Pacific Journal of Medical and Health Sciences
ISSN: 2456-7450 | Vol.5, No. 2, 2023, pp - 24 - 39

PJMHS
PACIFIC JOURNAL OF MEDICAL AND HEALTH SCIENCES

Review

Inhibition of α-Amylase Enzyme Activity through Plants: A Promising Approach for Diabetes Management

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ABSTRACT

A class of metabolic disorders known as diabetes is referred by hyperglycemia brought on by abnormalities in insulin production. Postprandial hyperglycemia is ultimately caused by the breakdown of starch by α-amylase, which also generates glucose. One possible treatment strategy for diabetes mellitus involves blocking the α-amylase enzyme to reduce postprandial increase in blood glucose levels. Many of the anti-diabetic drugs such as voglibose, acarbose, and miglitol act as α- amylase inhibitors. Nevertheless, their costs are high and their applications come with unfavourable consequences. Several studies demonstrated the efficacy, safety, and acceptance of natural products and medicinal plants as useful sources of novel anti-diabetic medicines with a strong ability to suppress α-amylase enzyme activity. Therefore, an overview is conducted to find out the plants having in vitro α-amylase inhibitory activity. The analysis of the data reveals that several plant extracts have α-amylase inhibitory activity, which is quite comparable to the standard anti-diabetic drug. Notably, most of the studies have been carried out in recent years indicating the growing interest among researchers to find safer and more effective α-amylase inhibitors from plants.

KEYWORDS: DNSA test, Diabetes, Medicinal Plants, Phytochemicals, Natural products

INTRODUCTION

Diabetes mellitus, a metabolic disorder associated with chronic hyperglycemia, is one of the most common health problems in the world[1-3]. It is predicted[4-5] that it will impact around 800 million adults by 2045. The conditions that cause hyperglycemia include insulin resistance at the cellular level, a decrease in the function of the pancreatic beta cells that secrete insulin, and abnormal metabolism of proteins, lipids, and carbohydrates[6,7]. Diabetic patients suffer from additional conditions badly impacting their health for example, high blood pressure, persistent increase in systemic adrenergic activity, dyslipidemia etc. eventually leading towards organ failure or malfunction, especially in the kidneys, eyes, nerves, blood vessels, and heart[8-11].

Therefore, management of stable blood glucose is the only strategy that is successful in treating diabetes. In this context, inhibition of two digestive enzymes, namely, α-glucosidase and α-amylase is an important strategy[12]. These inhibitors alter the environment in the body such that there is a delay in breakdown of carbohydrates and the bloodstream's absorption which decreases the level of blood glucose generated after a meal[13,14].

The α-1,4-glucan-4-glucanohydrolases; known as α–amylase (E.C. 3.2.1.1) is an important enzyme used for carbohydrate digestion; especially glycogen and starch. This enzyme is not only present in microorganisms, but also in plants and higher organisms. It is present in pancreatic juice and saliva. α–amylase is a calcium metalloenzyme which functions as a catalyst and makes it easier for polysaccharide molecules like amylose, amylopectin, glycogen, and other maltodextrins to hydrolyze their α-1,4 glycosidic linkages[15]. Inhibition of this enzyme lowers down the carbohydrate digestion and thereby reduces the entry of carbohydrates into bloodstream. This is eventually helpful for diabetic patients. Moreover, inhibition of α–amylase is treated as a prophylactic treatment for high blood sugar levels[16,17].

Conventional anti-diabetic drugs such as miglitol, acarbose, and voglibose are effective against the α-amylase enzyme. However, due to the lack of specificity associated with these drugs, several unwanted gastrointestinal side effects, including cramps, stomach distention, flatulence, and diarrhoea, have been reported[18,19]. Therefore, the search for new α-amylase and α-glucosidase inhibitors is essential for the control of blood sugar in diabetes mellitus. Natural compounds are widely used for the production of novel medications and are increasingly being used to produce hypoglycemic agents[20-22]. Several *in vitro* investigations have shown the inhibitory effect of medicinal plants on the activities of α-amylase and α-glucosidase. These therapeutic actions are attributed to the secondary metabolites found in the plants[17,23,24]. The present review aims to investigate the potential therapeutic benefits of plants and their phytochemical constituents for the inhibition of α-amylase to treat Diabetes mellitus.

METHODOLOGY

An exhaustive literature search was carried out on the online databases such as Pubmed, Google Scholar, Scopus, Springer Link, Science Direct, and Research gate using the keywords, α-amylase, diabetes mellitus, inhibition, medicinal plants, plant extract, antidiabetic, *in vitro* etc. to find out the suitable references during the period of last 20 years *i.e.* from 2004 to 2023. The relevant papers were studied in detail and results of those studies as *in vitro* α-amylase inhibition property of plants is given below.

In vitro α-amylase inhibition

Some of the plants traditionally used for treatment of diabetes in Africa and Europe were screened for their *in vitro* α-amylase inhibition potential. A moderate inhibition activity (45-75%) was found in *Camellia sinensis, Khaya senegalensis, Melissae officinalis, Rosamarinus officinalis* and *Balanites aegyptiaca.* Leaves of *Vaccinium myrtillus* exhibited more than 75% inhibition. Leaves of *Mitragyna inermis* exhibited an inhibition of 75% whereas leaves of *Tamarindus indica* demonstrated 90% inhibition. The standard drug acarbose demonstrated 85% inhibition[25].

In vitro antidiabetic efficacy of hexane extract of *Phyllanthus amarus* was evaluated by Ali *et al*.[18] By extracting and fractionating the hexane extract of *P. amarus*, dotriacontanyl docosanoate, triacontanol, and a combination of oleanolic acid and ursolic acid were obtained. Every compound underwent the α-amylase inhibition experiment; the findings indicated that the oleanolic acid and ursolic acid (2:1) combination was a potent α-amylase inhibitor, with an IC_{50} value of 2.01 μg/mL. α-amylase was found to be inhibited by lupeol, ursolic acid, and oleanolic acid.

Bhandari *et al*.[26] investigated *in vitro* anti-diabetic efficacy and identified the active components from *Pakhanbhed* (*Bergenia ciliata*). Two active compounds, (−)-3-O-galloylepicatechin and (−)-3-O-galloylcatechin, were isolated for the first time from this plant species. Against rat intestinal porcine pancreatic α-amylase, these isolated compounds exhibited strong dose-dependent enzyme inhibitory action, with an IC_{50} value of 739 μM for [(−)-3-O-galloylepicatechin] and 401 μM for [(−)-3-O-galloylcatechin]. According to Loizzo *et al*.[27], the methanol extracts of *Marrubium radiatum* and *Salvia acetabulosa* exhibited the strongest activity against α-amylase, with IC_{50} values of 61.1 and 91.2 mg/mL, respectively.

Subramanian *et al*.[28] evaluated *in vitro* and *in vivo* antidiabetic activity of ethanolic extract of *Andrographis paniculata* and its bioactive molecule, andrographolide. The extract revealed significant *in vitro* α-amylase inhibitory effect in a concentration-dependent manner (IC_{50}= 50.9 ± 0.17 mg/mL). However, andrographolide displayed strong inhibitory activity (IC_{50}=11.3 ± 0.29 mg/mL). The *in vivo* investigations showed that when oral starch and sucrose administration was given to diabetic rats, *A. paniculata* extract considerably ($P<0.05$) decreased the animals' peak blood glucose and area under the curve and andrographolide also significantly ($P<0.05$) decreased the area under the curve and peak blood glucose in diabetic rats.

In vitro α-amylase inhibitory activity against porcine pancreatic amylase (PPA) was assessed by Tamil *et al*.[29] using three extracts of *Phyllanthus amarus*: ethanol, hexane, and chloroform. A rotary evaporator was used to evaporate the extracts under low pressure after they were prepared sequentially with chloroform, ethanol, and hexane. The extracts were produced at different concentrations (10, 20, 40, 60, 80, and 100 μg/mL) and then subjected to the α-amylase inhibitory experiment using dimethyl sulfoxide (DMSO) as the substrate. The absorbance was noted at 595 nm. The activity of α-amylase was not inhibited by the chloroform extract. However, in comparison to the standard drug acarbose (IC_{50} 83.33 ± 0.34 μg/mL), the hexane and ethanol of *P. amarus* shown substantial α-amylase inhibitory activity, with IC_{50} values of 48.92 ± 3.43 μg/mL and 36.05 ± 4.01 μg/mL, respectively. Veeramani *et al*.[30] reported *in vitro* α-amylase inhibitory potential of ethanolic flower and leaf extracts of *Catharanthus roseus* (Fig. 3) using DNSA test. The flower and leaf extracts displayed IC_{50} values of 12.5mg/mL and 10 mg/mL, respectively.

In vitro α-amylase inhibition of three distinct isopropanol extracts was investigated by Sudha *et al*.[31]. IC_{50} value of 540 µg/mL was obtained for *Linum usitatissumum* seeds, IC_{50} of 8.9 µg/mL for *Ocimum tenuiflorum* leaves, and IC_{50} of 1440 µg/mL for *Morus alba* was observed. Acarbose, the reference drug, showed an IC_{50} value of 10.2 µg/mL.

Saha and Verma[32] evaluated the α-amylase inhibition test to determine the *in vitro* antidiabetic activity of three different plants: *Eugenia cumini* (seeds), *Terminalia arjuna* (stem bark), and leaves of *Aegle marmelos* (Fig. 1). Fifty percent methanolic extracts of *A. marmelos*, *E. cumini*, and *T. arjuna* showed α-amylase inhibitory action at 50-500 µg/mL concentrations, with IC_{50} values of 503 ± 0.28 µg/mL, 632 ± 0.21 µg/mL, and 302 ± 0.55 µg/mL, respectively. However, the lowest levels of inhibitory action were shown by the 100% methanol extracts of all the three plants.

Kazeem *et al*.[33] demonstrated *in vitro* α-amylase inhibitory activity of different extracts of *Morinda lucida*. Aqueous extract revealed the maximum α-amylase inhibitory with IC_{50} value of 2.30 mg/mL, when compared with ethanolic and acetone extracts. The n-hexane fraction obtained from hydro-methanolic (2:3) extract of *Salmalia malabarica* (Fig. 2) sepals revealed α-amylase inhibition with the IC_{50} value of 50.17 mg/L whereas the standard drug acarbose revealed an IC_{50}value of 47.19 µg/ml[34].

Prabhakar *et al*.[35] investigated *in vitro* α-amylase inhibitory efficacy of aqueous and methanolic extracts of different medicinal plants. The highest percentage of α-amylase inhibition was found in the aqueous extract of *Withania somnifera* (Fig. 4) leaves (92.7%) and the methanolic extract of *Ocimum sanctum* leaves (92.6%) followed by the methanolic bark extract of *Azadirachta indica* (91%), the aqueous extracts of *Curcuma longa* (90.9%), *O. sanctum* (90.3%), and the methanolic leaf extract of *A. indica* (90%). The extracts with the lowest levels of α-amylase inhibition were the methanolic extract of *W. somnifera* (65.1%) and the aqueous bark extract of *A. indica* (77%). *In vitro* α-amylase inhibitory activity of ethanolic extract of *Senna surattensis* (IC_{50} value 123.95 µg/mL) was studied by Thilagam *et al.*[36] using 3,5-Dinitrosalicylic acid assay (DNSA).

The butanolic extract of *Zhumeria majdae* was examined by Mirshafie *et al*.[37] for α-amylase inhibition at different concentrations (15-30 mg/mL), using acarbose as standard. The α-amylase enzyme was inhibited in a dose-dependent fashion by the butanol extract. The extract inhibited activity of the enzyme by 77.9 ± 2.1% at 30 mg/mL, while acarbose inhibited enzyme activity by 73.9 ± 1.9% at 20 mg/mL. IC_{50} values were found to be 24.5 ± 2.1 mg/mL and 6.6 ± 3.1 mg/mL for the butanol extract and acarbose, respectively.

Afrisham *et al*.[38] used the DNSA approach to test the *in vitro* α-amylase inhibitory effect of *Heracleum persicum* and *Ziziphus jujuba*. In comparison to the reference drug, acarbose (IC_{50} of 113µg/mL), the methanolic extracts of *Z. jujuba* and *H. persicum* demonstrated strong inhibitory efficacy against α-amylase, with IC_{50} values of 867µg/mL and 307µg/mL, respectively.

Poovitha and Parani[39] performed the DNSA test to inhibit the α-amylase enzyme to examine the possible antidiabetic properties of protein extracts from the fruits of two different types of bitter gourd (*Momordica charantia* L.). It was discovered that the α-amylase activity was inhibited in a dose-dependent manner between 0.5 and 2.5 mg/mL by *Momordica charantia* var. *charantia* (MCC), *Momordica charantia* var. *muricata* (MCM), and acarbose protein extracts. The highest inhibition of α-amylase activity by protein extracts from MCC, MCM, and acarbose was 66.5%, 67.0%, and 68.0% at a concentration of 2.5 mg/mL, respectively. The protein extracts of MCC, MCM, and acarbose have shown IC_{50} values of 0.267 ±0.024, 0.261 ±0.019, and 0.258 ±0.017 mg/mL, respectively.

Wickramaratne *et al*.[40] analysed *in vitro* α-amylase inhibitory potential of various extracts of leaves of *Adenanthera pavonina* employing DNSA method. The IC_{50} values of water, petroleum ether, ethanol and methanol extracts were found as 214.85 ± 9.72, 145.49 ± 4.86, 59.93 ± 0.25 and 16.16 ± 2.23 µg/mL, respectively and whereas the standard drug acarbose exhibited an IC_{50} value of 18.63 ± 1.21 µg/mL.

Jaiswal and Kumar[41] reported *in vitro* α-amylase inhibition potential of bark of *Albizia lebbeck* (L.) Benth. The maximum enzyme inhibition (43.50±0.17% to 73.43±0.08%) was showed by free flavonoid extract with IC_{50} value 0.6653 mg/mL followed by water, methanol and bound flavonoid extracts *i.e*., 26.67±0.12% to 32.07±0.17%, 26.70±0.15 to 32.67±0 .12% and 28.63±0.15% to 37.50±0.20%, with IC_{50} value of 22.28 mg/mL, 41.68 mg/mL and 7.36 mg/mL, respectively.

Bhosale *et al*.[42] screened *in vitro* α-amylase inhibitory activity of aqueous extracts of five plants *viz*., leaves and rhizome of *Curcuma longa* and leaves of *Azadirachta indica*, *Moringa oleifera*, *Murraya koenigii* (Fig. 5) and *Psidium guajava* using DNSA assay. Highest α-amylase inhibition activity was revealed by aqueous extract of leaves of *C. longa* with IC_{50} values of 0.53±0.10 followed by rhizome of *C. longa* 0.96±0.29, leaves of *Moringa oleifera* 1.24 ± 0.49, leaves of *Azadirachta indica* 1.54 ± 0.59, leaves of *Murraya koenigii* 1.57 ± 0.76 and leaves of *Psidium guajava* 4.50 ± 0.38 mg/mL, respectively in comparison with the standard drug acarbose having an IC_{50} value of 0.15 ± 0.11 mg/mL.

The antidiabetic efficacy of *Wedelia chinensis* methanolic extract was assessed by Thao *et al*.[43]. A bioactive compound jaceosidin had the strongest effect on α-amylase, with an IC_{50} value of 112.8 ± 15.1 µg/mL, and was found quite similar to acarbose, which had an IC_{50} value of 124.0 ± 21.3 µg/mL. Nevertheless, wednenic, pomonic, and pomolic acid showed a modest level of inhibition against α-amylase. Unuofin *et al*.[44] evaluated *in vitro* α-amylase inhibitory activity of aqueous and ethanol extracts of tuber of *Kedrostis africana* (L.) Cogn using the starch iodine assay and found the inhibition in a dose-dependent manner. At the concentrations 50, 100, and 200 µg/mL, aqueous and ethanol extracts exhibited inhibition

of 19.85 ± 0.37% to 31.64 ± 1.11% and 13.91 ± 1.55% to 20.14 ± 0.63%, respectively. However, the positive control acarbose revealed 92.98 ± 1.37% inhibition at 64 µg/mL. The IC_{50} values were 439.45 ± 1.95 and 949.75 ± 3.68 µg/mL for aqueous and ethanol extracts, respectively.

Ahmed *et al*.[45] reported *in vitro* α-amylase inhibitory property of miquelianin from *Euphorbia schimperi* with an IC_{50} value of 128.34 ± 12.30 µg/mL, and acarbose revealed IC_{50} value 64.20 ± 5.60 µg/mL. Methanolic stem bark extracts of *Maesobotrya dusenii* was evaluated by Mikailu *et al*.[46] for α-amylase inhibition activity. The extract exhibited a dose-dependent inhibition of α-amylase, even though the percentage of the standard drug, acarbose, was greater at 64 percent than that of crude methanol at 56.7% at 50 µg/mL. The methanol extract and acarbose were found to have IC_{50} values of 24 and 28µg/mL, respectively. *In vitro* and *in vivo* antidiabetic effects of *Terfezia claveryi* methanol extract were evaluated by AlAhmed and Khalil[47]. *T. claveryi* extract demonstrated a greater α-amylase inhibitory activity (IC_{50} = 38.7 µg/ml) than the positive control (IC_{50} value = 45.3 µg/ml) of acarbose. Moreover, the *T. claveryi* methanol extract, at a dosage of 200 mg/kg, also lowered the fasting plasma glucose level in the animal study.

The *in vitro* antidiabetic potential of three distinct extracts of leaves, stem bark, and root bark of *Alstonia boonei* was examined by Oyebode *et al*.[48]. The α-amylase enzyme was shown to be effectively inhibited by all of the extracts. However, ethanol extracts of leaves, stem bark, and root bark, demonstrated significant ($p < 0.05$) inhibition with IC_{50} values of 102.93, 16.78, and 129.06 µg/mL, respectively.

Hawash *et al*.[49] reported *in vitro* α-amylase inhibitory activities of hydrophilic and lipophilic fractions of leaves of *Arum palaestinum*, *Malva sylvestris, Plantago major*, *Centaurea iberica, Cichorium endivia, Bituminaria bituminosa* and *Sisymbrium irio*. The highest enzyme inhibition activity was found in lipophilic fractions of *S. irio* with an IC_{50} value of 7.72 µg/mL, followed by hydrophilic fraction of *C. endivia* (9.96 µg/mL). However, positive control, acarbose revealed an IC_{50} value of 10 µg/mL. The hydrophilic fractions of leaves of *P. major, M. sylvestris, A. palaestinum, S. irio, B. bituminosa and C. iberica* inhibited α-amylase with IC_{50} values of 352.31, 38.55, 573.72, 180.43, 180.43 and 12.33 µg/mL, respectively. The lipophilic fractions of leaves of *P. major, A. palaestinum, C. endivia,* and *B. bituminosa* inhibited the enzyme with IC_{50} values of 61.35, 25.34, 300.92, and 529.49 µg/mL, respectively.

The *in vitro* anti-α-amylase activity of different fractions of *Prosopis cineraria* pod extract was assessed by Kumar *et al*.[50]. The most effective fraction to inhibit α-amylase was n-butanol (IC_{50} 22.01 ± 0.92 µg/mL) followed by ethyl acetate fraction (IC_{50} of 28.23 ± 1.06 µg/mL). However, the IC_{50} of acarbose, the reference drug, was 39.26 ± 2.19 µg/mL. The *in vitro* α-amylase inhibition potential of leaf latex of *Aloe megalacantha* Baker and leaf of *Aloe monticola* Reynolds were examined using DNSA method. The IC_{50} values found for *A. megalacantha* and *A. monticola* were 74.76 ± 1.98 and 78.10 ± 1.88 µg/mL, respectively against the α-amylase. Acarbose, the standard drug, revealed an IC_{50} value of 16.49 ± 1.91 µg/mL[51].

Nine plants were evaluated for α-amylase inhibitory action by Hussein *et al*.[52]. With the highest percentage of inhibition (95.5%), *Raphanus raphanistrum* was found to be the most effective among them. Other plants that showed the highest percentage of inhibition were *Citrus limon*, *Matricaria chamomilla*, *Punica granatum*, *Allium sativum*, *Syzigium aromaticum*, *Zingiber officinale*, *Beta vulgaris* and *Nigella sativa* with corresponding inhibition percentages of 87.3, 83.5, 81, 72, 66.6, 60.6, 59.4 and 9.0.

Khadayat *et al*.[53] demonstrated α-amylase inhibition potential of *Swertia chirata*, *Dioscorea bulbifera,* and *Acacia catechu* with IC_{50} values 413.5, 296.1, and 49.9 µg/mL, respectively. However, the standard drug acarbose exhibited an IC_{50} value 6.1 µg/mL. Two triterpenes, namely, 3-oxolupenal and katononic acid were isolated from n-hexane fraction of the methanolic crude extract of *Nuxia oppositifolia* and evaluated for *in vitro* α-amylase inhibitory potential. The IC_{50} values of 46.2 µg/mL and 52.4 µg/mL were observed for 3-oxolupenal and katononic acid, respectively in comparison with control acarbose having IC_{50}of27.3 µg/mL[54].

According to Gök *et al*.[55], ethyl acetate extract of *Rhus coriaria* L. leaf inhibited α-amylase *in vitro* with an IC_{50} value of 20.810 ± 0.747 µg/mL, while acarbose showed an IC_{50} value of 26.993 ± 0.797 µg/mL. Notably, a bioactive compound, penta-*O*-galloyl-β-glucopyranose isolated from both fruit and leaf extracts of *R. coriaria* inhibited α-amylase with an IC_{50} value of 6.32 ± 0.18 µM. Hoang Anh *et al*.[56] demonstrated the α-amylase inhibitory property of *Clausena indica* fruits. The hexane and ethyl acetate extracts inhibited the α-amylase with IC_{50} values of 1.37 ± 0.01 and 8.56 ± 0.24 mg/mL, respectively. Remarkably, α-amylase suppression by acarbose was having an IC_{50} value of 0.07 ± 0.00 mg/mL. Jaradat *et al*.[57] reported that the acetone extract of *Nonea obtusifolia* leaves had a more effective α-amylase inhibitory effect when compared to acarbose, with IC_{50} values of 25.7 ± 0.08 µg/mL and 28.18 ± 1.22 µg/mL, respectively.

Kirisanth *et al*.[58] investigated *in vitro* α-amylase inhibitory activities of six different bryophyte species *viz*. *Calymperes motley*, *Fissidens* sp., *Hypnum cupressiforme*, *Marchantia* sp., *Plagiochila* sp. and *Sematophyllum demissum* using DNSA method. The ethyl acetate extract of *Fissidens* sp. exhibited the maximum inhibitory activity (39%) followed by *Marchantia* sp. (23%), *Plagiochila* sp. (12%) and *H. cupressiforme* (8%). However, positive control acarbose had shown 66% inhibitory activity. *C. motley* and *S. demissum* were found inactive for α-amylase inhibition activity.

Pandey *et al*.[59] evaluated *in vitro* α-amylase inhibitory activity of different extracts of *Bergenia pacumbis* using DNSA method. The methanol extract revealed the highest inhibition (IC_{50} = 14.03 ± 0.04 µg/mL) followed by ethyl acetate extract (29.91 ± 0.22 µg/mL), and water (43.77 ± 0.54 µg/mL). Acarbose, a standard drug showed an IC_{50} value of

20.12 ± 0.12 µg/mL.

Panigrahy *et al.*[60] evaluated *Hedychium coronarium* rhizome for α-amylase inhibition potential. The ethyl acetate fraction of rhizome inhibited α-amylase activity with IC_{50} value of 58.15 ± 1.23 µg/mL. The hypoglycemic properties of *Melilotus officinalis* and *Anchusa officinalis* were assessed by Paun *et al.*[61]. The highest α-amylase inhibitory activity was found in the crude extract of *M. officinalis* (IC_{50} = 1.32 ± 0.08 µg/mL), followed by that of *A. officinalis* (954.16 ± 7.46 µg/mL). However, the IC_{50} value of acarbose was 17.68 ± 1.24 µg/mL.

Momina and Rani[62] evaluated *in vitro* α-amylase inhibitory activity of methanolic leaf extracts of *Bambusa vulgaris*, *Lindernia ciliata* and *Phyllanthus reticulatus* (Fig. 6). At a concentration of 10mg/mL the methanolic extracts of *B. vulgaris*, *L. ciliata*, *P. reticulatus* and acarbose exhibited 69.5%, 83.1%, 72% and 97.3% α-amylase inhibitory activity, respectively. Among all the extracts, *L. ciliata* revealed significant α-amylase inhibition activity with IC_{50} 6.11 mg/mL which was quite comparable with an IC_{50} value of 5.03 mg/mL revealed by the standard drug acarbose. Quek *et al.*[63] investigated *in vitro* α-amylase inhibitory activity of different extracts of stem bark and leaves of *Melicope glabra*. The chloroform extract of leaves was obtained to be the most effective towards inhibition of α-amylase with IC_{50} of 303.64 µg/mL followed by chloroform extract of stem bark IC_{50} 975.80 ± 17.10, methanol leaves IC_{50} 2488.13 ± 231.54, methanol stem bark IC_{50} 3946.12 ± 143.21, hexane leaves IC_{50} 4230.12 ± 324.76, and hexane stem bark extracts IC_{50} 5447.01 ± 243.16 µg/mL.

Eriobotrya japonica leaves were tested for their *in vitro* antidiabetic potential by Mogole *et al.*[64]. Various extracts were tested against the activity of α-amylase, with acarbose serving as the control. Hexane extract had the greatest α-amylase inhibitory activity of 24% at a concentration of 1 µg/mL when compared to other extracts. *In vitro* α-amylase inhibitory activity of methanol extracts of *Oroxylum indicum* leaf (OIME) and *Rauvolfia tetraphylla* root (RTME) was shown by Swargiary and Daimari[65]. At a dose of 2 mg/mL of the extracts, the percent inhibitions for OIME, RTME, and acarbose were reported as 70.96%, 38.50%, and 59.80%, respectively.

Daoudi *et al.*[66] examined the α-amylase inhibitory effect of roasted (Roil) and unroasted (UnRoil) *Argania spinosa* seed oil *in vitro*, *in vivo*, and *in situ*. The findings demonstrated that, *in vitro*, pancreatic α-amylase was considerably ($p < 0.001$) inhibited by both Roil and UnRoil, with IC_{50} values of 2.17 ± 0.24 mg/mL and 0.78 ± 0.16 mg/mL, respectively. These were found quite comparable with acarbose (0.41 ± 0.015 mg/mL). Moreover, oral administration of these oils at a dosage of 2 mL/Kg reduced blood sugar in normal and STZ-diabetic rats.

Thengyai *et al.*[67] reported α-amylase inhibitory potential of the ethanol extract of the stem bark of *Vitex glabrata.* Six bioactive compounds *viz.,* α-amyrin, β-amyrin, betulin, betulinic acid, lupeol, and scopoletin *were isolated from V. glabrata stem bark* and the maximum α-amylase inhibitory activity was observed by β-amyrin (IC_{50} 32.33 µM). Rocamora *et al.*[68] reported *in vitro* α-amylase inhibition of essential oil derived from leaves of *Backhousia citriodora, Mentha piperita, Origanum vulgare,* and *Rosmarinus officinalis.* Inhibition of α-amylase found by *Mentha piperita* was (IC_{50} 0.41 mg/mL) followed by *Origanum vulgare* (IC_{50} 0.41 mg/mL), *Rosmarinus officinalis (IC_{50} 0.45 mg/mL),* and *Backhousia citriodora* (IC_{50}0.49 mg/mL).

Anigboro *et al.*[69] examined *in vitro* α-amylase inhibitory activity of leaf extract of *Justicia carnea* using DNSA method. A dose-dependent significant ($p<0.05$) reduction in α-Amylase activity (IC_{50} value 671.43±1.88 µg/mL) was exhibited by leaf extract. The IC_{50} value of standard acarbose was found to be 108.91±0.61 µg/mL. Quek *et al.*[70] reported α-amylase inhibitory activity of different extracts of *Melicope latifolia* bark. The maximum inhibition was revealed by chloroform extract with IC_{50} value of 1464.32 ± 312.19 µg/mL followed by methanol extract (2941.17 ± 113.72 µg/mL) and hexane extract (8113.15 ± 103.15 µg/mL).

Renganathan *et al.*[71] demonstrated *in vitro* antidiabetic potential of 70% ethanolic leaf extract of *Leucaena leucocephala* (Lam.) De Wit. The leaf extract inhibited α-amylase activity in a concentration-dependent way (IC_{50} = 288.01 µg/mL), while acarbose inhibited α-amylase with an IC_{50} value of 252.59 µg/mL. Choudhary *et al.*[72] analysed *in vitro* α-amylase inhibitory activity of various fractions of *Chenopodium album* L. The aerial parts of *C. album* were fractionated into different fractions, *i.e.,* alkaloid fraction (CAAF), flavonoid fraction (CAFF), saponin fraction (CASF) and tannin fraction (CATF). The *in vitro* assay revealed that CAFF was found to be more significant α-amylase inhibitory than the reference drug acarbose having IC_{50} values of 122.18 ± 1.15 and 812.83± 1.07 µg/mL, respectively. *In vivo* antidiabetic potential was screened using a high-fat diet and streptozotocin-induced diabetic mice. In both *in vitro* and *in vivo* diabetes models, the CAFF fraction was reported to have strong antidiabetic efficacy in a dose-dependent manner. On days 22 and 29, the levels of plasma glucose, total cholesterol, and total triglycerides were compared. The rise in glucose, cholesterol, and triglyceride levels, were reduced significantly after seven days administration of CAFF fraction at a dose of 500 mg/kg.

Abolaji *et al.*[73] examined *in vitro* antidiabetic potential of acetone extract of *Ziziphus mucronata* (AEZM) through determination of its α-amylase inhibition potential. The extract exhibited a dose-dependent rise in α-amylase inhibition. At a concentration of 1.0 mg/mL, AEZM and the standard drug, voglibose revealed (71.02%) and (83.47%) inhibition, respectively. Additionally, IC_{50} values for AEZM and voglibose were found as 0.62 and 0.42 mg/mL, respectively.

Methanolic extract of aerial parts of Phragmites *karka* was investigated for antidiabetic potential through α-amylase inhibition by Mazumder *et al.*[74]. Using the iodine starch and DNSA techniques, a significant inhibition of the enzyme was

shown in the α-amylase enzyme inhibitory test, with IC_{50} values of 2.05 and 2.08 mg/mL, respectively. Sani *et al*.[75] evaluated the α-amylase inhibitory activity of *Arachis hypogaea* and *Cinnamomum tamala*. The ethanol extract from peanut (*A. hypogaea*) seeds demonstrated α-amylase inhibition activity (67.68±8.67%) at 1.25 µg/mL concentration, with an IC_{50} value of 0.61µg/mL. This is extremely near to the standard α-amylase inhibitor acarbose (72.34±4.23%) with an IC_{50} value of 0.32 µg/mL. Similarly, the acetone extract from Indian bay (*C. tamala*) leaf showed α-amylase inhibition activity (47.75±1.63%) at 1.42 µg/mL at the same concentration.

Sen *et al*.[76] reported *in vitro* α-amylase inhibitory activity of the essential oil obtained from the aerial parts of *Centaurea pterocaula* Trautv. An IC_{50} value of 79.66 ±0.43 µg/mL was found for α-amylase inhibition. However, the standard drug acarbose had an IC_{50} value of 11.6 ± 0.18 µg/mL. Silva *et al*.[77] reported *in vitro* α-amylase inhibition of hexane fraction from Brazilian *Morus nigra* leaves. The α-amylase inhibitory activity of hexane fraction was found with an IC_{50} value of 13.05 mg/mL whereas acarbose had an IC_{50} value of 0.21 mg/mL.

Saraswathi *et al*.[78] evaluated *in vitro* α-amylase inhibition of aqueous and ethanolic and aqueous extracts of *Solanum virginianum* dried fruits at different doses (20–120 µg/mL). In a concentration-dependent manner, the aqueous extract (54.12 ± 0.44–86.80 ± 0.27%) showed a considerably ($P<0.05$) greater rate of α-amylase inhibition than the ethanolic extract (23.07 ± 0.47–81.61 ± 0.43%). At all the doses, α-amylase was considerably ($P<0.05$) more inhibited by standard drug acarbose (58.36 ± 0.30–88.24 ± 0.16%) rather than by aqueous and ethanolic extracts. According to Prasathkumar *et al*.[79], methanolic extract of *Senna auriculata* (L.) Roxb. leaves showed α-amylase inhibition with an IC_{50} value of 49.45 µg/mL.

Yashoda *et al*.[80] investigated the ability of methanolic extracts of *Achyranthes aspera* and *Catharanthus roseus* to inhibit the α-amylase enzyme using DNSA test. The inhibition of α-amylase by *A. aspera* and *C. roseus* was determined to be 97.60±1.11 µg/mL and 94.05±1.18 µg/mL, respectively, in comparison to the IC_{50} of 68.13±0.46 µg/mL of reference drug acarbose. Bello *et al*.[81] observed that the *Eucalyptus globulus* plant's both leaf DEE ethanol extract (hexane defatted) and NEE ethanol extract (non-defatted) exhibited α-amylase inhibitory action. When compared to acarbose, the extracts showed a discernible suppression of α-amylase. The α-amylase inhibition IC_{50} values for DEE, NEE, and acarbose were 23.6 ± 1.2 µg/mL, 14.8 ± 1.2 µg/mL, and 5.2 ± 1.3 µg/mL, respectively.

In vitro α-amylase inhibitory activity of crude methanolic extract of *Pastinaca sativa* (CEPS) was determined by starch iodine test. IC_{50} values for CEPS and acarbose were found as 91.69 ± 1.5 µg/mL and 83.25 ± 1.28 µg/mL, respectively. CEPS also exhibited *in vivo* blood sugar lowering effect in alloxan-induced diabetic rats. Blood glucose levels decreased from 208.33 mg/dL to 106.38 mg/dL and from 209.82 mg/dL to 111.65 mg/dL after administration of 200 and 400 mg/kg CEPS, respectively. These results were comparable to standard drug glibenclamide (0.5 mg/kg) which exhibited a significant drop from 205.55 mg/dL to 84.88 mg/dL on the seventh day[82].

Mechchate *et al*.[83] observed that the hydroethanolic leaf extract of *Withania frutescens* L. significantly inhibited α-amylase in dose-dependent manner. Notably, the plant extract (IC_{50} 0.40 ± 0.124 mg/mL) demonstrated higher *in vitro* α-amylase inhibition as compared to acarbose (0.717 ± 0.054 mg/mL). The ethanolic extract of *Moringa oleifera* flower demonstrated a significant ($p < 0.05$) dose-dependent inhibition against α-amylase (IC_{50} = 37.63 mg/mL) as compared to the standard drug acarbose[84]. Shanak *et al*.[85] reported α-amylase inhibitory potential of methanolic extract of aerial parts of *Ocimum basilicum*. A 500 µg/mL concentration, the plant extract demonstrated 25.4% ± 3.3 α-amylase inhibition.

Siegień *et al*.[86] screened α-amylase inhibitory potential of aqueous and ethanolic extracts of twelve plants *viz*., *Hibiscus sabdariffa, Chaenomeles japonica, Hippophae rhamnoides, Berberis vulgaris, Rosa canina, Quercus spp., Sorbus aucuparia, Juglans regia, Sambucus nigra, Aronia melanocarpa, Artemisia dracunculus,* and *Humulus lupulus. H. sabdariffa* flower revealed the highest inhibitory activity of α-amylase with IC_{50} values of 35.81 ± 3.660 and 40.22 ± 2.898 µg/mL for aqueous and ethanolic extracts, respectively followed by *C. japonica* fruit aqueous extract (53.61 ± 5.074) and ethanolic extract (48.69 ± 4.993), *H. rhamnoides* fruit aqueous extract (83.01 ± 7.840) and ethanolic extract (92.99 ± 7.804), *B. vulgaris* fruit aqueous extract (252.9 ± 27.59) and ethanolic extract (378.0 ± 44.94), *R. canina* fruit aqueous extract (823.3 ± 107.6) and ethanolic extract (401.9 ± 71.97), *Quercus* spp. fruit aqueous extract (1123 ± 133.3) and ethanolic extract (1550 ± 129.7), *S. aucuparia* fruit aqueous extract (1236 ± 177.0) and ethanolic extract (973.9 ± 61.60), *J.regia* fruit aqueous extract (1479 ± 183.6) and ethanolic extract (295.0 ± 74.04), *S. nigra* fruit aqueous extract (2091 ± 160.1) and ethanolic extract (2259 ± 344.4), *A. melanocarpa* fruit aqueous extract (2632 ± 208.5) and ethanolic extract (1130 ± 91.19), *A. dracunculus* herb aqueous extract (6778 ± 405.4) and ethanolic extract (2824 ± 273.0) and *H. lupulus* flower aqueous extract (9249 ± 525.0) and ethanolic extract (7215 ± 784.7) µg/mL. However, IC_{50} for the reference drug was found as 2.4 ± 0.4 µg/mL.

In vitro α-amylase inhibitory activity of *Catunaregam spinosa* leaf and bark methanol extracts was conducted by Timalsina *et al*.[87]. The α-amylase inhibitory activity of the bark methanol extract was also evaluated for the hexane, dichloromethane, ethyl acetate, and water-soluble fractions. The IC_{50} value of the crude bark extract (94.66 ± 2.19 µg/mL) was lower than that of the crude leaf methanolic extract (119.7 ± 2.79 µg/mL), suggesting that the former was more potent. The ethyl acetate and dichloromethane fractions exhibited IC_{50} values 116 ± 1.60 and 77.17 ± 1.75 and µg/mL, respectively whereas the standard acarbose revealed an IC_{50} of

6.34 ± 0.07 µg/mL.

Bakshi *et al*.[88] screened *in vitro* α-amylase inhibitory potential of methanol extracts of *Azadirachta indica*, *Bauhinia variegata*, *Dalbergia sissoo*, *Psidium guajava*, and *Syzygium cumini* leaves. Notably, *S. cumini* and *B. variegata* exhibited strong inhibitory effects against α-amylase with IC_{50} values of 24.69 ± 0.91and 27.28 ± 6.11 µg/mL, respectively.

Acetone extracts of *Artemisia pallens* Wall ex DC. leaf and bud were evaluated for their *in vitro* α-amylase inhibitory action[89]. The extract efficiently suppressed PPA with an IC_{50} of 388.05 µg/mL, while acarbose, a positive control and known inhibitor of pancreatic amylase, had an IC_{50} of 9.71 µg/mL. The plant extract at increasing concentration of 62.5 µg/mL, 125 µg/mL, 187.5 µg/mL, 250 µg/mL, and 312.5 µg/mL demonstrated 28.36%, 35.05%, 38.93%, 43.45%, and 46.19% inhibitory activity in an increasing manner.

Dar *et al*.[90] examined the α-amylase inhibitory activity of the methanolic heartwood extract of *Pterocarpus marsupium* (MHPM). A strong dose-dependent α-amylase inhibitory action was shown by MHPM, with an average inhibition of 66.441 ± 3.459% at 500 µg/mL and an IC_{50} value of 158.663 ± 10.986 µg/mL. At 500 µg/mL, the percentage inhibition of the positive control, acarbose, was 78.410 ± 4.005%, while the IC_{50} value was 56.060 ± 4.465 µg/mL.

Hassan *et al*.[91] evaluated *in vitro α*-amylase inhibition activity of various extracts of *Veronica biloba*. Water extract showed highest inhibition with IC_{50} value of 110.25 *µ*g/mL, followed by ethyl acetate 121.09, dichloromethane 123.68, and n-hexane 148.01 *µ*g/mL extracts. Interestingly, acarbose had an *α*-amylase inhibition activity with IC_{50} value of 138.79*µ*g/mL However, the bound phenolics of *V. biloba* revealed IC_{50} = 219.66 *µ*g/mL.

Karray *et al*.[92] demonstrated *in vitro* α-amylase inhibitory activity of different extracts of *Moringa oleifera* leaf. The methanol extract disclosed the highest α-amylase inhibitory activity (65.6 ± 4.93%), followed by hexane extract (52.3 ± 2.5%). The extracts of water, ethylene acetate, and ethanol showed much lower amylase inhibitory activity, with inhibition rates of 43.3 ± 2.3%, 36 ± 2.6%, and 33 ± 2.6%, respectively. Olaokun *et al*.[93] reported *in vitro* hypogylcemic effect of *Englerophytum magalismontanum.* The crude methanol extract displayed an IC_{50} value 16.16 ± 2.23 µg/mL, while the methanol fraction and standard acarbose revealed $IC_{50\ of}$ 10.76 ± 1.33 and 1.24 ± 1.64 µg/mL. α-Amylase was inhibited by the phenolic compound that was extracted and identified as naringenin, with an IC_{50} of 5.81 ± 2.14 µg/mL. The methanolic leaf extract of *Morus alba* exhibited a dose-dependent α-amylase inhibition (78.55 ± 2.53%) at a dose of 500 µg/mL and an IC_{50} of 74.76 ± 6.76 µg/mL. Nonetheless, at 500 µg/mL, acarbose had 87.67 ± 3.67% inhibition, and the IC_{50} was 35.34 ± 4.87 µg/mL[94].

Prakash[95] examined the potential inhibitory effects of leaf extracts from *Rhododendron arboreum* and *Rhododendron campanulatum* on porcine α-amylase, with concentrations ranging from 0.2 to 1.0 mg/mL. At a dose of 1 mg/mL, *R. arboreum* showed 51.10, 44.00, and 35.40% inhibition for methanol, acetone, and aqueous leaf extracts, respectively. In similar dose of 1 mg/mL, *R. campanulatum* demonstrated α-amylase inhibition of 21.15, 18.25, and 15.85% for methanol, acetone, and aqueous extracts, respectively. Ahmed *et al*.[96] 2022 investigated *in vitro* anti-diabetic activity of *Calligonum polygonoides*; an important desert shrub of Rajasthan. They observed that 80% methanolic extract of *C. polygonoides* whole plant inhibited α-amylase by 70% at a concentration of 1 mg/ml with an IC_{50} of 610 µg/ml However, the standard tagipmet showed an IC_{50} of 424 µg/ml.

Benrahou *et al*.[97] evaluated *in vitro* and *in vivo* α-amylase inhibitory activity of different extracts of *Erodium guttatum.* All three extracts exhibited significant inhibitory impact ($P < 0.05$) on α-amylase, with the methanolic extract of *E. guttatum* exhibiting the strongest effect, showing an IC_{50} of 479.20 ± 0.81 µg/mL. The IC_{50} values of the aqueous and ethanolic extracts were 781.30 ± 0.54 and 498.5 ± 0.81 µg/mL, respectively. Acarbose, the positive control, revealed an IC_{50} of 44.75 ± 0.54 µg/mL. Blood sugar levels were reported to be affected by *E. guttatum* extracts and metformin. The diabetic mice treated with the three extracts plus metformin showed significantly different blood sugar levels on day one compared to the diabetic mice in the normal group who were not treated ($P < 0.05$). Conversely, there was no discernible difference ($P>0.05$) between the groups receiving metformin plus plant extract treatment and the diabetic group receiving no treatment. The results showed that blood sugar levels were considerably lower in the group of diabetic mice treated with *E. guttatum* extracts plus metformin after 30 days ($P < 0.05$).

Shreya Reddy *et al*.[98] reported *in vitro* α-amylase inhibitory ability of ethanolic extracts of *Andrographis paniculata* and *Andrographis echioides*. In a dose-dependent manner (100-500µg/mL), both the extracts significantly ($p<0.05$) increased the α-amylase inhibitory activity. By inhibiting α-amylase *in vitro*, Nisar *et al*.[99] evaluated the antidiabetic effect of *Picrorhiza kurroa* roots. The highest inhibitory activity of root against the α-amylase enzyme was shown by the methanol extract, with an IC_{50} value of 0.39 ± 0.41 mg/mL. Ethanolic and aqueous extracts trailed methanolic extract in terms of highest inhibitory efficacy against α-amylase. The *in vitro* α-amylase inhibitory activity of the ethyl acetate fraction of *Erythropalum scandens* was examined by Adhikari *et al*.[100] showing an IC_{50} value of 44.51 ± 0.12 µg/mL.

Das *et al*.[101] evaluated *in vitro* antidiabetic potential of ethanolic extract of *Coscinium fenestratum* (Gaertn.) Colebr seeds through DNSA method by inhibiting *α*-amylase activity. The percentages of enzyme inhibition activity were found to be 19.46%, 38.19%, 52.09%, and 61.22% at doses of 100, 200, 300, and 400 µg/mL, respectively. For the reference drug, acarbose at the same doses, higher activity was observed (36.11%, 52.10%, 64.28%, and 76.2%). The IC_{50} values for the seed extract and standard were determined to be 3.02 and 1.96 µg/mL, respectively. Interestingly, both the

extract and the standard showed a dose-dependent inhibition of *α*-amylase.

Mariadoss *et al*.[102] investigated the α-amylase inhibitory activity of *Lespedeza cuneata* fractions in methanol, ethyl acetate, and hexane solvents. With an IC_{50} of 205.32 ± 23.47 µg/mL, the ethyl acetate fraction of *L. cuneata* (Lc-EAF) demonstrated the most high α-amylase inhibitory activity among them. An *in vivo* study revealed that administering 100 mg/kg of Lc-EAF maintained blood glucose levels, decreased insulin levels, and enhanced the lipid profile, hepatic, and renal indicators in streptozotocin-induced diabetic rats. Recently, Omar *et al*.[103] have shown that methanolic extract of *Phyllanthus emblica* L. leaves possess significant α-amylase inhibition activity (98.37 ±1.09%).

In vitro α-amylase inhibitory effect of methanol extract of *Phoenix pusilla* ripened fruits (PPRF) was reported by Srinivasan *et al*.[104] on porcine pancreatic α-amylase having an IC_{50} value of 69.86 µg/mL. Ullah *et al*.[105] assessed the *in vitro* α-amylase inhibitory activity of ethanol and aqueous extracts of the seed, root, stem, flower, and gum, of *Acacia modesta*. When the Starch-iodine test was used, the aqueous extract of gum showed the highest inhibitory potential against α-amylase with an IC_{50} value of 91.8 ±0.05 µg/mL. This was nearly three times more than that of the control, acarbose (286.8 ± 0.04 µg/mL). In addition, the gum's ethanolic extract demonstrated strong activity, with an IC_{50} value of 100.4 ± 0.04 µg/mL.

The hypoglycemic effectiveness of raspberry (*Rubus corchorifolius* L.) leaf was reported by Li *et al*.[106]. Using affinity ultra filtration in conjunction with HPLC-MS/MS, eight major bioactive chemicals were identified, including epigallocatechin gallate, delphinidin-3-O-glucoside, cyanidin-3-rutinoside, isoorientin, procyanidin C3, dihydromyricetin, rutin, and isovitexin. Confirmation tests revealed that these compounds were in-charge of α-amylase's inhibitory actions. According to molecular docking studies, it was found that through hydrogen bonding or van der Waals force, these inhibitors may effectively interact with α-amylase. Different leaf extracts were evaluated *in vitro* for their potential to inhibit α-amylase. The extracts with the highest inhibiting activity were 70% ethanol (IC_{50} = 1.26 ± 0.03 mg/mL) and 70% methanol (IC_{50} = 1.47 ± 0.05 mg/mL) followed by aqueous extracts (IC_{50} = 4.39 ± 0.17 mg/mL). The positive control acarbose revealed an IC_{50} of 5.12 ± 0.42 mg/mL. Notably, the extracts of ethyl acetate and acetone showed poor inhibitory action (IC_{50} > 20.00 mg/mL).

Remok *et al*.[107] performed *in vitro* α-amylase inhibitory ability of aqueous extract of *Salvia lavandulifolia* Vahl leaf with an IC_{50} value of 0.99 ± 0.00 mg/mL which was found comparable with the standard drug, acarbose (IC_{50} = 0.52 ± 0.01 mg/mL). Yang *et al*.[108] analysed 16 phenolic compounds found in the ethyl acetate fraction of *Sterculia nobilis* Smith pericarp extract (EAF) using the LC-ESI-MS/MS-MRM technology. Apigegetrin, epicatechin gallate, and luteolin-7-O-glucoside were the main phenolics in the EAF. EAF exhibited reversible and uncompetitive inhibition of α-amylase activity, with an IC_{50} value of 2.151 ± 0.044 mg/mL.

CONCLUSION

Inhibition of alpha-amylase enzyme is a promising strategy towards management of high blood glucose in diabetes mellitus. The present review indicates the therapeutic potential of several plant species through inhibition of alpha-amylase activity and suggests the possibility of developing cheaper and safer plant-derived novel hypoglycaemic molecules. Interestingly, several of these studied plants are used in food for example, *Aegle marmelos, Allium sativum*, *Murraya koenigii, Curcuma longa, Citrus limon*, *Punica granatum*, *Zingiber officinale*, *Phyllanthus emblica, Momordica charantia, Eugenia cumini, Syzigium aromaticum, Moringa oleifera, Psidium guajava, Tamarindus indica, Ziziphus jujuba* etc. This further opens up the avenue for development of some nutraceuticals effective for the treatment of diabetes.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

Figure 1: *Aegle marmelos*

Figure 2: *Bombax ceiba*

Figure 3: *Catharanthus roseus*

Figure 4: *Withania somnifera*

Figure 5: *Murraya koenigii*

Figure 6: *Phyllanthus reticulatus*

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