in streptozocin induced diabetic rats. International Journal of Pharma Sciences and Research. 2011, 2(3):107-109.