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# *In Vitro* anti-diabetic activity of aqueous extract of the medicinal plants *Nigella sativa, Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre*

**Arunachalam Sathiavelu[1*], Sundaramoorthy Sangeetha[1], Rastogi Archit[1], Sathiavelu Mythili[1]**
[1]School of Bio Sciences and Technology, VIT University, Vellore – 632 014, Tamil Nadu, India.

**Abstract**

Objective: *In vitro* analysis of the anti-diabetic effect of aqueous extracts of the medicinal plants *Nigella sativa, Eugenia jambolana, Andrographis paniculata, Gymnema sylvestre*. Methods: Aqueous extracts of the plants were prepared by maceration. They were then tested for inhibition of α-amylase activity by DNSA colour reagent. They were tested for their ability to hinder diffusion of glucose across a dialysis membrane. Results: The aqueous extract of Nigella sativa showed maximum inhibition of α-amylase activity and a strong hindrance to diffusion of glucose across a dialysis membrane. Andrographis paniculata showed both a strong inhibition of α-amylase and a significant hindrance to the diffusion of glucose across the dialysis membrane. Gymnema sylvestre showed low inhibition of α-amylase activity, but it showed maximum hindrance to the diffusion of glucose across the dialysis membrane. Nigella sativa was found to possess maximum anti-diabetic properties. Conclusions: The findings indicate that all the above plants possess anti-diabetic properties too varying degrees. They can be used to develop natural drugs which may be used in lieu of commonly used strong allopathic drugs which possess a number of harmful side effects.

*Corresponding author, Mailing address:
**Sathiavelu A**
Associate Professor, Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore – 632 014, Tamil Nadu, India.
E-mail: asathiavelu@vit.ac.in

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*Nigella sativa, Gymnema sylvestre,* Phytotherapy, Anti-diabetic plants, Plant preparations, α-amylase inhibition, Glucose diffusion inhibition, Dialysis membrane, Phytochemistry, Hypoglycaemic plants, Hyperglycaemia.

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## INTRODUCTION

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency,resulting in hyperglycaemia leading to acute and chronic complications.[14]The incidence of diabetes mellitus is on rise allover the world.Control of plasma glucose

concentrations is vital to decrease the incidence and severity of longterm diabetes effects.[6]Synthetic drugs are likely to give serious effects in addition they are not suitable for intake during conditions like pregnancy.[9]Apart from conventional diabetes therapy, several studies have shown that some plants used in traditional medicine have beneficial effects in diabetic patients.[13,11]More than 400 plants worldwide have been documented as beneficial in the treatment of diabetes.[3,4,12]The majority of traditional anti-diabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control. *Nigella sativa* is a grassy plant with green- to blue-coloured flowers and small black seeds that grows in temperate and cold climates.[2] It is commonly used in Asian cuisine as a spice and is a home remedy for the respiratory problems and the common cold. *Andrographis paniculata* is an herb native to India belonging to the family *Acanthaceae*. Because of its extremely bitter taste, it is known as the "King of Bitters" and is a common Ayurveda treatment for various diseases. *Gymnema sylvestre* is an herb native to tropical Indian forests and has been used in herbal medicine as a treatment for diabetes for nearly two millennia, but there is insufficient scientific evidence to draw definitive conclusions about its efficacy.[15]*Eugenia jambolana* is the member of Myrtaceae family. It is also known in Hindi as jamun, jambo, jambul, jamhool, in English as black plum, purple plum, black berry and, nerudu in Telugu.[10]

## MATERIALS AND METHODS

**Chemicals and reagents:** The dialysis membrane and 1-4,α-D-Glucan-glucanohydrolase (α-amylase) were purchased from HiMedia Laboratories, Mumbai, India. All other chemicals, reagents, kits and solvents used in this study were of analytical grade and procured locally.

**Plant material and extract powder:** Fresh leaves of the above plants were collected from Gudiyattham District, Tamil Nadu, India. Plant material was shade dried and ground into a powder. Extract was prepared by successive maceration of the powder (10g) at room temperature with aqueous extract (100ml) in shaker for 2 days. The final extract obtained was filtered and the filtrate was subjected to lyophilisation (Shimadzu Analytical (India) Pvt Ltd,Mumbai, India) to obtain powdered extract which was used for the following assays.

**Inhibition assay for α-amylase activity (DNSA):** Four concentrations of plant extract were prepared by dissolving in double distilled water. These were 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml. A total of 500µl of plant extract and 500 µl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (0.5mg/ml) were incubated for 10 minutes at 25°C.After pre-incubation,500 µl of 1% starch solution in 0.02M sodium phosphate buffer (pH6.9 with 0.006M sodium chloride)was added to each tube at 5s intervals. This reaction mixture was then incubated for 10 minutes at 25°C.1ml of DNSA colour reagent was added to stop the reaction. These testtubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally this reaction mixture was again diluted by adding 10ml distilled water following which absorbance was measured at 540nm.[5]

$$\%\ Inhibition = \frac{A_{540CONTROL} - A_{540EXTRACT}}{A_{540CONTROL}} \times 100$$

**Glucose diffusion inhibitory study:** An aqueous extract of all the plants was prepared by maceration at 37°C. 1ml of the extract was then placed in a dialysis membrane (12000MW, HiMedia Laboratories, Mumbai, India) along with a glucose solution (0.22mM in 0.15 M sodium chloride).It was then tied at both ends using thread and it was immersed in a beaker containing 40ml of 0.15 M sodium chloride and 10ml of distilled water. The control contained 1ml of 0.15M sodium chloride

containing 22mM glucose and 1ml of distilled water. The beakers were then placed on orbital shaker (The I L E Company, Chennai, India) and kept at room temperature. The movement of glucose into the external solution was monitored every half hour. Three replications of this were done for 3 hours.[4,12]

## RESULTS

**Inhibition assay for α-amylase activity (DNSA):** The results of the DNSA study are summarized in Figure 1 and Table 1.All the above four plants showed varying effect on glucose utilization. At all concentrations, *Nigella sativa* showed maximum inhibition of the enzyme with the highest value of 84% seen at 100mg/ml concentration of plant extract. *Eugenia jambolana* showed the next highest value of 71.71% seen at 100mg/ml concentration of plant extract. *Andrographis paniculata* showed the third highest value of 65.78% seen at 100mg/ml concentration of the plant extract and *Gymnema sylvestre* showed the least inhibition of enzyme with a highest value of only 49.34% seen at 100mg/ml concentration of plant extract.

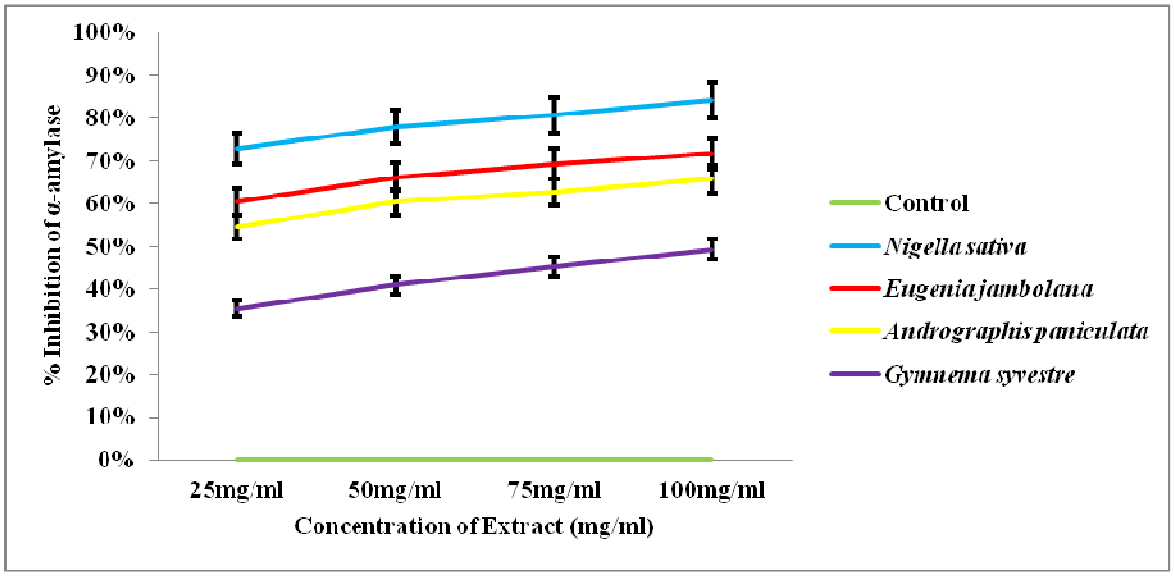


**Figure 1.**Effects of aqueous extracts of *Nigella sativa, Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre* at varying concentrationson α-amylase activity as compared to an aqueous extract. The figures are in percentage with the control showing 0% inhibition. The errors in measurement are indicated by the black bars.

**Table 1:** % Inhibition of α-amylase enzyme brought about by aqueous extracts of varying concentrations of *Nigella sativa,Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre* as compared to an aqueous control.

**% Inhibition of α-amylase**

| Concentration (mg/ml) | Control %* | *Nigella sativa* %* | *Eugenia jambolana* %* | *Andrographis paniculata* %* | *Gymnema sylvestre* %* |
|---|---|---|---|---|---|
| **25** | 0% | 73.02% | 60.52% | 54.60% | 35.52% |
| **50** | 0% | 77.92% | 66.23% | 60.39% | 40.90% |
| **75** | 0% | 80.66% | 69.33% | 62.66% | 45.33% |
| **100** | 0% | 84% | 71.71% | 65.78% | 49.34% |

*$p < 0.05$

**Glucose diffusion inhibitory test:** The results of the glucose diffusion inhibitory test are given in Tables 2 and 3.They have been compared in Figures 2 and 3. All plants showed significant inhibitory activity with *Gymnema sylvestre* showing maximum inhibition to the diffusion of glucose and *Eugenia jambolana* showing least inhibition to the diffusion of glucose.

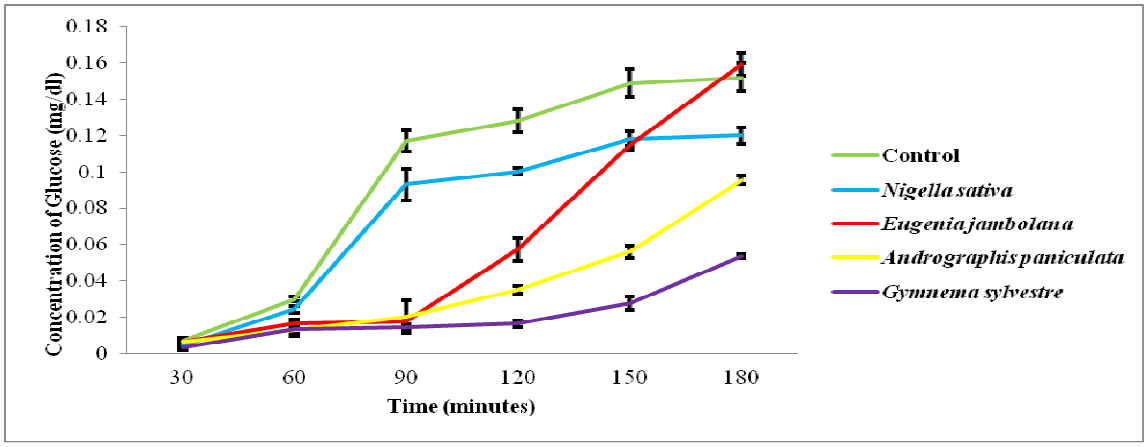


**Figure 2.** Effect of aqueous extracts of *Nigella sativa, Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre* on the diffusion of glucose out of a dialysis membrane as compared to aqueous control. Values are Means ± SEM for groups of 3 observations with their standard errors indicated by vertical bars.

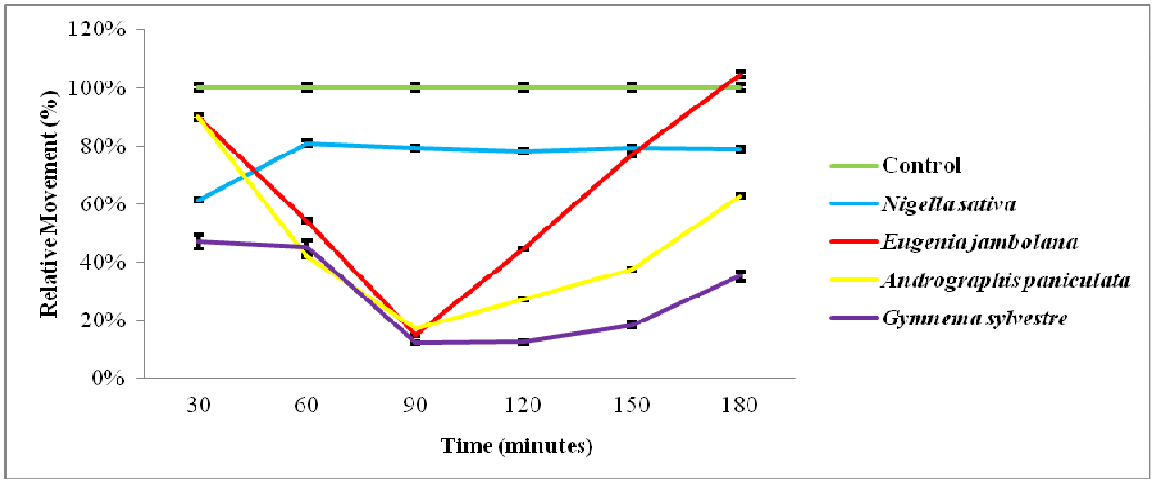


**Figure 3.** Relative movement of glucose across dialysis membrane with respect to aqueous control under the influence of aqueous extracts of *Nigella sativa, Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre*. Values are percentages with their standard errors indicated by vertical bars.

**Table 2:** Effect of aqueous extracts (100g/l) of *Nigella sativa* and *Eugenia jambolana* on diffusion of glucose out of a dialysis membrane over 180 minutes.

**Concentration of Glucose (mg/dl)**

| Time (Minutes) | Control Mean±SEM* | *Nigella sativa* Mean±SEM* | Relative Movement %** | *Eugenia jambolana* Mean±SEM* | Relative Movement %** |
|---|---|---|---|---|---|
| **30** | 0.007±0.0003 | 0.004±0.0015 | 61.43% | 0.0063±0.0017 | 90% |
| **60** | 0.03±0.0015 | 0.024±0.0017 | 81% | 0.0163±0.0022 | 54.33% |
| **90** | 0.117±0.0058 | 0.093±0.0085 | 79.48% | 0.0176±0.0017 | 15.04% |
| **120** | 0.128±0.0064 | 0.1±0.002 | 78.36% | 0.0573±0.0062 | 44.76% |
| **150** | 0.149±0.0074 | 0.118±0.0042 | 79.40% | 0.115±0.0026 | 77.18% |
| **180** | 0.152±0.0076 | 0.12±0.0046 | 78.94% | 0.1593±0.0064 | 104.60% |

Values are Means ± SEM for groups of 3 observations.
*$p < 0.05$
**$p<0.01$

**Table 3:** Effect of aqueous extracts (100g/l) of *Andrographis paniculata* and *Gymnema sylvestre* on diffusion of glucose out of a dialysis membrane over 180 minutes.

**Concentration of Glucose (mg/dl)**

| Time (Minutes) | Control Mean±SEM* | *Andrographis paniculata* Mean±SEM* | Relative Movement %** | *Gymnema sylvestre* Mean±SEM* | Relative Movement %** |
|---|---|---|---|---|---|
| **30** | 0.007±0.0003 | 0.0063±0.0022 | 90% | 0.0033±0.0017 | 47.14% |
| **60** | 0.03±0.0015 | 0.0126±0.0028 | 42% | 0.0136±0.0027 | 45.33% |
| **90** | 0.117±0.0058 | 0.0203±0.0089 | 17.35% | 0.0146±0.0013 | 12.47% |
| **120** | 0.128±0.0064 | 0.035±0.002 | 27.34% | 0.0163±0.0017 | 12.73% |
| **150** | 0.149±0.0074 | 0.056±0.0033 | 37.58% | 0.0276±0.0037 | 18.52% |
| **180** | 0.152±0.0076 | 0.0956±0.0024 | 62.89% | 0.0536±0.0011 | 35.26% |

Values are Means ± SEM for groups of 3 observations.
*$p < 0.05$
**$p<0.01$

## DISCUSSION

Plants serve as an excellent source of various therapeutic agents. One of the major advantages of using plants is that they seldom show the deleterious side effects commonly associated with other allopathic drugs. This study investigated the ability of *Nigella sativa, Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre* to serve as effective anti-diabetic agents.

*Eugenia jambolana* is reported to have hypolipedemic effect; it reduces blood cholesterol, triglycerides and free fatty acids.[10]Aqueous extract of *Nigella sativa* has been shown to decrease insulin resistance in diabetic *Meriones shawi*.[1]Aqueous extract of *Andrographis paniculata* is known to exhibit antioxidant action in terms of free radical xanthine oxidase inhibition and anti-lipid peroxidation.[7]*Gymnema sylvestre* has been reported to possess significant anti-diabetic activity and a hypolipidemic activity in alloxan induced and normal fasting rats.[8] All these tests were done *in vivo* and this is the first *in vitro* study for these plants.

*Nigella sativa* showed the maximum inhibition of α-amylase activity. It also showed a strong inhibition towards the flow of glucose across a dialysis membrane. *Eugenia jambolana* showed the second most inhibition of α-amylase activity but it showed the least inhibition to diffusion of glucose across a dialysis membrane. It effectively inhibited the diffusion of glucose only for 150 minutes. *Andrographis paniculata* showed a strong inhibition of α-amylase activity and a strong inhibition towards flow of glucose across a dialysis membrane. It showed very strong anti-diabetic properties in both tests. The glucose diffusion inhibitory test showed *Gymnema sylvestre* to have the maximum inhibitory effect to the diffusion of glucose across a dialysis membrane but it showed the least inhibition of α-amylase activity. In the end *Nigella sativa* was found to have maximum anti-diabetic activity.

All the plants used in this study are of Indian origin and are well known by herbal pharmacologists for their medicinal properties. This study provides scientific evidence of their anti-diabetic effect. These plants are very affordable to the common man and can be easily incorporated in their daily diets. They can be further analysed to develop anti-diabetic drugs free from harmful side effects.

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## REFERENCES

1) Benhaddou-Andaloussi A, Martineau L, Vuong T, Meddah B, Madiraju P, *et al.,*. The *in vivo* antidiabetic activity of *Nigella sativa* is mediated through activation of the AMPK pathway and increased muscle Glut4 content. Evid Based

Complement Alternat Med.2011; Article ID 538671, 9 pages.

2) Boskabady MH, Shirmohammadi B. Effect of *Nigella sativa* on isolated guinea pig trachea. Arch Iranian Med. 2002; 5(2):103-107.
3) Day C, Bailey CJ.Hypoglycemic agents from traditional plant treatments for diabetes.Int Ind Biotech.1998;50:5-8.
4) Gray AM, Flatt PR. Nature's own pharmacy: The diabetes perspective. ProcNutr Soc. 1997;56:507-517.
5) Jayasri MA, Radha A, Mathew TL.α-amylase and α-glucosidase inhibitory activity of *Costuspictus*D. Don in the management of diabetes. Journal of Herbal Medicine and Toxicology.2009; 3(1):91-94.
6) Larner J. Insulin and Oral Hypoglycemic Drugs; Glucagon. In: Gilman AG, Goodman LS, Rall TW, Murad F (eds).The Pharmacological Bases for Therapeutic, ed 7, New York, USA, Macmillan, 1985, pp 149-151.
7) Lin FL, Wu SJ, Lee SC, Ng LT.Antioxidant, antiedema and analgesic activities of *Andrographis paniculata* extracts and their active constituent andrographolide. Phytother Res.2009; 23:958-964.
8) Mall GK, Mishra PK,Prakash V. Antidiabetic and hypolipidemic activity of *Gymnema sylvestre* in alloxan induced diabetic rats. Global Journal of Biotechnology & Biochemistry.2009; 4(1):37-42.
9) Rao K, Giri R, Kesavelu MM Apparao C. Herbal Medicine in the management of diabetes mellitus. ManpharVaidhyaPatrica,1997; 1:33-35.
10) Sagrawat H, Mann AS, Kharya M D. Pharmacological potential of *Eugenia jambolana*: a review. Pharmacognosy Magazine.2006; 2(6):96-105.
11) Soumyanath A. Traditional Medicines for Modern Times: Antidiabetic Plants.Oregon, USA, CRC Press, 2006.
12) Swanston-Flatt SK, Flatt PR, Day C, Bailey CJ. Traditional dietary adjuncts for the treatment of Diabetes mellitus. ProcNutr Soc.1991; 50:641-650.
13) Valiathan MS. Healing plants. Current Science.1998; 75(11):1122-1127.
14) Yadav JP, Saina S, Kalia AN, Dangi AS. Hypoglycemic and Hypolipidemic activity of ethanolic extract of *Salvadoraoleoides* in normal and alloxan-induced diabetic rats.Ind. J. Pharmacol.2008; 40(1);23-27.
15) Yeh GY, Kaptchuk TJ, Eisenberg DM and Phillips R S. Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care.2003; 26 (4): 1277-1294.