

# Evaluation of $\alpha$ -Glucosidase Inhibitory Potential of Some Homeopathic Mother Tinctures

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## Author's Contribution

All the authors contributed significantly to the research that resulted in the submitted manuscript.

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## ABSTRACT

**Objectives:** Mother tinctures are frequently prescribed in Homeopathy for different diseases especially for control of diabetes. This study was done to evaluate  $\alpha$ -glucosidase inhibitory potential of 8 homeopathic mother tinctures.

**Materials and Methods:** 8 marketed prepared mother tinctures were tested against  $\alpha$ -glucosidase. These include *Achillia millefolium*, *Allium sativum*, *Atropa belladonna*, *Cinchona officinalis*, *Hamamelis virginiana*, *Pulsatilla nigrican*, *Rhus toxidendron* and *Strychnos Nux-vomica*. Acarbose was taken as positive control.

**Results:** All mother tinctures showed more or less  $\alpha$ -glucosidase inhibitory potential. *S. Nux-vomica* had maximum inhibition (98.22 $\pm$ 0.15%) followed by *A. belladonna* (82.63 $\pm$ 0.14%).

**Conclusion:** This study reveals that the tested mother tinctures have sufficient  $\alpha$ -glucosidase inhibitory potential.

**Keywords:**  $\alpha$ -Glucosidase, homeopathic mother tinctures, diabetes.

## INTRODUCTION

Diabetes is an endocrine metabolic disorder that is one of leading cause of morbidity and mortality [1]. The different synthetic drugs used in managing this syndrome have certain side effects [2]. Homeopathic mother tinctures prepared from plants are the hydro-alcoholic extracts of medicinal plants [3] that are further used for preparation of ultrahigh dilutions [4]. Homeopathic medicines (mother tinctures and ultrahigh dilutions) are frequently used for many ailments as antioxidants [2], antibacterial [3], urinary tract infections [5], antipyretic [6-9], anti-epileptic [10], etc. The extracts of medicinal plants contain secondary metabolites including alkaloids, saponins,

glycosides, phenols, flavonoids and various others. They can produce anti-diabetic and various other therapeutic effects [11-13]. *Achillia millefolium* contain polyphenol compounds, essential oil, and some of flavonoids such as quercetin, apigenin, fatty acids, borneol, sesquiterpenes, betaine, lactones, linalool, luteolin, cineol and tannins [14]. *Allium sativum* contain many sulphur compounds such as alliin, cysteine sulfoxides, ajoene, ajoenes, vinylidithiins and Sulfide. Allicin and thiosulfinates are non-sulfur compounds [15]. *Atropa belladonna* contains alkaloids, main of which is hyoscyamine. *N*-methyl pyrroline and pyridine are present in small quantities. Leaves contain calcium oxalate and methyl aesculetin [16]. Quinine is the major alkaloid in *Cinchona*

*officinalis* [17]. *Hamamelis virginiana* contain tannin, proanthocyanidins, hydrolyzable tannins, hamamelitannin and pentagalloyl glucose, terpenoids, polypeptides or lectins, pseudo tannins or hydrolysable tannins, hamamelitannin and gallic acid [18]. *Pulsatilla nigricans* contain polyphenolic flavonoids, anthocyanidins, auxin and cytokinin and dihydroxy-isosteviol methyl [19]. *Rhus toxicodendron* is also rich in flavonoids [20]. Alkaloids are the main constituents in *Strychnos Nux-vomica*. Brucine and strychnine are main constituents [21].

$\alpha$ -Glucosidase inhibitors are used in treatment of diabetes as it is the key enzyme in biosynthesis of glycoproteins and cleavage of glycosidic bond [22]. The previous researches proved that inhibition of  $\alpha$ -glucosidase is an ideal strategy to combat diabetes [23], as  $\alpha$ -glucosidase inhibitors decreased postprandial hyperglycemia [22]. Previously reported study suggested that alkaloids and flavonoids are the main component in plant materials for amelioration of diabetes mellitus [24]. Traditionally used anti-diabetic plants are a good source of alpha glucosidase inhibitors [25]. Selected mother tinctures may have potential to inhibit  $\alpha$ -glucosidase as all the used plants contain alkaloids, flavonoids, terpenoids, etc. The present study aimed to study alpha glucosidase potential of 8 homeopathic mother tinctures (hydroalcoholic extracts) of plant origin.

## MATERIALS AND METHODS

### Chemicals and Mother Tinctures

$\alpha$ -Glucosidase and Acarbose (Sigma Aldrich). Mother tinctures were purchased from local market. 8 mother tinctures were tested for  $\alpha$ -glucosidase inhibitory potential 1) *Achilea millefolium*, 2) *Allium sativum*, 3) *Atropa belladonna*, 4) *Cinchona officinalis*, 5) *Hamamelis virginiana*, 6) *Pulsatilla nigricans*, 7) *Rhus toxicodendron*, 8) *Strychnos Nux-vomica*.

### $\alpha$ -Glucosidase Inhibition Assay

$\alpha$ -Glucosidase inhibitory assay was performed with the method of Moradi-Afrapoli *et al.* [26] with some modification. 10 $\mu$ L of mother tinctures, 70 $\mu$ L of 0.1 M phosphate buffer pH 6.8 and 10 $\mu$ L of  $\alpha$ -glucosidase (0.5 unit/mL) per well of 96 well plate were incubated for 15 min at 30°C. 10 $\mu$ L of p-Nitrophenyl- $\alpha$ -D-glucopyranoside substrate solution was added and incubated for an additional 30 min. Absorbance was measured with HT BioTek microplate reader at 405

nm. The reaction system without sample (only methanol) was used as control and acarbose was used as positive control. Each experiment was conducted in triplicate. The enzyme inhibitory rates of samples were calculated as follows:

$$\% \text{ inhibition} = 100 - (A_{m.t} / A_c) * 100$$

Where,

A<sub>c</sub> = Absorbance of negative control

A<sub>m.t</sub> = Absorbance of mother tincture

## RESULTS

Table 1 showed  $\alpha$ -glucosidase inhibitory potential of 8 homeopathic mother tinctures and acarbose. *S. Nux-vomica* had maximum inhibition (98.22 $\pm$ 0.15%) followed by *A. belladonna* (82.63 $\pm$ 0.14%). *R. toxicodendron* (68.27 $\pm$ 1.32%) and *H. virginiana* (66.20 $\pm$ 0.62%) showed moderate  $\alpha$ -glucosidase inhibitory potential. *A. millefolium* and *P. nigricans* had less activity against  $\alpha$ -glucosidase. *A. sativum* and *C. officinalis* showed least activity among the tested mother tinctures.

**Table 1. Results of mother tinctures and acarbose against  $\alpha$ -glucosidase. Results are expressed in terms of mean  $\pm$  S.D (n=3).**

Homoeopathic Mother Tinctures	%age Inhibition of $\alpha$ -Glucosidase Activity
<i>Cinchona officinalis</i>	5.67 $\pm$ 3.66
<i>Allium sativum</i>	11.80 $\pm$ 0.29
<i>Strychnos Nux-vomica</i>	98.22 $\pm$ 0.15
<i>Pulsatilla nigricans</i>	32.95 $\pm$ 2.90
<i>Atropa belladonna</i>	82.63 $\pm$ 0.14
<i>Hamamelis virginiana</i>	66.20 $\pm$ 0.62
<i>Rhus toxicodendron</i>	68.27 $\pm$ 1.32
<i>Achilea millefolium</i>	31.42 $\pm$ 0.44
Acarbose (Positive control)	93.5 $\pm$ 0.05

## DISCUSSION

The present study reveals that various tested mother tinctures possess  $\alpha$ -glucosidase inhibition *in vitro*. These mother tinctures contain several secondary metabolites including phenolic and flavonoid contents. Previously reported study suggested that alkaloids and flavonoids are the main component in plant materials for amelioration of diabetes mellitus [13,

24]. *S. Nux-vomica* and *A. belladonna* are rich in alkaloids and also contain flavonoids, so it may be assumed that highest activity of these mother tinctures even greater than positive control was due to presence of these metabolites. *R. toxidendron* and *H. virginiana* also contain flavonoids and other metabolites so they also possess sufficient  $\alpha$ -glucosidase inhibition. However, other mother tinctures can't inhibit  $\alpha$ -glucosidase up to 50% and so are considered to be weak anti diabetics as  $\alpha$ -glucosidase inhibitors are ideal anti diabetics [22]. The mechanism of action of these mother tinctures in diabetes is not fully understood yet. However, it could be partially understood by the presence of various active metabolites and  $\alpha$ -glucosidase inhibition.

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## CONCLUSION

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It is concluded from the study that *S. Nux-vomica* exhibited higher efficacy than acarbose followed by *A. belladonna*. These two homeopathic mother tinctures showed excellent inhibition, while *R. toxidendron*, *H. virginiana*, *A. millefolium* and *P. nigrican* were found to be moderately effective. *A. sativum* and *C. officinai*s showed least activity. Hence, most of the homeopathic mother tinctures proved to be effective in inhibiting the *in vitro* growth of clinically important enzyme  $\alpha$ -glucosidase.

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