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Research Article

Invitro evaluation of phytochemical profile and antidiabetic potential of *Syzygium aromaticum* Linn.

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ABSTRACT

DIABETES consist of a group of diseases that are caused due to high level of glucose aka sugar in the blood, either due to the deficiency of insulin due to insufficient production or due to inappropriate response of the cells to secreted insulin or both. A search for effective medication with lesser or no side effects is the need of the hour. Hence the current study is focussed at evaluating the antidiabetic potential of *Syzygium aromaticum.* The cloves of *S. aromaticum* were subjected to solvent extraction with methanol, ethyl acetate and hexane and analysed by various *in vitro* assays such as inhibition of carbohydrate digesting enzymes, non-enzymatic glycosylation of haemoglobin, glucose diffusion and uptake of glucose by yeast cells. The best screened extract was further evaluated for its phytochemical profile by qualitative and quantitative phytochemical analysis. The extracts were also subjected to TLC. The results of the study suggest that *Syzygium aromaticum* possesses significant hypoglycemic potential. With further mechanistic studies it can be proved as a better source of natural antidiabetic agents.

Keywords: *Syzygium aromaticum*, in vitro antidiabetic activity, phytochemical analysis, thin layer chromatography

INTRODUCTION

Diabetes Mellitus (DM) is a systemic metabolic disease characterized by hyperglycemia, abnormal elevated levels of lipid and fat in blood and hypoinsulinaemia [12]. According to WHO, the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 majorly in the developing countries [9]. India presently has the largest number of diabetic patients in the world and has been infamously known as the 'diabetic capital of the world' [1]. The classical symptoms of type 1 diabetes are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger) and weight loss [11].

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In recent years, drug therapies have been in use for the treatment of diabetes. Some of the standard synthetic drugs used for the treatment of diabetes are sulfonylureas, biguanides, α-glucosidase inhibitors and glinides etc. These drugs lend to cause side effects like nausea, vomiting, abdominal pain, diarrhoea, head ache, abnormal weight gain, allergic reaction, low blood glucose, darkurine, fluid retention or swelling. Moreover, they are not safe for use during pregnancy [3]. Active research has been performed on traditional available medicinal plants for discovery of new antidiabetic drug as an alternative for synthetic drugs. Hence the current study is focussed to evaluate the antidiabetic potential of *S. aromaticum.*

MATERIALS AND METHODS

Extraction of Plant Material

The matured fruits of *S. aromaticum* were collected from the local market and cleaned. Coarsely powdered fruits were subjected to direct extraction using solvents of varying polarity such as hexane, ethyl acetate and methanol by following the method of Eloff [5].

Evaluation of Antidiabetic Potential

α- Amylase inhibition

In α -amylase inhibition method, 1ml of the extract of various concentrations (250, 500, 750, 1000 µg/ml) and 1ml of enzyme solution (0.5mg/ml) were mixed together and incubated at 25°C for 10min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10min.

The reaction was then stopped by adding 2ml of DNS reagent and heating the reaction mixture in a boiling water bath (5min). After cooling, the absorbance was measured spectrophotometrically at 565 nm (Narkhede *et al.*, 2011).

The inhibition percentage was calculated using the given formula,

$$\%\text{inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}} \times 100}{\text{Abs}_{\text{Control}}}$$

Glucose uptake by Yeast cells

1mL of glucose solution (5, 10 and 25 mM) was added to various concentrations of methanol extract (250, 500, 750 and 1000µg) and incubated for 10 min at 37°C. Reaction was started by adding 100µl of yeast suspension (Cirillo, 1962), vortexed and further incubated at 37°C for 60 min. The reaction mixture was centrifuged (2,500×g, 5 min) and glucose was estimated in the supernatant [4]. The percentage increase in glucose uptake by yeast cells was calculated using the following formula-

$$\%\text{inhibition} = \frac{\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}} \times 100}{\text{Abs}_{\text{Sample}}}$$

Glucose diffusion assay

This assay was performed as described by Gallagher *et al*., [6] with some modifications. 2 ml of 0.15 M NaCl containing 0.22mM D-glucose was loaded into a dialysis tube containing plant extract (50g/L) and the sealed tube was then placed in a centrifuge tube containing 45 ml of 0.15 M NaCl and kept in an orbital shaker at room temperature. The diffusion of glucose into the external solution was monitored by measuring the glucose in the external solution every 60minutes.

Qualitative Phytochemical Analysis

The methanol extract of *S. aromaticum* was subjected to various biochemical tests to screen for the presence of phytochemicals such as alkaloids, flavonoids, phenols, saponins, tannins, carbohydrates and glycosides following the methods described by Harborne [8].

Quantitative Phytochemical Analysis

Determination of total phenols

The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR) following the assay provided by McDonald *et al.*, [10]**.** The total phenolic content of the extract was expressed as Gallic Acid Equivalents/g sample.

Estimation of total flavonoids

Total flavonoid content was determined by Aluminium chloride method using Quercetin as a standard [15].

Estimation of total alkaloids

The total alkaloid content of *S. aromaticum* was estimated using the method specified by Harborne as mentioned by Soladoye *et al.*, [13].

Thin Layer Chromatography

The methanol extract of *S. aromaticum* was further subjected to TLC to study its compound profile. The extract was spotted on precoated silica plates and developed with methanol: chloroform mixture in varying ratio. The run TLC plates were visualized under UV illumination and Iodine vapors. The ratio in which distinct bands appeared was optimized and Rf values of the bands was calculated [7].

RESULTS AND DISCUSSION

Extraction

Extracts of the *S. aromaticum* was obtained using coarse powdered fruits extracted with different solvents such as chloroform and methanol. These extracts were filtered, re extracted with same solvents respectively, condensed to dryness to obtain crude extracts.

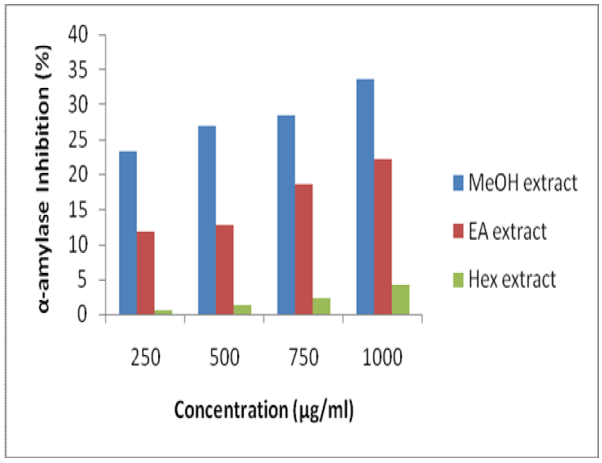
Inhibition of α-Amylase Activity

The results of in vitro inhibitory assay of α-amylase suggest that methanol extract showed higher inhibitory activity compared to the ethylacetate and hexane extracts. The percentage inhibition varied from 23 to 33 in the concentration range of 250µg/ml to 1000µg/ml (Fig. 1).

Hence the methanol extract was selected for further investigation. Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha linked polysaccharide such as starch to yield high levels of glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide (Ponnusamy *et al.*, 2011).

Hence, retardation of starch digestion by inhibition of enzymes such as α -amylase plays a key role in the control of diabetes. Inhibitors of pancreatic α -amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels.

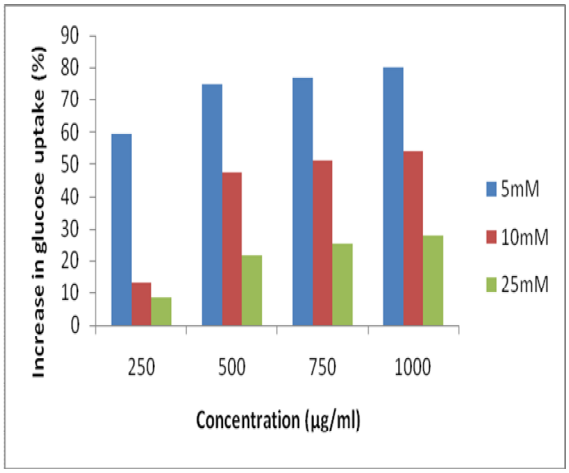
Figure 1: Inhibitory effect of *S. aromaicum* extracts on alpha amylase activity



Glucose Uptake by Yeast Cells

The rate of glucose uptake rate increased with increasing concentration of the plant extract and decreased with increasing extracellular glucose concentration. The methanol extract of *S. aromaticum* showed upto 80% increase in glucose uptake by yeast cells (Fig. 2). The glucose transport across yeast cell membrane is proposed to occur by facilitated diffusion down the concentration gradient and only if the intracellular glucose is effectively reduced [2]. The amount of glucose lingering in the medium after a specific time serves as a marker of the glucose uptake by the yeast cells. The data obtained suggests that the plant extract is capable of effectively enhancing glucose uptake.

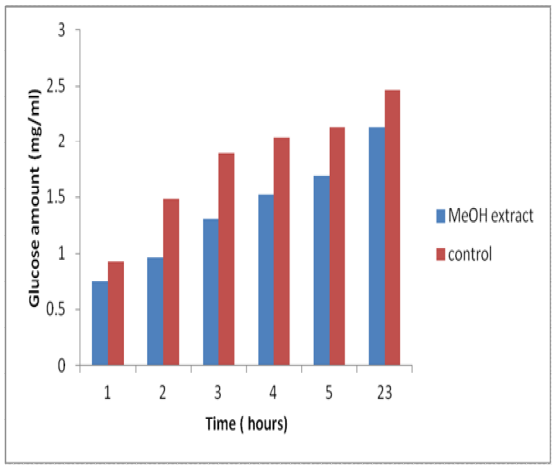
Figure 2: Effect of methanol extract of *S. aromaticum* on glucose uptake by yeast cells



Glucose Diffusion Assay

The results of the glucose diffusion assay are depicted in Fig. 3. The data proves that *S. aromaticum* is capable of limiting the diffusion of glucose across the cell membrane which in turn states that the plant is capable of controlling post prandial glucose levels. Antihyperglycemic behaviors of most efficient plants were in part explicated by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and to stimulate insulin secretion.

Figure 3: Inhibition of glucose diffusion by methanol extract of *S. aromaticum*



PHYTOCHEMICAL SCREENING

Qualitative Screening Analysis

The phytochemical analysis of methanol extract of *S. aromaticum* showed the presence of alkaloids, flavonoids, phenols, tannins, sterols, fats and oils (Table 1) and the major phytocompounds were quantified. Quantitative tests proved that total alkaloids, total flavonoids, total phenols and total tannins were estimated to be 0. 438mg/g sample, 17.91µg/ml, 66.92µg/ml and 132.57µg/ml, respectively (Table 2).

Table 1: Qualitative phytochemical analysis

S.No	PHYTOCHEMICALS	RESULTS
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Phenols	+
5	Proteins	-
6	Saponins	-
7	Sterols	+
8	Reducing sugars	+
9	Fixed oils and Stains	+

+ Present phytochemical – Absence of phytochemical

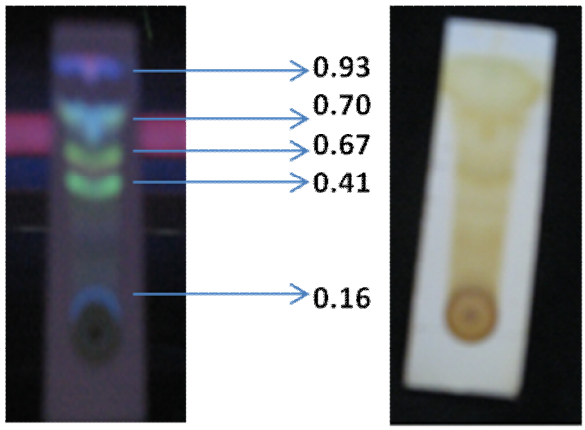
Table 2: Quantitative phytochemical estimation

PHYTO COMPOUND	COMPOSITION
Total Alkaloids	0. 438 mg/g sample
Total Flavonoids	17.91 µg/ml
Total Phenolics	66.92 µg/ml
Total Tannins	132.57 µg/ml

Thin Layer Chromatography

In the ratio 0.25:9.75 (methanol: chloroform v/v), the separation of compounds was most distinct and clear. 5 distinct bands of Rf values 0.93, 0.70, 0.67, 0.41 and 0.61 were observed under both UV and iodine. These compounds might be responsible for antidiabetic activity of plant *Syzigium aromaticum*.

Figure 4: Compound separation by thin layer chromatography



REFERENCES

1. Abate, N., Chandalia, M. (2007). Ethnicity, type 2 diabetes & migrant Asian Indians.The Indian Journal of Medical Research, 125(3):251-258.
2. Ahmed F, Sudha S. Asna U (2009). Effect of various ayurvedic formulations and

medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells – an in vitro study. Journal of Pharmacy Research. 2(3):563–568.

3. Anbu, N., Musthafa, Velpandian, V. (2012). Anti-Diabetic Activity of Polyherbal Formulation *Aavaraiyathi churnam* in Alloxan Induced Diabetic Rats. International Journal of Toxicological and Pharmacological Research, 4(4):77-80.
4. Chandrashekar, Daksha G, Lobo R, Yogendra, Nilesh G (2012). In-vitro Antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. Der Pharmacia Lettre.4(2):614–619.
5. Eloff JN(1988). Which extractant should be used for the screening and isolation of antimicrobial component from plants .J. Ethnopharm ; 60(1): 1–8.
6. Gallagher, A.M., Flatt, P.R., Duffy, G., Abdel, Y.H.A. (2002) .The effects of traditional antidiabetic plants on *in vitro* glucose diffusion. Nutrition Research, 23: 413–424.
7. Hao *et al.,* (2004). Insulin releasing and alpha-glucosidase inhibitory activity of ethyl acetate fraction of Acorus calamus in vitro and in vivo. Journal of Ethanopharmacology. 48(1):154-159.
8. J.B. Harborne, (1988).Phytochemical methods. A Guide to Modern Techniques of Plants Analysis,2nd ed., Chapman and Hall, London, 1–226
9. Jayaprasad, B,. Thamayandhi, B., Shravanan, P.S. (2011). Traditionally Using Antidiabetic Medicinal Plants in Tamil Nadu**.** International Journal of Research in Pharmaceutical and Biosciences, 2(1):1-8.
10. McDonald, S., Prenzler, P.D., Autolovich, M., Robards K. (2001). Phenolic content and antioxidant activity of olive extracts. Food Chem. 73:73-84.
11. Plevyak *et al.,* (2011). Association of Gestational weight gain with caesarian delivery rate after labor induction. The Journal of Reproductive Medicine. 56: 95-102.
12. Rao *et al.,* (2010). Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of Syzygium alternifolium seeds in streptozotocin induced diabetic rats . Food and Chemical Toxicology-Elsevier. 48: 1078–1084.
13. Soladoye, M.O., Chukwuma, E.C. (2012). Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill. & Perr. (Vitaceae). Scholars Research Library, 4 (1): 200-206.
14. Vincent P.Cirillo (1962). Mechanism of glucose transport across the yeast cell membrane. J. Bacteriol. 84(3): 485-491.
15. Yadav *et al.,* (2011). Antidiabetic effect of Raphanus sativus root juice. Pharmaceutical Biology. 49(1):32-37.